



# National Toxicology Program

U.S. Department of Health and Human Services

# Annual Report for Fiscal Year 2019



## Letter from the NIEHS and NTP Director

In fiscal year (FY) 2019, NTP continued to advance toxicology and inform public health policy by providing information to decision makers and the public about substances in our environment. NTP scientists published more than 249 peer-reviewed research studies and reports on substances of public health concern, including per- and polyfluoroalkyl substances (PFAS), the organophosphorus nerve agent sarin, and others.

Other notable research achievements in FY 2019 included a pioneering systematic review of whether exposure to traffic-related air pollution (TRAP) during pregnancy is associated with hypertensive disorders of pregnancy. NTP also published final monographs on antimony trioxide and *Helicobacter pylori*, both of which are now proposed for listing in the 15th Report on Carcinogens.

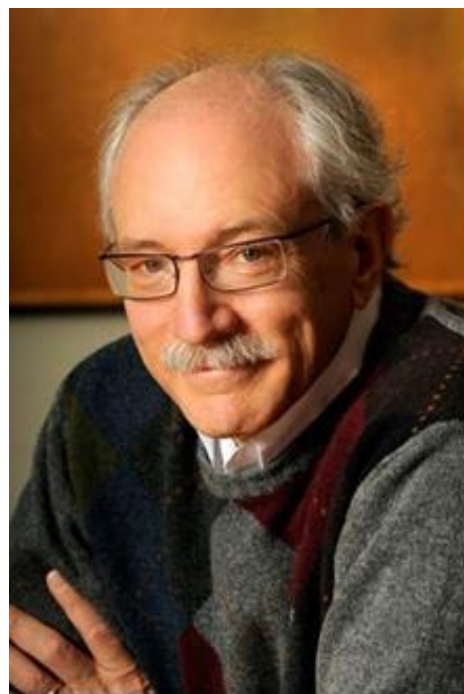
Noteworthy events included a workshop to review alternative methods for human and veterinary rabies vaccine potency testing, a workshop which converged on cancer topics, and a webinar for alternative inhalation toxicity testing methods that emphasized in vitro and in silico methodologies.

I invite you to read this report to learn about our work and what we accomplished in FY 2019 toward advancing toxicology and safeguarding public health by generating and communicating trusted scientific information.

On October 3, 2019, Linda Birnbaum, Ph.D., DABT, ATS retired after 40 years as a federal scientist including 10 years (2009-2019) as Director of the NIEHS and NTP. We recognize and applaud Linda's many accomplishments and her global leadership in toxicology and environmental health research. I am privileged to serve as Acting Director of both NIEHS and NTP and work with NTP Associate Director Brian R. Berridge, D.V.M., Ph.D., DACVP and our NTP agency partners at the National Institute for Occupational Safety and Health and the Food and Drug Administration. Together we will continue NTP's critical role in the generation, interpretation, and communication of toxicological information that strengthens the science base and informs decisions that protect the health of Americans.

Rick Woychik, Ph.D.

Acting Director, NIEHS and NTP



Rick Woychik, Ph.D., Acting Director, NIEHS and NTP.

(Photo courtesy of Steve McCaw.)

“

**Under Linda's leadership, NIEHS became a world leader in toxicology and environmental health research.**

*Francis Collins*

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## Table of Contents

<b>1. FY 2019 at a Glance</b>	<b>1</b>
1.1. Timeline	1
1.2. Completed NTP Reports and Publications	2
1.3. NTP Public Health Impact	2
1.4. Highlights from 40 Years of Work	6
1.5. Dr. Linda Birnbaum Announces Retirement	8
1.6. PFAS in the Spotlight	9
1.7. New Interagency Memorandum of Understanding Aims to Improve Cardiovascular Safety of Pharmaceuticals	9
1.8. NTP Converges on Cancer	10
1.9. NTP Improving Tests of Developmental Neurotoxicity	11
1.10. Peer Review of Draft NTP Monograph on Organophosphorus Nerve Agent Sarin	11
1.11. Additional Activities	12
<b>2. About NTP</b>	<b>13</b>
2.1. Organizational Structure and Oversight	13
2.2. Interagency Agreements	14
2.3. Funding	16
2.4. Program Contact Information	18
<b>3. Scientific and Public Input Opportunities</b>	<b>19</b>
3.1. NTP Board of Scientific Counselors	19
3.2. Scientific Advisory Committee on Alternative Toxicological Methods	21
3.3. Scientific Panels	23
3.3.1. NTP Developmental and Reproductive Toxicity Reports Peer Reviewed in FY 2019	23
3.3.2. NTP Monographs Peer Reviewed in FY 2019	24
3.3.3. NTP Report on Carcinogens Monographs Peer Reviewed in FY 2019	24
3.4. Training Opportunities	25
<b>4. Research and Testing</b>	<b>27</b>
4.1. Tox21	27
4.2. Testing and Toxicology Studies	31
4.3. NICEATM	31
4.3.1. NICEATM Webinars and Workshops	32
4.3.2. NICEATM Support of Tox21	33
4.3.3. Additional NICEATM Activities	33
4.4. ICCVAM	38
4.4.1. Progress toward Strategic Roadmap Goals	39
4.4.2. ICCVAM Meetings	40
4.4.3. ICCVAM Test Method Evaluation Activities	40
4.4.4. ICCVAM International Validation Activities	41
<b>5. Literature Analysis</b>	<b>43</b>
5.1. Noncancer Research	43

## 2019 Annual Report – National Toxicology Program

5.2. Report on Carcinogens.....	47
5.2.1. Evaluating Cancer Hazards .....	48
<b>6. Partner Agency Research .....</b>	<b>50</b>
6.1. NTP at NIEHS.....	50
6.1.1. Biomolecular Screening .....	50
6.1.2. NTP Laboratory.....	51
6.2. NTP at NCTR .....	55
6.2.1. NTP at NCTR: Interagency Agreement Projects .....	64
6.3. NTP at NIOSH .....	66
6.3.1. NTP at NIOSH: Immunotoxicology Research .....	72
6.3.2. NTP at NIOSH: Occupationally Relevant Exposures.....	72
<b>Appendix I: NTP Publications in FY 2019 .....</b>	<b>76</b>
<b>Appendix II: Testing and Toxicology Studies in FY 2019 .....</b>	<b>76</b>

# 1. FY 2019 at a Glance

This section provides an overview of notable events and other NTP activities and accomplishments during FY 2019. Following a timeline of events, this section includes a summary of completed NTP reports and other publications, a listing of federal and state public health actions affected by NTP data and recommendations, and synopses of featured research activities, milestones, and other highlights.

## 1.1. Timeline

Meetings, workshops, and other events bring together scientists from government, academia, industry, and the public to discuss scientific research conducted by NTP and other important topics in the fields of toxicology and public health. A timeline of notable meetings and events that took place in FY 2019 is listed below.

Month, Year	Event
October 2018	<b>Implementing Nonanimal Approaches to Human and Veterinary Vaccine Testing: Achieving Scientific and Regulatory Success for Rabies and Beyond</b> This <a href="#">October 11–13 workshop</a> , co-organized by NICEATM and the International Alliance for Biological Standardization (IABS), examined alternative methods for human and veterinary rabies vaccine potency testing and other animal reduction and replacement initiatives.
December 2018	<b>NTP Board of Scientific Counselors Meeting</b> At its <a href="#">December 12 meeting</a> , the BSC provided updates to the NTP Translational Toxicology Pipeline Plan to translate science to support decisions in the regulatory, governmental, and scientific arenas.
January 2019	<b>Friends of NIEHS Discuss Hot Topics with Birnbaum</b> The Friends of NIEHS held a <a href="#">January 15 meeting</a> to discuss the latest laboratory and clinical environmental health research projects, recent community forums, and other institute activities.
January 2019	<b>ICCVAM Communities of Practice Webinar: Nonanimal Approaches for Inhalation Toxicity Testing</b> This <a href="#">January 22 webinar</a> presented alternative methods for inhalation toxicity testing with an emphasis on in vitro and in silico methodologies.
February 2019	<b>Peer Review of Draft NTP Monograph on Sarin</b> At the <a href="#">February 4 peer review meeting</a> , panelists reviewed and provided comment on the <i>Draft NTP Monograph on the Systematic Review of Evidence of Long-Term Neurological Effects Following Acute Exposure to the Organophosphorus Nerve Agent Sarin</i> .
February 2019	<b>NTP Board of Scientific Counselors Meeting</b> At the <a href="#">February 15 meeting</a> , the board further explored the theme “The Changing Toxicology Landscape: Challenges and the Future of Risk Assessment” as participants discussed a shift in NTP’s scientific direction and capabilities.
April 2019	<b>NTP Workshop: Converging on Cancer</b> At the <a href="#">April 29–30 workshop</a> in Washington, DC, scientists discussed a clear path forward for evaluating interactions between environmental exposures and cancer biology using the latest tools in toxicology and identified areas for further research.

Month, Year	Event
May 2019	<b>ICCVAM Public Forum</b> At the <a href="#">May 23 forum</a> , attendees and webcast viewers heard presentations by ICCVAM members on current activities related to the development and validation of alternative test methods and approaches.
June 2019	<b>NTP Board of Scientific Counselors Meeting</b> At the <a href="#">June 17–18 meeting</a> , the board revisited the strategic realignment of NTP research and current projects as examples of translational toxicology at NTP.
July 2019	<b>Peer Review Meeting: Developmental and Reproductive Effects of Four Chemicals</b> At the <a href="#">July 31 peer review meeting</a> , four chemicals were the focus of a rigorous peer-review process to evaluate NTP conclusions regarding the chemicals' developmental and reproductive toxicity.
September 2019	<b>Scientific Advisory Committee on Alternative Toxicological Methods Meeting</b> At the <a href="#">September 19–20 meeting</a> , the committee heard presentations on the goals of the U.S. Strategic Roadmap for new approaches to safety and risk assessment.

## 1.2. Completed NTP Reports and Publications

The findings of NTP studies and research projects are published in the following types of NTP reports.

- [NTP technical reports](#), which document long-term toxicology and carcinogenicity studies, generally of 2 years' duration
- [NTP toxicity reports](#), which document shorter-term studies, generally up to 13 weeks' duration
- [NTP research reports](#), which provide the results of research studies, rapid communications, and literature surveys that do not fall under the scope of the first two report series
- [NTP monographs](#), which assess the evidence that exposure to a substance causes adverse health effects
- [NTP Report on Carcinogens monographs](#), which are prepared for candidate substances selected for review and consist of a cancer evaluation and substance profile to support NTP's policy decision to list the substance in the Report on Carcinogens

All published NTP reports are peer reviewed by experts who are screened for conflicts of interest before their service. In 2019, NTP completed three technical reports, three toxicity reports, seven research reports, two NTP monographs, and two Report on Carcinogens monographs. Full citations for these reports are provided in [Appendix I](#), and all [NTP reports](#) completed in FY 2019, and in prior years, are available on the NTP website.

NTP scientists published more than 250 journal articles and book chapters in FY 2019. Citations for these publications are provided in [Appendix I](#).

## 1.3. NTP Public Health Impact

Federal and state regulatory agencies use NTP study data and recommendations in considering the need to regulate and test specific chemicals for the protection of human health. The NTP data and recommendations used by other agencies in FY 2019 are listed below. A [full listing](#) is available on the NTP website.

*Use of NTP Study Data or Recommendations by Federal and State Health Regulatory and Research Agencies in FY 2019*

## Notice, Summary, and NTP Information Cited

### FDA Removes 7 Synthetic Flavoring Substances from Food Additives List

October 05, 2018

21 CFR Parts 172 and 177

#### Summary of Notice

FDA partially granted a petition by amending food additive regulations to no longer authorize the use of benzophenone, ethyl acrylate, eugenyl methyl ether, myrcene, pulegone, and pyridine as synthetic flavorings in food. FDA took this action because the petitioners provided data that demonstrated that these additives induced cancer in laboratory animals so, as a matter of law, FDA cannot list these synthetic flavorings in the food additive regulations. Because of evidence that benzophenone causes cancer in animals, FDA also prohibited the use of benzophenone as a plasticizer in rubber articles intended for repeated use with food.

#### NTP Information Cited

- [NTP \(1986\) Carcinogenesis Studies of Ethyl Acrylate in F344/N Rats and B6C3F1 Mice \(Gavage Studies\). TR 259.](#)
- [NTP \(2006\). Toxicology and Carcinogenesis Studies of Benzophenone in F344/N Rats and B6C3F1 Mice \(Feed Studies\). TR 533.](#)
- [NTP \(2000\). Toxicology and Carcinogenesis Studies of Methyleugenol in F344/N Rats and B6C3F1 Mice \(Gavage Studies\). TR 491.](#)
- [NTP \(2010\). Toxicology and Carcinogenesis Studies of  \$\beta\$ -Myrcene in F344/N Rats and B6C3F1 Mice \(Gavage Studies\). TR 557.](#)
- [NTP \(2011\). Toxicology and Carcinogenesis Studies of Pulegone in F344/N Rats and B6C3F1 Mice \(Gavage Studies\). TR 563.](#)
- [NTP \(2000\). Toxicology and Carcinogenesis Studies of Pyridine in F344/N Rats, Wistar Rats, and B6C3F1 Mice \(Drinking Water Studies\). TR 470.](#)

### Notice of Intent to List p-Chloro- $\alpha,\alpha,\alpha$ -trifluorotoluene (para-Chlorobenzotrifluoride, PCBTF)

November 23, 2018

Proposition 65

#### Summary of Notice

The California Office of Environmental Health Hazard Assessment announced its intent to list p-chloro- $\alpha,\alpha,\alpha$ -trifluorotoluene (also known as PCBTF) as known to the state to cause cancer under the Safe Drinking Water and Toxic Enforcement Act of 1986.

#### NTP Information Cited

- [NTP \(2018\). Toxicology and Carcinogenesis Studies of p-Chloro- \$\alpha,\alpha,\alpha\$ -Trifluorotoluene in Sprague Dawley Rats \(Hsd:Sprague Dawley SD\) and B6C3F1/N Mice \(Inhalation Studies\). TR 594.](#)

### Chlorate; Pesticide Exemptions from Tolerance

December 26, 2018

83 FR 66138

#### Summary of Notice

EPA established an exemption from the requirement of a tolerance for residues of chlorate in or on cantaloupe and tomato under the Federal Food, Drug, and Cosmetic Act (FFDCA).

## Notice, Summary, and NTP Information Cited

### NTP Information Cited

- Risk Assessment of Tomato and Cantaloupe Fumigation with Sodium Chlorite 3.2% (Chlorine Dioxide Gas).
- Inorganic Chlorates Human Health Assessment Scoping Document in Support of Registration Review (Docket ID No. EPA-HQ-OPP-2016-0080-0008)
- Revised Inorganic Chlorates. HED Chapter of the Reregistration Eligibility Decision Document (RED) (Docket ID No. EPA-HQ-OPP-2005-0507-0004).

### Amendment to Section 25705 No Significant Risk Level for Bromodichloroacetic Acid

February 13, 2019  
Proposition 65

#### Summary of Notice

On February 5, 2019, the California Office of Administrative Law approved an amendment of Title 27, California Code of Regulations, section 25705, that established a no significant risk level of 0.95 micrograms per day for bromodichloroacetic acid.

#### NTP Information Cited

- [NTP \(2015\) Toxicology Studies of Bromodichloroacetic Acid \(CAS No. 71133-14-7\) in F344/N Rats and B6C3F1/N Mice and Toxicology Carcinogenesis Studies of Bromodichloroacetic Acid in F344/NTac Rats and B6C3F1/N Mice \(Drinking Water Studies\). TR 583.](#)

### Amendment to Section 25705 No Significant Risk Level for Bromochloroacetic Acid

February 13, 2019  
Proposition 65

#### Summary of Notice

On February 5, 2019, the California Office of Administrative Law approved an amendment of Title 27, California Code of Regulations, section 25705, that established a no significant risk level of 0.70 micrograms per day for bromochloroacetic acid.

#### NTP Information Cited

- [NTP \(2009\). Toxicology and Carcinogenesis Studies of Bromochloroacetic Acid \(CAS No. 5589-96-8\) in F344/N Rats and B6C3F1 Mice \(Drinking Water Studies\). TR 549.](#)

### Sunscreen Drug Products for Over-the-Counter Human Use

February 26, 2019  
84 FR 6204

#### Summary of Notice

FDA issued a proposed rule to put into effect a final monograph for nonprescription, over-the-counter (OTC) sunscreen drug products. This proposed rule described the conditions under which OTC sunscreens are generally recognized as safe and effective and not misbranded. It was published as part of the ongoing FDA review of OTC drug products and to comply with the Federal Food, Drug, and Cosmetic Act, as amended by the Sunscreen Innovation Act.

#### NTP Information Cited

- [NTP \(2004\). Toxicology and Carcinogenesis Studies of Triethanolamine in B6C3F1 Mice \(Dermal Study\). TR 518.](#)



## Notice, Summary, and NTP Information Cited

- NTP (1999). Toxicology and Carcinogenesis Studies of Triethanolamine in F344/N Rats and B6C3F1 Mice (Dermal Studies). TR 449.
- NTP (1992). Technical Report on the Toxicity Studies of 2-Hydroxy-4- Methoxybenzophenone (Cas No. 131-57-7) Administered Topically and in Dosed Feed to F344/N Rats and B6c3f1 Mice. TOX21.
- Testing Status of 2-Hydroxy-4- methoxybenzophenone 10260–S.

### Current Proposition 65 No Significant Risk Levels (NSRLs) Maximum Allowable Dose Levels (MADLs)

March 25, 2019

Proposition 65

#### Summary of Notice

Under Proposition 65, California has established safe harbor levels, which include NSRLs for cancer-causing chemicals and MADLs for chemicals causing reproductive toxicity for many listed chemicals. Exposure levels and discharges to drinking water sources that are below the safe harbor levels are exempt from the requirements of Proposition 65. In some instances, enforcement actions may have resulted in negotiated exposure levels relative to specific settlement agreements.

#### NTP Information Cited

- NCI (1980). Bioassay of 4,4'-Oxydianiline for Possible Carcinogenicity. TR 205.
- NTP (1982). Toxicology and Carcinogenesis Studies of D & C Red 9 in F344/N Rats and B6C3F1 Mice (Feed Study). TR 225.
- NTP (1983). Toxicology and Carcinogenesis Studies of 4,4'-Methylenedianiline Dihydrochloride in F344/N Rats and B6CJF1 Mice (Drinking Water Studies). TR 248.
- NTP (1985). Toxicology and Carcinogenesis Studies of Chlorodibromomethane in F344/N Rats and B6C3F1 Mice (Gavage Studies). TR 282.
- NTP (1986). Toxicology and Carcinogenesis Studies of Chlorinated Paraffins (Cl2. 60% Chlorine) in F344/N Rats and B6C3F1 Mice (Gavage Studies). TR 308.
- NTP (1986). Toxicology and Carcinogenesis Studies of 3-Chloro-2-methylpropene in F344/N Rats and B6C3F1 Mice (Gavage Studies). TR 300.
- NTP (1986). Toxicology and Carcinogenesis Studies of C.I. Basic Red 9 Monohydrochloride in F344/N Rats and B6C3F1 Mice (Feed Studies). TR 285.
- NTP (1986). Toxicology and Carcinogenesis Studies of Diglycidyl Resorcinol Ether in F344/N Rats and B6C3F1 Mice (Gavage Studies). TR 257.
- NTP (1986). Toxicology and Carcinogenesis Studies of Dimethylvinyl Chloride (1-chloro-2-methyl-propene) in F344/N Rats and B6C3F1 Mice (Gavage Studies). TR 316.
- NTP (1986). Toxicology and Carcinogenesis Studies of Disperse Blue 1 (a commercial dye containing approximately 50% 1,4,5,8-tetra amino anthraquinone, 30% other compounds structurally related to 1,4,5,8-tetra amino anthraquinone and 20% water) in F344/N Rats and B6C3F1 Mice (Feed Studies). TR 299.
- NTP (1986). Toxicology and Carcinogenesis Studies of HC Blue 1 in F344/N Rats and B6C3F1 Mice (Feed Studies). TR 271.
- NTP (1986). Toxicology and Carcinogenesis Studies of Toluene Diisocyanate in F344/N Rats and B6C3F1 Mice (Gavage Studies). TR 251.
- NTP (1987). Toxicology and Carcinogenesis Studies of Chlorendic Acid in F344 Rats and B6C3F1 Mice (Feed Studies). TR 304.
- NTP (1989). Toxicology and Carcinogenesis Studies of Hexachloroethane in F344/N Rats (Gavage Studies). TR 361.

## Notice, Summary, and NTP Information Cited

- NTP (1991). Chemical Status Report.

### Restrictions on Discontinued Uses of Asbestos; Significant New Use Rule

April 25, 2019  
84 FR 17345

#### Summary of Notice

Under the Toxic Substances Control Act (TSCA), EPA promulgated a rule to ensure that any discontinued uses of asbestos cannot re-enter the marketplace without EPA review. This closed a loophole in the regulatory regime for asbestos.

#### NTP Information Cited

- [NTP \(2016\). 14th Report on Carcinogens.](#)
- [NTP \(1980\). First Annual Report on Carcinogens.](#)

### Review of the Dust-Lead Hazard Standards (DLHS) and the Definition of Lead-Based Paint (LBP)

February 13, 2019  
Proposition 65

#### Summary of Notice

As part of efforts to reduce childhood lead exposure, EPA evaluated the current DLHS and the definition of LBP. Informed by this evaluation, the final rule revised the DLHS from 40 µg/ft<sup>2</sup> and 250 µg/ft<sup>2</sup> to 10 µg/ft<sup>2</sup> and 100 µg/ft<sup>2</sup> on floors and window sills, respectively. EPA also finalized its proposal to make no change to the definition of LBP because insufficient information existed to support such a change at this time.

#### NTP Information Cited

- [NTP \(2012\). NTP Monograph: Health Effects of Low-Level Lead.](#)

\*CASRN = Chemical Abstracts Service Registry Number.

## 1.4. Highlights from 40 Years of Work

In 1978, the environmental disaster at Love Canal, New York, focused national attention on the public health impacts of exposure to toxic chemicals. Three months after a federal health emergency was declared at Love Canal, the Department of Health, Education, and Welfare (now Health and Human Services, HHS) established NTP. Four decades after its founding, NTP is the most trusted source of toxicology knowledge worldwide.

Throughout 2019, NTP celebrated four decades of [history and milestones](#) through continued progress in the field of toxicology. Highlights from the first 40 years of work at NTP are listed below.

### 1980 – 1989

The first [Report on Carcinogens](#) is published, with 26 listings.

NTP initiates developmental toxicity testing.

The Department of Health and Human Services (HHS) transfers the National Cancer Institute (NCI) Carcinogenesis Testing Program to NIEHS/NTP.



NTP expands its testing strategy to include immunotoxicology testing, a comprehensive testing battery to evaluate immune system alterations.

NTP publishes its first systematic evaluation, on the predictability of various genetic toxicity screens for cancer.

## 1990 - 1999

NIEHS/NTP establishes an interagency agreement with Centers for Disease Control/National Institute for Occupational Safety and Health (CDC/NIOSH) for immunotoxicity of workplace xenobiotics.

Kenneth Olden, Ph.D., is named Director of NIEHS and NTP.

NTP initiates its [Toxicity Report](#) series.

NIEHS/NTP establishes an interagency agreement with Food and Drug Administration/National Center for Toxicology Research (FDA/NCTR) for conducting comprehensive toxicological evaluations of substances of concern to the FDA.

The international workshop on validation and regulatory acceptance of alternative toxicological methods is convened with an ad hoc [Interagency Coordinating Committee on the Validation of Alternative Methods](#) (ICCVAM).

NTP establishes a formal process for removing a listing from the Report on Carcinogens. established.

NIEHS/NTP initiates an agreement with the CDC/National Center for Environmental Health (NCEH) to provide funding for expanded biomonitoring of environmental toxicants in the National Health and Nutrition Examination Survey.

NIEHS/NTP establishes an interagency agreement with CDC/NIOSH to characterize and evaluate adverse effects of complex occupational exposures.

NTP establishes the [Interagency Center for the Evaluation of Alternative Toxicological Methods](#) (NICEATM) to convene scientific panels to evaluate alternative toxicological methods.

ICCVAM achieves regulatory acceptance of the Murine Local Lymph Node Assay and Corrositex® for dermal safety testing.

## 2000 – 2009

ICCVAM becomes permanent under NICEATM with passage of the ICCVAM Authorization Act of 2000.

NIEHS/NTP formalizes collaborations with the European Ramazzini Foundation of Oncology and Environmental Sciences.

NTP celebrates a quarter century of toxicology for public health and unveils a new [Toxicology for the 21st Century](#) (Tox21) federal interagency program.

David A. Schwartz, M.D., Ph.D., is named Director of NIEHS and NTP.

Linda S. Birnbaum, Ph.D., is named Director of NIEHS and NTP.

NIEHS/NTP establishes the [International Cooperation on Alternative Test Methods](#) (ICATM), a formal agreement to cooperate on alternative test methods with Europe, Japan, and Canada.

## 2010 – 2019

NTP creates the [Chemical Effects in Biological Systems](#) (CEBS), a new database for housing, integrating, and managing data.

ICCVAM achieves regulatory acceptance by EPA of the BG1 luciferase estrogen receptor transactivation test method and the first OECD performance-based test guidelines for estrogen receptor agonists (PBTG 455).

NTP launches the [Nonneoplastic Lesion Atlas](#) as guide for standardizing terminology in toxicologic pathology.

The National Institute of Standards and Technology (NIST) joins ICCVAM.

ICCVAM publishes [A Strategic Roadmap for Establishing Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States](#) published.

## 1.5. Dr. Linda Birnbaum Announces Retirement

Linda S. Birnbaum, Ph.D., effective October 3, 2019.

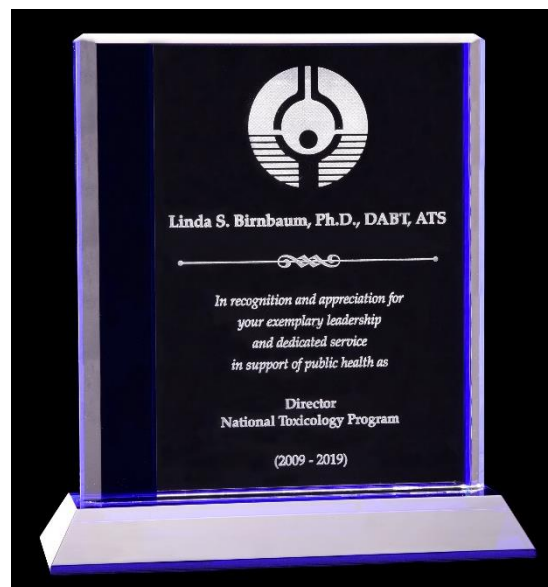
Dr. Birnbaum retired after nearly four decades of federal services including just over 10 years as director of NIEHS and NTP. Dr. Birnbaum was the first board-certified toxicologist and the first woman to serve as director of NIEHS and NTP. She was an active supporter of the NIH Women in Biomedical Research Program, an NIH-wide effort to remove barriers for women in science and to promote entry, recruitment, retention, and advancement of women in biomedical and research careers.

“Under Linda’s leadership, the NIEHS became a world leader in toxicology and environmental health research, with NIEHS science inspiring health policy and safety standards in the United States and abroad,” wrote Francis Collins, M.D., Ph.D., Director of the National Institutes of Health.

Under Dr. Birnbaum’s leadership, NTP accomplishments include the following:

- The NTP Executive Committee was reinvigorated, bringing interagency collaboration to highly consequential issues, such as the effects of per- and polyfluoroalkyl substances (PFAS) and flame retardants.
- NTP developed the Consortium Linking Academic and Regulatory Insights on Bisphenol A Toxicity (CLARITY-BPA) research program, a collaboration between NIEHS, FDA, and academia to better understand the potential health effects of long-term exposure to bisphenol A.
- The Tox21 collaboration grew, advancing the field of toxicology with the implementation of new methods.
- NTP adopted and applied systematic review methodologies for literature-based assessments, such as identifying immune system hazards of PFOA and Perfluorooctanoic acid (PFOS) and linking acute sarin exposure with long-term neurological effects.

During the March 28, 2019, hearing on PFAS, Dr. Birnbaum testified before the Senate Committee on Environment and Public Works, summarizing the growing body of literature on PFAS, as well as its array of cancer and noncancer health effects. She spoke about new research and emphasized the importance of considering PFAS chemicals as a class and as mixtures.





Dr. Birnbaum has been recognized with numerous awards in her career. In 2010, she was elected to the Institute of Medicine (now the National Academy of Medicine). In 2016, Dr. Birnbaum received the distinguished North Carolina Award in Science, the highest civilian honor given by the state's governor. In 2018, she was named the Distinguished Toxicology Scholar by the Society of Toxicology and earned the Mildred S. Christian Career Achievement Award from the Academy of Toxicological Sciences.

## 1.6. PFAS in the Spotlight

Per/polyfluoroalkyl substances, or PFAS, are a class of manmade chemicals that are highly persistent in the environment and are associated with numerous health effects related to immune function, hormone disruption, neurodevelopment, and cancer. PFAS are found in a range of common products (e.g., food packaging, clothing, stain-resistant furniture). In addition, they have been used in firefighting foam and in industrial processes, which have contaminated surface and groundwater drinking water sources.



During FY 2019, PFAS continued to gain increasing public, scientific, and political attention. In March 2019, NTP and NIEHS Director Linda Birnbaum, Ph.D., testified before the Senate Committee on Environment and Public Works. Dr. Birnbaum provided the scientific perspective on PFAS, particularly human exposure and human effects research. She also informed Congress of ongoing PFAS research and challenges in addressing real-world human exposure to PFAS.

The North American division of the Society of Environmental Toxicology and Chemistry (SETAC) focused on PFAS risk assessment at its August 2019 annual conference. NTP scientists and other researchers presented on and discussed several topics as they relate to PFAS, including methods for detection and measurement, dietary exposure, drinking water systems, reproductive and developmental toxicity, and animal effects.

NTP is at the forefront of research providing new insights into the human health effects of PFAS. Some of the notable NTP contributions to work on PFAS in FY 2019 include publication of two toxicity reports, TOX-96 and TOX-97, which describe 28-day toxicity studies on rats for seven PFAS chemicals. In addition, NTP continued its collaborative research efforts with EPA through the Responsive Evaluation and Assessment of Chemical Toxicity (REACT) Program, which conducts research on subclasses of PFAS chemicals with similar properties and toxicity.

## 1.7. New Interagency Memorandum of Understanding Aims to Improve Cardiovascular Safety of Pharmaceuticals

In February 2019, NTP entered into a memorandum of understanding (MOU) with the U.S. Food and Drug Administration (FDA) Center for Drug Evaluation and Research (CDER) and the Cardiac Safety Technical Committee of the Health and Environmental Sciences Institute (HESI). This MOU was established with the primary goal of improving the cardiovascular safety of pharmaceuticals. The three organizations will mobilize experts in cardiovascular toxicology and risk assessment and use their collective resources to accomplish three primary public health objectives:

- *Improve toxicity testing and safety monitoring in drug development.* By aligning environmental toxicology interests with FDA's process for determining drug safety, NTP can begin to develop expertise in this area.

- *Identify, test, and validate new technologies in pharmaceutical development using nonanimal approaches.* Animal toxicology studies are costly, time-consuming, and inconsistent in predicting human health outcomes. Projects in this area will aim to build a collection of assays that more precisely identify the potential for cardiovascular harm in people from pharmaceuticals.
- *Apply predictive toxicology methods, such as the Tox21 program.* The ability to predict or discover a harmful side effect from a new drug as early as possible may reduce research, regulatory, and policy challenges for pharmaceutical development. Through the Tox21 program, innovative test methods are developed that can better predict how chemicals will affect biological responses.

Activities under this MOU are expected to continue for 5 years.

## 1.8. NTP Converges on Cancer

More than 90 researchers working in areas of cancer biology, assay development, mixtures toxicology, in silico modeling, and cancer risk assessment gathered April 29–30, 2019, in Washington, DC, for the [Converging on Cancer Workshop](#)—an effort that was organized by NTP, the University of California, Berkeley, and others. Hundreds more attendees from around the world participated in the workshop via webcast. This workshop aimed to provide a clear path forward for evaluating interactions between environmental exposures and cancer biology using the latest tools in toxicology and identifying knowledge gaps where research is needed.



Cancer is a leading cause of mortality worldwide and is a complex disease with multiple etiologies and target tissues. Diverse environmental factors have been associated with the development and progression of various types of cancer.

Workshop presentations and discussion sessions focused on three general topics:

- *Improving carcinogenicity testing.* This includes building a framework for incorporating mechanistic data into cancer risk assessment.
- *Defining hallmarks and key characteristics of cancer development.* Potential applications include developing effective screening tools to detect the carcinogenic potential of environmental chemicals, engineering safer products, and designing more effective multi-target therapeutics.
- *Better understanding carcinogenic potential of environmental chemicals, including mixtures.* This includes interaction between multiple carcinogens that act on different biological pathways as well as noncarcinogens that might be promoting cancer.

In addition to a webcast, breakout discussions, and a poster session, the workshop included a real-time polling event. During one poll, participants weighed in on which technologies hold the most promise for modern cancer assessment. Genomics, transcriptomics, and 3D bioprinted tissues and organs were among the top responses.

## 1.9. NTP Improving Tests of Developmental Neurotoxicity

NTP scientists and other researchers convened to evaluate alternative toxicological methods to predict the effects of various chemicals on developmental neurotoxicity.



NTP hosted a collaborative project with approximately 40 participants from academia, industry, government, and regulatory agencies. The goal was to evaluate a battery of cell-based assays and nontraditional animal models to predict the effects of various chemicals on development of the human neurological system.

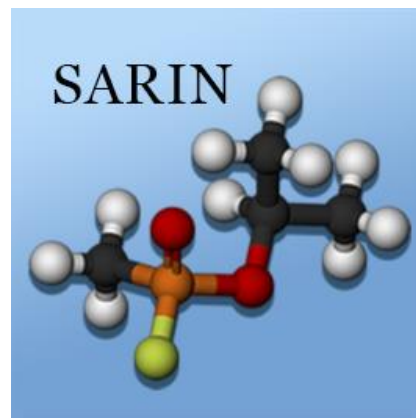
NTP provided participants with a compound library that consisted of known and suspected neurotoxicants that included drugs, flame retardants, industrial chemicals, polycyclic aromatic hydrocarbons, and pesticides. Groups of researchers independently tested these chemicals using their respective cell-based assays or alternative animal models.

Cell-based assays were used by researchers to measure neuronal proliferation, differentiation, neurite outgrowth, migration, and neuronal network formation. Alternative animal models (e.g., zebrafish, planaria) were used to measure early development, movement, and mortality. Freshwater zebrafish is widely used in developmental biology and toxicology studies. Their small size and rapid development allow study of how early life exposures influence normal body processes in later life.

NTP developed a data analysis system to combine and compare assays across researchers. Following this effort, a collection of [eight journal articles](#) were published that described different aspects of the initiative.

## 1.10. Peer Review of Draft NTP Monograph on Organophosphorus Nerve Agent Sarin

An international panel met February 4, 2019, to peer review the conclusions of the [Draft NTP Monograph on Systematic Review of Long-term Neurological Effects Following Acute Exposure to the Organophosphorus Nerve Agent Sarin](#). The project was initiated after sarin was nominated by the NIH Countermeasures Against Chemical Threats (CounterACT) Program. The CounterACT program requested a systematic review of potential long-term neurological effects of sarin to inform decisions about the need to develop treatments.



The monograph concluded that acute sarin exposure is known to be a neurological hazard to humans in the initial time period of more than 24 hours to 7 days after exposure because of suppression of cholinesterase and is suspected to be a neurological hazard to humans in the intermediate time period of 8 days to 1 year after exposure and in the extended time period of more than 1 year after exposure.

The [peer review committee voted](#) on the animal and human conclusions for the three time periods after exposure—initial, intermediate, and extended—for each of four main effects:

- Changes in levels of the enzyme cholinesterase
- Effects on eyes and vision



- Effects on learning, memory, and intelligence
- Changes in nervous system tissues

The committee generally agreed with NTP conclusions, including the ultimate hazard identification conclusions. The [final monograph](#) was completed in June 2019.

## 1.11.Additional Activities

During FY 2019, NTP attended several meetings with stakeholders in the scientific community. At the 2019 annual meeting of the Society of Toxicology in Baltimore, Maryland, staff from NTP and NIEHS showcased its hard work through scientific and poster presentations, an NIH grant funding workshop, hands-on demonstrations, and awards.

Presentations and discussions covered a range of topics, including the health effects of electronic waste recycling and PFAS chemicals. High honors went to NTP's own Nicole Kleinstreuer, Ph.D., deputy director of NICEATM. She received the 2019 Society of Toxicology Achievement Award for leadership in alternative toxicological methods and computational toxicology. The full program, including all NTP and NIEHS activities, can be found at the [Society of Toxicology](#) website.



NTP regularly hosts symposia and workshops to discuss the state of the science or issues of public health concern. Experts from NIEHS and the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) became available on August 15 via the [Reddit Ask me Anything](#) (AMA) platform to answer questions from the public related to air pollution exposures faced by parents before and during pregnancy and the health of children. This effort was created to introduce a way for experts to share their expertise through online question and answer sessions.

NTP also hosted [two workshops and webinars](#) in FY 2019—one aimed at identifying better tools to assess interactions between environmental exposures and cancer biology and another related to alternative methods development:

- [Webinar Series on the Utility of Zebrafish Models for Toxicology](#)
- [Converging on Cancer](#) (highlighted in Section 1.8)
- [Achieving Scientific and Regulatory Success for Rabies and Beyond](#)



## 2. About NTP

The U.S. Department of Health, Education, and Welfare, now the U.S. Department of Health and Human Services, established NTP in 1978 in response to concerns about the potential human health effects of chemicals in our environment. NTP goals are to:

- Coordinate toxicology testing programs within the federal government.
- Strengthen the science base in toxicology.
- Develop and validate improved testing methods.
- Provide information about potentially toxic chemicals to health agencies, regulatory agencies, research agencies, scientific communities, medical communities, and the public.

### NTP MISSION:

To evaluate agents of public health concern by developing and applying the tools of modern toxicology and molecular biology

NTP provides scientific data to regulatory agencies and other health-related research groups and interpretation and guidance in their appropriate use. The American people and government agencies, at state and federal levels, rely on NTP to provide a strong scientific basis for decisions aimed at protecting public health. In the past 40 years, NTP has studied and shared information on the health effects of more than 2,800 substances, including dietary supplements, industrial chemicals, consumer products, and complex mixtures.

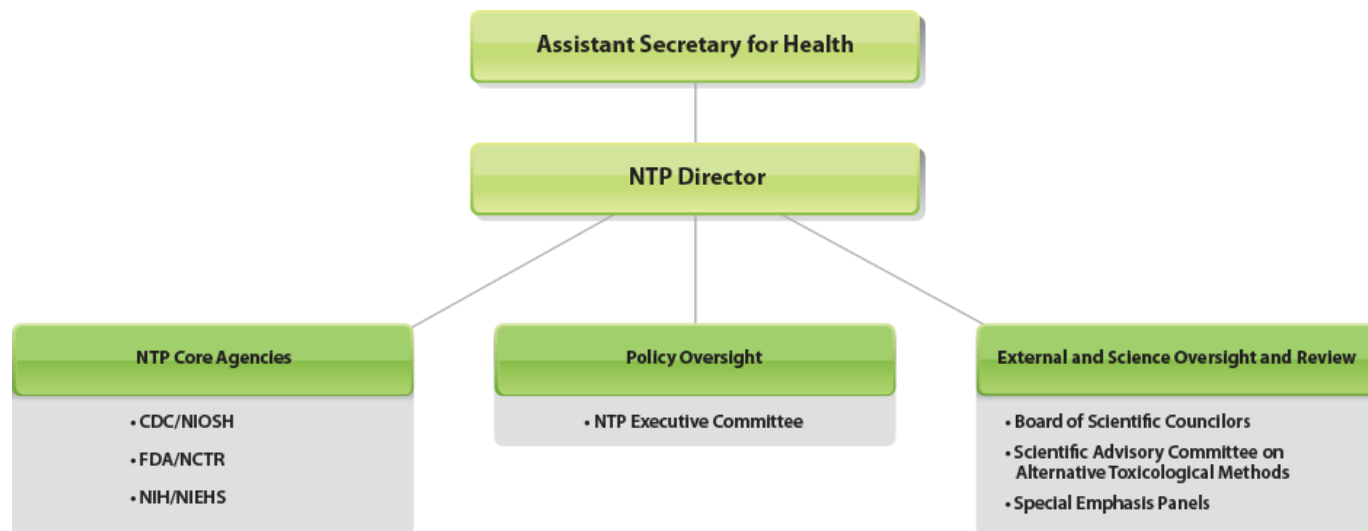
In following government-wide efforts to increase access to the results of federally funded scientific research, NTP maintains open communication and dialogue with the public, federal and state agencies, industry, nongovernmental organizations, and academic institutions. The [NTP website](#) provides the public with a variety of information, including Federal Register notices, status of and data from NTP studies, access to NTP reports and journal publications, notifications through media releases, a calendar of upcoming events, and a newsletter, the [NTP Update](#).

The public and other interested parties can stay abreast of NTP activities and events by [subscribing](#) to receive emails of news. In addition, requests for information can be made through the Central Data Management office (984-287-3211 or [ntpwebrequest@niehs.nih.gov](mailto:ntpwebrequest@niehs.nih.gov)) and an [online contact form](#).

NTP welcomes input on its programs and priorities. This input can be submitted in response to formal requests for public comment in Federal Register notices or through informal submissions to the Office of Liaison, Policy, and Review (984-287-3209 or the [online contact form](#)).

### 2.1. Organizational Structure and Oversight

Three agencies form the core for NTP: National Institute for Occupational Safety and Health (NIOSH) of the Centers for Disease Control and Prevention (CDC); U.S. Food and Drug Administration (FDA), primarily through the National Center for Toxicological Research (NCTR); and National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health (NIH).



NTP is located administratively at NIEHS, and Linda Birnbaum, Ph.D., was director of both NIEHS and NTP since 2009. Upon her retirement in October 2019, Rick Woychik, Ph.D. was named acting director of NIEHS and NTP. Brian Berridge, D.V.M., Ph.D., serves as NTP associate director and director of the NTP Division at NIEHS, herein referred to as NIEHS/NTP, which is a focal point for many [NTP activities](#). NIEHS and NTP espouse best research practices, embrace developments in technology to discover how the environment affects people and to maintain leadership in the field of environmental health sciences by applying innovative research to address public health issues.

John Howard, M.D., is director of NIOSH. Staff from two NIOSH divisions participate in NTP activities. Elizabeth Whelan, Ph.D., chief of the Field Research Branch, and Cheryl Estill, Ph.D., Exposure Assessment Team Lead, manage NTP activities within the Division of Field Studies and Engineering. Donald Beezhold, Ph.D., from the Health Effects Laboratory Division (HELD), is the principal investigator for the Immunotoxicity of Workplace Xenobiotics project through a NIOSH-NIEHS interagency agreement.

NIOSH's participation in NTP is consistent with its mandate to protect worker health and safety under the Occupational Safety and Health Act and the Federal Mine Safety and Health Act.

William Slikker Jr., Ph.D., director of FDA/NCTR, provides management oversight and coordination of NTP activities within NCTR. NCTR's scientists collaborate with other researchers in FDA, other government agencies, academia, and industry to produce innovative technologies, methods development, vital scientific training, and technical expertise. NCTR conducts an array of studies that reflect the NTP mission and are critical in supporting FDA product centers and their regulatory roles.

## 2.2. Interagency Agreements

In FY 2019, NIEHS provided support for NTP activities through interagency agreements with other federal agencies.

### *FDA/NCTR*

Under an interagency agreement, NIEHS provided fiscal resources to the U.S. Food and Drug Administration (FDA) to conduct toxicology studies on FDA-regulated agents of concern and on issues of mutual interest to NIEHS and FDA at the [National Center for Toxicological Research \(NCTR\)](#). Gonçalo Gamboa da Costa, Ph.D., Division of Biochemical Toxicology, is FDA project officer for the FDA-NIEHS interagency agreement and provides oversight and coordination of NCTR activities funded under this agreement with NIEHS. These studies are designed to provide FDA and

other regulatory agencies with hazard identification and dose-response data to support risk assessment and risk management decisions that could affect public health. The interagency agreement supports studies on endocrine-active agents, dietary supplements, food additives and contaminants, therapeutics and medicines, cosmetic ingredients, and nanoscale materials and on the development of novel toxicological approaches. Studies in these areas have produced 18 published NTP technical reports and more than 250 peer-reviewed journal publications since 1993, when this interagency agreement was established. The studies have led to an increased understanding of the pharmacokinetics, mode-of-action, and dose-response relationships of the substances and to refinements of risk assessment models. Further information about NTP, NCTR, and current research can be found in the [Partner Agency Research](#) section of this Annual Report.

#### *CDC/NIOSH*

NIEHS/NTP provides support to the [National Institute for Occupational Safety and Health \(NIOSH\)](#) of the [Centers for Disease Control and Prevention \(CDC\)](#) through two interagency agreements. Studies under the interagency agreement on immunotoxicity of workplace xenobiotics have assessed the potential toxicity of exposures to substances such as fungi, mycotoxins, volatile organic compounds, lead, latex, nickel, isocyanates, nanomaterials, and beryllium in occupationally exposed populations, such as miners, farmers, health care workers, autoworkers, and firefighters. The second interagency agreement supports the development of methods to assess complex mixtures, such as asphalt fumes, welding fumes, and tungsten fibers, and to conduct occupational exposure assessments to identify toxicologically relevant exposures. Research under these agreements in FY 2019 evaluated occupational exposure to alternative flame retardants, graphene, and other two-dimensional nanomaterials, molds and mycotoxins, and polycyclic aromatic hydrocarbons in coal tar sealants. For more information, see the [Partner Agency Research](#) section of this Annual Report.

#### *NIH/NCATS/DPI*

This interagency agreement supports ongoing and anticipated studies conducted at the National Institutes of Health/National Center for Advancing Translational Sciences (NCATS)/Division of Pre-Clinical Innovation (DPI) to evaluate high-throughput and high-content screening assays in support of [Tox21](#). Tox21 is a collaboration among federal agencies to characterize the potential toxicity of chemicals by using cells and isolated molecular targets instead of laboratory animals. This interagency agreement between NIEHS/NTP and NCATS/DPI produces data for information-poor substances to help prioritize them for further studies, including toxicological evaluation, mechanisms of action investigation, and development of predictive modeling for biological response.

#### *DOE/ORNL*

Under this interagency agreement, NIEHS/NTP is working with the U.S. Department of Energy's Oak Ridge National Laboratory (ORNL) to develop tools for evaluating the use and outcomes of its work. For example, ORNL is developing publication mining tools that help to evaluate NTP's effect across the agencies and with stakeholders to whom the work is disseminated and that extract and organize data (e.g., potential outcomes and impacts) from NTP's large inventory of documents (e.g., publications and progress reports).

#### *EPA/NCCT*

With projects under this interagency agreement, the U.S. Environmental Protection Agency's National Center for Computational Toxicology works to accelerate the development and use of advanced methods and models for quantitative and qualitative risk assessments of environmental chemicals. Specifically, research in FY 2019 provided products for standardization across transcriptomic

screening assays, understanding on how diverse biology affects results using our current screening methods, and a collaborative chemical management to reduce redundancies.

### *EPA/NCEA*

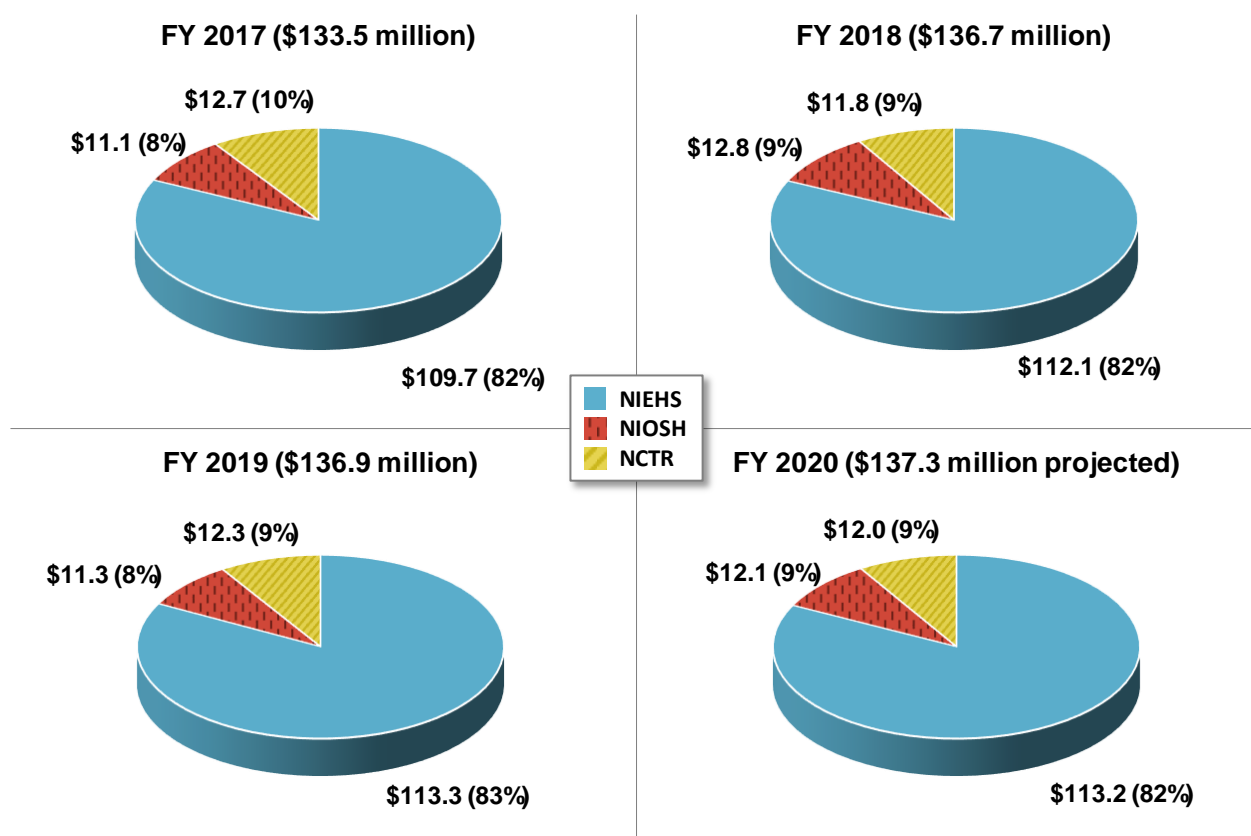
This interagency agreement between NIEHS/NTP and the U.S. Environmental Protection Agency's National Center for Environmental Assessment facilitates communication and coordination to foster common practices in human health assessments and to minimize the differences in hazard assessment methodologies. Through exchange of scientific expertise and peer review of work products, the agencies increase the quality and integrity of toxicological assessments while ensuring efficient use of federal resources and avoiding duplicative effort.

### *NIST*

Through this interagency agreement to support the experimental study of cell phone radiofrequency radiation exposure, NIEHS/NTP and the National Institute of Standards and Technology (NIST) collaborated to develop a new exposure system that addresses the limitation in existing systems.

## 2.3. Funding

NTP relies on voluntary allocations from the three core agencies—NIEHS, FDA/NCTR, and CDC/NIOSH—to support its activities. These allocations are specified after annual appropriations have been determined. The total NTP budget for FY 2019 was \$136.9 million.



NTP conducts its research through in-house studies at the three core agencies or through contract laboratories or [interagency agreements](#) with other agencies. In FY 2019, NIEHS funded 40 contracts, listed below, held [two workshops](#), [three peer review meetings](#), [two Board of Scientific Counselors](#)

meetings, and [one scientific advisory meeting](#). CDC and FDA may have additional contracts that support some of their voluntary NTP efforts.

### *NIEHS Contracts That Supported NTP Activities in FY 2019*

Description	Contractor
Administrative and scientific staff	Kelly Scientific
Analytical chemistry services	Battelle Memorial Midwest Research Institute Research Triangle Institute
Archives and specimen repository	Experimental Pathology Laboratories
Bioinformatics methylation project	Laboratory Corporation of American Holdings
Bioinformatics support	Sciome, LLC.
Collaborative Work with Ramazzini Institute	DOE with Oak Ridge Institute for Science and Education
Evaluation of alternative toxicological methods	Integrated Laboratory Systems
Evaluation of toxicity following early life exposure	Southern Research Institute
Evaluation of the toxicity of selected chemicals	Battelle Memorial
Genetic toxicity testing support services	Integrated Laboratory Systems
Immunotoxicity	Burleson Research Technologies
In-life data collection and management system	Instem
NTP information systems support	Signature Consulting Group
NTP technical reports preparation support services	Biotechnical Sciences, Inc.
Pathology support	Experimental Pathology Laboratories Integrated Laboratory Systems PAI/Charles River Laboratories
Production of B6C3F <sub>1</sub> mice	Taconic Biosciences
Provision for animals and specialized services	Charles River Laboratories The Jackson Labs Taconic Biosciences
Quality assessment support/audits and inspections	CSS-Dynamac Corporation
Reproductive assessments by continuous breeding	Research Triangle Institute
Scientific information management and literature-based evaluations for NTP	ICF International, Inc.

Description	Contractor
Statistical support	Social and Scientific Systems
Support for toxicological data	Vistronix
Support services for clinical research studies	Social and Scientific Systems
Toxicological and carcinogenic potential of chemicals	Battelle Memorial

## 2.4. Program Contact Information

**For general inquiries, contact:**

NTP Web Team

P.O. Box 12233, MD K2-05

Research Triangle Park, NC 27709

984-287-3211

[ntpwebrequest@niehs.nih.gov](mailto:ntpwebrequest@niehs.nih.gov) (or use [contact form](#))

A [staff directory](#) is available.



### 3. Scientific and Public Input Opportunities

NTP calls on a variety of advisory groups to provide rigorous scientific peer review and advice on programs and activities.

#### 3.1. NTP Board of Scientific Counselors

The NTP [Board of Scientific Counselors \(BSC\)](#), a federally chartered advisory group whose members are appointed by the Secretary of Health and Human Services, oversees the scientific merit of NTP programs and activities. The BSC includes scientists, primarily from the public and private sectors, with expertise relevant to NTP activities. The [current roster and meeting minutes](#) are available on the NTP website. In FY 2019, Mary Wolfe, Ph.D., served as the designated federal officer for the BSC. A list of FY 2019 members follows.

The Board met four times in FY 2019: October 9, 2018; December 12, 2018; February 15, 2019; and June 17, 2019. Materials and highlights from each meeting can be accessed on the [Past NTP Board of Scientific Counselors Meetings](#) web page. At the October meeting, Dr. Brian Berridge provided an update on strategic realignment at NTP and discussed the Translational Toxicology Pipeline Plan, which is central to the strategic realignment. Also, at this meeting, Office of the Report on Carcinogens members presented peer-review findings for two substances recommended for listing in the RoC—*Helicobacter pylori* and antimony trioxide. During the December meeting, discussions of strategic realignment at NTP continued, and Dr. Berridge presented the refined NTP vision and mission statements. In February, Dr. Berridge reviewed the translational toxicology pipeline and described the Health Effects Innovations (HEIs) program that focuses on three areas—cardiovascular hazard assessment, developmental neurotoxicity modeling, and carcinogenicity testing. Discussion of the HEIs program continued at the final BSC meeting, which focused on broadening the traditional focus of NTP to include analyzing how chemical exposures influence disease development.



NTP Board of Scientific Counselors members and NTP staff

*NTP Board of Scientific Counselors Membership Roster FY 2019*

<b>Name and Title</b>	<b>Affiliation</b>	<b>Term End Date</b>
Cynthia A. Afshari, Ph.D., D.A.B.T. Global Head and Vice President, Nonclinical Safety	Janssen Pharmaceutical Companies of Johnson & Johnson San Diego, California	6/30/19
Norman J. Barlow, D.V.M., Ph.D. Head of Nonclinical Sciences	Seattle Genetics Bothell, Washington	12/28/19
David M. Berube, Ph.D. Director, PCOST Professor, Department of Communication	North Carolina State University Raleigh, North Carolina	6/30/22
Paul W. Brandt-Rauf, Dr.P.H., M.D., Sc.D. Distinguished University Professor and Dean	Drexel University Philadelphia, Pennsylvania	6/30/20
Weihshueh A. Chiu, Ph.D. Professor, College of Veterinary Medicine and Biomedical Science	Texas A&M University College Station, Texas	6/30/22
Myrtle Davis, D.V.M., Ph.D. Executive Director, Discovery Toxicology, Pharmaceutical Candidate Optimization	Bristol-Myers Squibb Princeton, New Jersey	6/30/20
David L. Eaton, Ph.D., D.A.B.T. Dean and Vice Provost Emeritus, The Graduate School	University of Washington Seattle, Washington	6/30/22
Susan P. Felter, Ph.D. Research Fellow, Central Product Safety	Procter & Gamble Mason, Ohio	6/30/22
Daniel Kass, M.S.P.H. Senior Vice President, Environmental Health	Vital Strategies New York, New York	6/30/19
Kenneth E. McMartin, Ph.D. Board Chair Professor, Pharmacology, Toxicology, and Neuroscience	Louisiana State University Health Science Center Shreveport, Louisiana	12/28/19
David M. Michaels, Ph.D. Professor, Departments of Environmental and Occupational Health and Epidemiology and Biostatistics	George Washington University Washington, District of Columbia	6/30/22
Kenneth S. Ramos, M.D., Ph.D. Assistant Vice Chancellor for Health Services	The Texas A&M University System Houston, Texas	12/28/19
Anne M. Ryan, D.V.M., Ph.D.	Pfizer, Inc.	6/30/22



Name and Title	Affiliation	Term End Date
Executive Director, Drug Safety Research and Development	Groton, Connecticut	
Jennifer Sass, Ph.D. Senior Scientist	Natural Resources Defense Council Washington, District of Columbia	6/30/20
James Stevens, Ph.D. President	Paradox Found Consulting Services, LLC Apex, North Carolina	12/28/19
Donald G. Stump, Ph.D., D.A.B.T. Vice President, Nonclinical Safety Science	WIL Research Ashland, Ohio	6/30/20
Susan C. Tilton, Ph.D. Assistant Professor, Department of Environmental and Molecular Toxicology	Oregon State University Corvallis, Oregon	6/30/22
Katrina Waters, Ph.D. Deputy Director, Biological Sciences Division	Pacific Northwest National Laboratory Richland, Washington	2/28/19

### 3.2. Scientific Advisory Committee on Alternative Toxicological Methods

The [Scientific Advisory Committee on Alternative Toxicological Methods \(SACATM\)](#) is a federally chartered advisory committee established on January 9, 2002, in response to the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Authorization Act of 2000 (42 U.S.C. 285I-3[d]). SACATM advises ICCVAM, the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), and the director of NIEHS and NTP on the statutorily mandated duties of ICCVAM and activities of NICEATM. SACATM provides advice on priorities and activities related to the development, validation, scientific review, regulatory acceptance, implementation, and national and international harmonization of new, revised, and alternative toxicological test methods. The [SACATM charter](#) and [current roster](#) are available on the NTP website, and the roster for FY 2019 is listed below. SACATM typically meets once a year and members serve rotating terms of up to 4 years. Elizabeth Maull, Ph.D., served as the FY 2019 designated federal officer and manager of SACATM.

SACATM met [September 19–20, 2019](#), at the Crown Plaza Crystal City–Washington, DC, in Arlington, Virginia. The SACATM meeting focused on implementation of ICCVAM Strategic Roadmap approaches to validation, new computational tools, and microphysiological systems. Examples of the nine meeting presentations include:

- Evaluation of a Proposed Approach to Refine the Inhalation Risk Assessment for Point of Contact Toxicity: A Case Study Using a New Approach Methodology (NAM)
- The Future of Computational Toxicology: Balancing Machine Learning and Mechanistic Modeling
- The NIH Microphysiological Systems Program: Tissue Chips for Drug Safety and Efficacy Studies



**Members of SACATM and ICCVAM and staff from NIEHS and NTP**

### *SACATM Membership Roster FY 2019*

<b>Name and Title</b>	<b>Affiliation</b>	<b>Term End Date</b>
Michael B. Bolger, Ph.D. Chief Scientist	Simulations Plus, Inc. Lancaster, California	11/30/20
Joseph L. Charest, Ph.D. Biomedical Solutions Program Manager	The Charles Start Draper Laboratory, Inc. Cambridge, Massachusetts	11/30/22
Amy Clippinger, Ph.D. Director	PETA International Science Consortium Ltd. (PISC) PETA Regulatory Testing Department Washington, DC	11/30/22
Kelly P. Coleman, Ph.D., D.A.B.T., R.A.C. Distinguished Scientist and Technical Fellow	Medtronic PLC Minneapolis, Minnesota	11/30/20
K. Nadira De Abrew, Ph.D. Senior Scientist (Toxicologist)	The Procter & Gamble Company Cincinnati, Ohio	11/30/22
Sean C. Gehen, Ph.D., D.A.B.T. Regulatory Sciences Team Leader	Corteva Agriscience Agriculture Division of DowDuPont Dow AgroSciences LLC Indianapolis, Indiana	11/30/22
Hisham K. Hamadeh, Ph.D. D.A.B.T., M.B.A. Vice President	Global Head of Data Sciences Genmab U.S., Inc.	11/30/19
Lawrence M. Milchak, Ph.D., D.A.B.T. Senior Manager, Toxicology and Strategic Services	3M Medical Department St. Paul, Minnesota	11/30/19
Pamela J. Spencer, Ph.D., D.A.B.T.	ANGUS Chemical Company	11/30/19

Name and Title	Affiliation	Term End Date
Committee Chair Director of Regulatory and Product Stewardship	Buffalo Grove, Illinois	
ClarLynda Williams-Devane, Ph.D. Associate Professor, Discipline Coordinator of Bioinformatics	Fisk University Nashville, Tennessee	11/30/20
Hao Zhu, Ph.D. Assistant Professor, Department of Chemistry	Rutgers University Camden, New Jersey	11/30/19

### 3.3. Scientific Panels

NTP convenes ad hoc scientific panels, referred to as special emphasis panels, to provide independent scientific peer review and advice on targeted issues, such as agents of public health concern, new and revised toxicological test methods, and others. All panel reviews provide the opportunity for public comment. These panels help ensure that NTP receives transparent, unbiased, and scientifically rigorous input for its use in making credible decisions about human health hazards, setting research and testing priorities, and evaluating test methods for toxicity screening.

#### 3.3.1. NTP Developmental and Reproductive Toxicity Reports Peer Reviewed in FY 2019

NTP Developmental and Reproductive Toxicity (DART) Reports document the evaluation of the developmental and reproductive toxicity of selected substances in laboratory animals. For each DART report, the panel is charged with reviewing the scientific and technical elements and the presentation of the study and with determining whether the study's experimental design and conduct support NTP conclusions regarding the carcinogenic activity of the substance tested.

The draft DART reports, listed below, underwent peer review in FY 2019. Additional information about past technical reports' peer review meetings can be found on the [NTP DART Reports Peer Review Panels](#) web page.

Report	Report Number	Uses of Studied Substance	Peer Review Information
Peer Review of Draft NTP Technical Report on the Prenatal Developmental Toxicity Studies of Tris(chloropropyl) Phosphate (TCPP)	<a href="#">DART-01</a>	As a flame retardant within textiles, furniture (flexible polyurethane foam), and other related products	<a href="#">Actions</a> <a href="#">Peer Review Report</a>
Peer Review of Draft NTP Technical Report on the Prenatal Developmental Toxicity Studies of 4-Methylcyclohexanemethanol (MCHM)	<a href="#">DART-02</a>	To reduce impurities in mined coal	<a href="#">Actions</a> <a href="#">Peer Review Report</a>

Report	Report Number	Uses of Studied Substance	Peer Review Information
<b>Peer Review of Draft NTP Technical Reports on the Prenatal Developmental Toxicity Studies of Vinpocetine</b>	<a href="#">DART-03</a>	As a dietary supplement for cognitive enhancement, Alzheimer's disease, dementia, and ischemic stroke	<a href="#">Actions</a> <a href="#">Peer Review Report</a>
<b>Peer Review of Draft NTP Technical Reports on the Prenatal Developmental Toxicity Studies of Dimethylaminoethanol Bitartrate (DMAE)</b>	<a href="#">DART-04</a>	As a dietary supplement to improve memory and general cognitive function	<a href="#">Actions</a> <a href="#">Peer Review Report</a>

### 3.3.2. NTP Monographs Peer Reviewed in FY 2019

NTP monographs are prepared for each candidate substance selected for review and consist of a noncancer evaluation component and a substance profile. For each monograph, the panel is charged with reviewing and assessing whether the draft health outcome evaluation component is technically correct and clearly stated, whether NTP objectively presents and assesses the scientific evidence, and whether the scientific evidence is adequate for applying the listing criteria.

The draft monograph listed below underwent peer review in FY 2019. Additional information about past monographs' peer review meetings can be found on the [NTP Research Reports Peer Review Panels](#) web page.

Chemical/Topic	CASRN*	Subject	Peer Review Information
<a href="#">Sarin: Potential Long-term Neurological Effects</a>	107-44-8	Reviewed the evidence for long-term neurological effects in humans and nonhuman animals following acute exposure to sarin.	<a href="#">Actions</a> <a href="#">Peer Review Report</a>

### 3.3.3. NTP Report on Carcinogens Monographs Peer Reviewed in FY 2019

The Report on Carcinogens monographs are prepared for each candidate substance selected for review and consist of a cancer evaluation component and a substance profile. For each monograph, the panel is charged with reviewing and assessing whether the draft cancer evaluation component is technically correct and clearly stated, whether NTP objectively presents and assesses the scientific evidence, and whether the scientific evidence is adequate for applying the listing criteria. For the draft substance profile, the panel is charged with assessing whether the scientific justification presented supports the preliminary NTP policy decision on the Report on Carcinogens listing status.

The draft Report on Carcinogens monograph, listed below, underwent peer review in FY 2019. Additional information about past Report on Carcinogens monographs' peer review meetings can be found on the [NTP Report on Carcinogens Monographs Peer Review Panels](#) web page.

Chemical/Topic	CASRN*	Subject	Peer Review Information
Night Shift Work and Light at Night	—	Reviewed the potential carcinogenic hazard from working night shifts and being exposed to aberrant lighting conditions at night.	<a href="#">Actions</a> <a href="#">Peer Review Report</a>

\*CASRN = Chemical Abstracts Service Registry Number.

### 3.4. Training Opportunities

NIEHS/NTP offers a limited number of postdoctoral training fellowships to prepare trainees for careers in pharmaceutical and chemical industries, regulatory agencies, and academia. In FY 2019, NIEHS/NTP staff mentored 21 postdoctoral fellows at NIEHS in seven focal areas (below). Full details on opportunities, benefits, and the application process can be found on the [NIEHS training website](#).

The training program has seven focal areas:

- (1) Alternative Toxicological Methods
- (2) Applied Toxicology and Carcinogenesis
- (3) Biomolecular Screening and Computational Toxicology; Alternative Methods
- (4) Health Assessment and Translation
- (5) Laboratory Animal Medicine
- (6) Systems and Mechanistic Toxicology
- (7) Toxicological Pathology

#### *NIEHS/NTP Training Program Postdoctoral Fellows in FY 2019*

Training Program	Fellow
Alternative Toxicological Methods	Shagun Krishna
Applied Toxicology and Carcinogenesis	Anika Dzierlenga Madelyn (Mimi) Huang Troy Hubbard AtLee Watson
Biomolecular Screening and Computational Toxicology	Alex Borrel Katelyn Lavrich Arif Rahman Sreenivasa Ramaiahgari Dahea You
Health Assessment and Translation	None
Laboratory Animal Medicine	Ian Chen David Crizer Jingli Liu Xian Wu
Systems and Mechanistic Toxicology	Gopi Gadupudi

Training Program	Fellow
	Janice Harvey Donna Webb-Wright Miaofel Xu
Toxicological Pathology	Daven Jackson-Humbles Gregory Krane Eui Jae Sung



## 4. Research and Testing

NTP evaluates substances and circumstances for a variety of cancer and noncancer health-related effects, usually (but not always) using rodent models. In addition, NTP is a leader in research to develop alternatives to animal testing and new ways to rapidly and efficiently test whether substances adversely affect human health.

### 4.1. Tox21

Toxicology in the 21st Century ([Tox21](#)) is a unique federal collaboration among the [National Institutes of Health](#) (NIH), including the [National Toxicology Program](#) (NTP) at the [National Institute of Environmental Health Sciences](#) (NIEHS) and the [National Center for Advancing Translational Sciences](#) (NCATS), the [U.S. Environmental Protection Agency](#) (EPA), and the [U.S. Food and Drug Administration](#) (FDA). Its purpose is to develop new methods to rapidly test whether chemicals adversely affect human health. Tox21 uses new approaches including high-throughput and high-content methods to evaluate large numbers of chemicals quickly and efficiently to provide insight into potential human health effects. Bioinformatics and computational toxicology support for Tox21 is provided by Tox21 members, including members of the Biomolecular Screening Branch and the [NTP Interagency Center for the Evaluation of Alternative Toxicological Methods](#).



Listed below are the NTP Tox21 projects completed in FY 2019 along with associated NIEHS/NTP staff.

#### *Assay Development in FY 2019*

Project Study Scientists	Project Summary
<b>Use of HepaRG cells for high-content screening</b> Stephen Ferguson, Sreenivasa Ramaiahgari	<ul style="list-style-type: none"> <li>Examined the response of metabolically competent human HepaRG liver cells (derived from a human hepatic progenitor cell line) to exposures of xenobiotic compounds using multiplex, high-content screening assays and high-throughput transcriptomics to help characterize liver-like responses of these cultures.</li> </ul>
<b>Testing of gene signatures and profiles in NTP archival tissues</b> Alex Merrick, Julie Foley	<ul style="list-style-type: none"> <li>Determined whether RNA and DNA extracted from fixed tissue and frozen tissue blocks can be used to measure gene signatures and mutational profiles based on studies of chemical exposure to toxic compounds.</li> </ul>
<b>High-throughput assays and computational models to replace current EPA Endocrine Disruptor Screening Program Tier 1 tests</b> Warren Casey, Nicole Kleinstreuer	<ul style="list-style-type: none"> <li>Developed an approach for using validated ToxCast and Tox21 high-throughput assays and an associated computational model to replace Tier 1 tests currently used to assess endocrine activity in the EPA Endocrine Disruptor Screening Program.</li> </ul>

Project Study Scientists	Project Summary
<b>Liquid biopsy: Circulating cell-free DNA as a predictor of chemical toxicity</b> Julie Foley, Alex Merrick	<ul style="list-style-type: none"> <li>Developed methods for extracting circulating cell-free DNA (ccfDNA) from human, rat, and mouse plasma to serve as a liquid biopsy to help predict or better describe toxicity in affected tissues during chemical exposure.</li> </ul>
<b>Tox21 cross-partner project: In vitro pipeline to assess population toxicodynamic variability for chemicals suspected to cause developmental neurotoxicity</b> Alison Harrill, Mamta Behl, Dahea You, Kristine Witt, Richard Paules	<ul style="list-style-type: none"> <li>Developed a computational and cellular testing framework for assessing genetic susceptibility to chemical agents suspected of causing developmental neurotoxicity using ~200 Diversity Outbred mouse neural progenitor cell lines exposed to a chemical test battery and assessing cellular effect potency using high-content imaging techniques.</li> </ul>
<b>Tox21 cross-partner project: Evaluate expansion of pathway coverage by Tox21 qHTS assays for better prediction of adverse effects from exposures</b> Kristine Witt, Stephen Ferguson	<ul style="list-style-type: none"> <li>Improved the prediction of adverse effects from exposures to drugs and chemicals by using additional assays that can probe toxicologically important targets and pathways that are not captured in current Tox21 testing.</li> </ul>
<b>Tox21 cross-partner project: Retrofitting existing Tox21 HTS assays with metabolic capability</b> Kristine Witt, Stephen Ferguson	<ul style="list-style-type: none"> <li>Refined existing methods to (1) imbue Tox21 assays with metabolic capability, (2) screen the Tox21 10,000-compound collection using these new methods to identify chemicals that are either bioactivated or detoxified by liver cytochrome P450s and cofactors, and (3) identify the particular CYPs responsible for observable shifts in bioactivity.</li> </ul>

### Data Analysis in FY 2019

Project Study Scientist	Project Summary
<b>Analysis of Tox21 quantitative high-throughput screening assay data</b> Jui-Hua Hsieh	<ul style="list-style-type: none"> <li>Developed computational tools and data analysis pipelines for Tox21 quantitative high-throughput screening data to determine the activity of compounds in assays, considering compound potency, efficacy, and data reproducibility.</li> </ul>
<b>Prioritization of Tox21 compounds for genotoxicity</b> Jui-Hua Hsieh, Kristine Witt, Stephanie Smith-Roe, Scott Auerbach, Alex Merrick	<ul style="list-style-type: none"> <li>Developed a prioritization approach for genotoxicity testing that includes using compounds that show clear evidence of activity in the quantitative high-throughput screening genotoxicity assays and compounds that are weakly active based on this analysis.</li> </ul>
<b>Design of Tox21 data exploration graphical user interface</b> Jui-Hua Hsieh	<ul style="list-style-type: none"> <li>Developed two graphical user interfaces for viewing Tox21 data; one used to explore the concentration-response data in a line chart, and the second is used to explore compound similarity relationships in terms of their chemical structures and activities in Tox21 quantitative high-throughput screening assays.</li> </ul>
<b>Data-driven analysis of Tox21 assay data project</b>	<ul style="list-style-type: none"> <li>Developed unsupervised data analysis approaches focused on methods (data organization based on patterns and performed by software) to identify chemicals that exhibit biological properties similar</li> </ul>



Project Study Scientist	Project Summary
Scott Auerbach, Nicole Kleinstreuer	<p>to those of well-characterized toxicants from the quantitative high-throughput screening assays used to screen the 10,000-compound library.</p> <ul style="list-style-type: none"> <li>Continued to update a web interface for multiple integrated tools, along with a supporting manuscript. A chemical structure-based map of the Tox21 chemical library, with the ability to overlap chemical properties and toxicity data, is available <a href="#">here</a>.</li> <li>Work is ongoing to map Tox21 assay targets to tissue types to identify where chemicals may have toxicological effects in the body, and a web application (BodyMap) is planned for release in FY 2020.</li> </ul>
<b>Tox21 cross-partner project: Cell line selection for Tox21 Screening</b> Nisha Sipes	<ul style="list-style-type: none"> <li>Developed a data-driven approach to choose cell lines to maximize biological diversity using a content maximization approach to pick a diverse set of cell types that are based on publicly available gene expression data and baseline gene expression profiling.</li> </ul>
<b>Aggregated hit-call of Tox21 data</b> Nisha Sipes	<ul style="list-style-type: none"> <li>Identified higher-confidence chemical-assay actives by developing an aggregated hit-call function of Tox21 data analysis methods and a web-based tool for public access to the data and visualizations.</li> </ul>
<b>Next-generation sequencing in toxicology</b> Alex Merrick, Kristine Witt, Stephanie Smith-Roe	<ul style="list-style-type: none"> <li>Developed bioinformatic pipelines for genomic and transcriptomic gene expression analysis and mutational analysis on a genome-wide level using next-generation sequencing technologies to build signatures of toxicity and chemical exposure.</li> <li>Expanded to evaluate gene expression changes in frozen tissue samples from brain subregions obtained from genetic toxicity studies conducted as part of the NTP Cell Phone Radio Frequency Radiation study. Deep Duplex Sequencing is also being investigated for mutational analysis.</li> </ul>
<b>Semi-automated extraction of literature using machine-learning methods</b> Warren Casey, Nicole Kleinstreuer	<ul style="list-style-type: none"> <li>Created a comprehensive database (described in a manuscript published in FY 2016) of high-quality in vivo data from over 1,000 scientific articles describing uterotrophic assay experiments for more than 2,660 distinct combinations of chemicals, studies, and protocols.</li> <li>Used database as a training set for developing a semi-automated approach to extracting literature data, which is being applied to developmental toxicity studies.</li> </ul>
<b>Evaluation and qualification of in silico methods for predicting metabolism</b> Stephen Ferguson	<ul style="list-style-type: none"> <li>Evaluated various in silico methods for predicting the extent of xenobiotic metabolism and identifying metabolites for prioritizing chemicals in the Tox21 10,000-compound library.</li> </ul>
<b>Selection of a target set of genes for use in a high-throughput transcriptomics screen</b> Richard Paules, Scott Auerbach, Elizabeth Maull, Alex Merrick, Nisha Sipes	<ul style="list-style-type: none"> <li>Determined the best target set of genes representing humans, rats, mice, and zebrafish to detect patterns of exposure-induced biological responses to characterize toxicity and disease pathways and facilitate extrapolation of findings from model species to humans.</li> </ul>

Project Study Scientist	Project Summary
<b>Development of quantitative structure-activity relationship (QSAR) models to predict androgen receptor binding and activity</b> Warren Casey, Nicole Kleinstreuer	<ul style="list-style-type: none"> <li>Developed QSAR models to predict androgen receptor binding and activity using the computational model of the androgen receptor pathway. These QSAR models are currently being refined, with a goal of using them to predict androgen receptor pathway activity of chemicals in the EPA Endocrine Disruptor Screening Program.</li> </ul>
<b>Tox21 assay target mapping and machine learning</b> Warren Casey, Nicole Kleinstreuer	<ul style="list-style-type: none"> <li>Mapped Tox21/ToxCast assay targets to known modes of action for developmental toxicity, acute toxicity, and carcinogenicity.</li> <li>Mapped assays are being combined with in silico features to build machine-learning models to provide predictions of chemical hazard.</li> <li>Future updates of the NICEATM Integrated Chemical Environment (ICE) will include grouping of Tox21 assays based on this target mapping.</li> </ul>
<b>Curation of high-throughput screening data</b> Warren Casey, Nicole Kleinstreuer	<ul style="list-style-type: none"> <li>Curated high-throughput screening data from Tox21 and EPA ToxCast HTS program (available through the NICEATM ICE database) to identify and exclude low-confidence activity calls, considering factors such as chemical stability and purity information, robustness of concentration-response curve fits, and contextualization of active concentrations relative to testing range.</li> </ul>
<b>Development of software and methods for performing genomic dose-response analysis</b> Scott Auerbach	<ul style="list-style-type: none"> <li>Developed methods and software for performing genomic dose-response analysis to identify sensitive screening-level potency estimates.</li> </ul>
<b>Evaluation of the in vivo genomic dose-response approach for identifying biological effect points of departure</b> Mike DeVito, Will Gwinn, Scott Auerbach, Fred Parham	<ul style="list-style-type: none"> <li>Determined if dose-response modeling of toxicogenomics data from short-term, in vivo studies can be used to identify biological effect points of departure that are comparable in potency to those derived from long-term toxicity studies.</li> </ul>
<b>Tox21 cross-partner project: Performance-based validation of Tox21 assays</b> Nicole Kleinstreuer	<ul style="list-style-type: none"> <li>Developed semi-automated approaches to identify reference chemicals for Tox21/ToxCast assay targets with EPA.</li> </ul>

### Testing Projects in FY 2019

Project Study Scientist	Project Summary
<b>Tox21 Cross-partner project: Predictive modeling of developmental toxicity with human pluripotent stem cells</b> Nicole Kleinstreuer	<ul style="list-style-type: none"> <li>Evaluated approximately 80 chemicals in a metabolomic biomarker-based assay using human pluripotent stem cells using chemicals that were selected based on ICH guidance and reference NTP in vivo studies and that are relevant to the program.</li> </ul>

Project Study Scientist	Project Summary
<p><b>Tox21 Cross-partner project: Development of a common reference chemical data set for interpretation of high-throughput transcriptomic screening data</b></p> <p>Stephen Ferguson, Richard Paules, Suramya Waidyanatha</p>	<ul style="list-style-type: none"> <li>Identified reference chemicals with a rich legacy of molecular target interaction knowledge (e.g., IC<sub>50</sub>, K<sub>i</sub>, K<sub>D</sub>, EC<sub>50</sub>) that are being leveraged to create a contextualized biological-response space in MCF-7 cells and 3D HepaRG spheroid culture models as a framework for the interpretation of future transcriptomic screening studies.</li> </ul>

## 4.2. Testing and Toxicology Studies

The NTP testing program evaluates substances for a variety of health-related effects, generally using rodent models. For each test article, a study team develops an appropriate testing strategy to address the identified research needs, and a project review committee evaluates the strategy. Reports and summaries of NTP toxicity studies, including carcinogenicity and effects on development and reproduction, are available on the NTP website.

The following Division of NTP branches at NIEHS are involved in the testing program:

- Biomolecular Screening Branch, led by acting chief Richard S. Paules, Ph.D.
- Cellular and Molecular Pathology Branch, led by chief Robert Sills, D.V.M., Ph.D.
- NTP Laboratory, led by acting chief Michael DeVito, Ph.D.
- Program Operations Branch, led by chief Michelle Hooth, Ph.D.
- Toxicology Branch, led by acting chief Nigel Walker, Ph.D.

Information about the more than 500 studies initiated, ongoing, or completed in 2019 are listed in [Appendix II](#).

## 4.3. NICEATM

The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) focuses on the development and evaluation of alternatives to animal use for chemical safety testing.

NICEATM activities include:

- Conducting and publishing analyses and evaluations of data from new, revised, and alternative testing approaches
- Providing curated data and information to test method developers, regulators, and regulated industry through its website and other communications and by organizing workshops and symposia
- Coordinating and providing scientific and operational support for the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) meetings, webinars, teleconferences, working groups, and public forums
- Providing bioinformatics and computational toxicology support to NIEHS/NTP projects, especially those related to Tox21

Warren Casey, Ph.D., is director of NICEATM, and Nicole Kleinstreuer, Ph.D., is deputy director. NICEATM receives contract support from Integrated Laboratory Systems, Inc.

### 4.3.1. NICEATM Webinars and Workshops

#### *Workshop: [Converging on Cancer](#)*

Cancer, a leading cause of mortality worldwide, is a complex disease with varied presentations that involves dysregulation of multiple interconnected signaling pathways. Diverse environmental factors have been associated with the development and progression of various cancer types. A critical question in the field of environmental health is how to harness what is known about cancer biology and associated environmental exposures to improve public health outcomes.



The Converging on Cancer Workshop, held April 29–30, 2019, aimed to provide a clear path forward for evaluating the interactions between environmental exposures and cancer biology using the latest tools in toxicology and identifying knowledge gaps that require research attention. The workshop was preceded by four webinars that provided an overview of current activities and introduced the concepts of “key characteristics of carcinogens” and “hallmarks of cancer.” At the workshop, more than 100 in-person attendees considered how to assess potential carcinogenicity of low doses of chemicals in mixtures. Workshop participants, including approximately 500 online viewers, suggested:

- Initial efforts should focus on breast, liver, colon, and lung cancers
- Investigating effects of mixtures of carcinogens should be prioritized over mixtures of noncarcinogens
- Disease-centered and pathway-based approaches should be prioritized over animal models

A workshop report is in preparation and will be submitted for publication in FY 2020.

#### *Workshop: [Implementing Nonanimal Approaches to Human and Veterinary Vaccine Testing: Achieving Scientific and Regulatory Success for Rabies and Beyond](#)*

Vaccines improve human and animal health and welfare by preventing the spread of infectious diseases. However, testing to ensure effectiveness and safety of these products often requires the use of large numbers of animals. Technological advances have led to the development of methods that could reduce or eliminate the need for animal testing for vaccines.

In coordination with the International Alliance for Biological Standardization – North America (IABS-NA), NICEATM organized an October 16–17, 2018 workshop to bring together experts from government, academia, and industry to advance alternative methods for human and veterinary rabies vaccine testing. Presentations detailed the current state of the science of nonanimal alternatives to traditional animal-based rabies virus vaccine potency and safety tests. Breakout group discussions focused on the steps necessary for implementing alternatives for veterinary and human rabies virus vaccine potency testing. Participants identified actions and data



needed for further progress and laid the foundation for a roadmap toward successful implementation of alternative tests.

More [information about the workshop](#) is available on the IABS-NA website.

#### 4.3.2. NICEATM Support of Tox21

The Tox21 federal research initiative aims to improve regulatory hazard assessment of substances potentially harmful to humans and the environment. Tox21 uses in vitro high-throughput screening assays to evaluate the biological activity of compounds in a 10,000-compound library and to relate observed activities to toxicological endpoints.

NICEATM provides support to the Tox21 effort primarily through its computational toxicology group. Specific NICEATM projects in support of Tox21 are included in the [Tox21](#) section.

#### 4.3.3. Additional NICEATM Activities

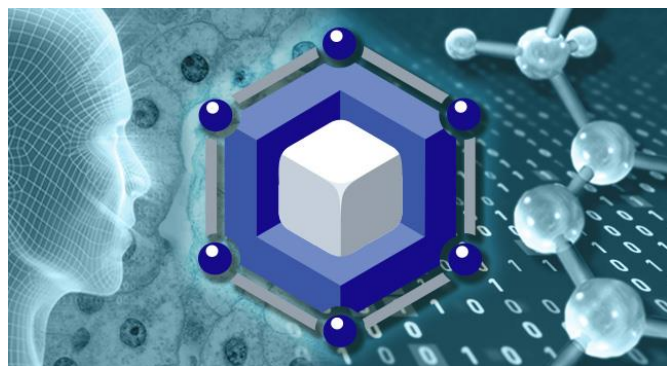
##### *Integrated Chemical Environment*

Successful computational toxicology projects depend on freely available, high-quality data that are formatted for use in computational workflows. The NICEATM [Integrated Chemical Environment](#) (ICE) resource, launched in 2017, provides data from NICEATM and its partners as well as other resources and tools in an environment designed to support chemical safety assessment and new method development and evaluation.

ICE 2.0, launched in May of 2019 and updated in July, includes:

- A new home page consistent with the layout of the NTP website
- A new “Search” module that replaces the original “Integrator” and simplifies assay selection
- Ability to include chemicals in a search with the same core structure, such as salts and stereoisomers
- Updates and improvements to the high-throughput in vitro to in vivo extrapolation (IVIVE) tool
  - New Tox21 data and curation updates based on analytical chemistry quality control, concentration-response curve flag integration, and tagging of assay endpoints
  - Computational model predictions of androgen and estrogen receptor agonist, antagonist, and binding activities and acute oral systemic toxicity
  - Simplified outputs for the machine-learning tool
  - Revised, easier-to-use user guides

Updates in early FY 2020 will improve the user interface, make it easier for users to download key data sets, update Tox21 data, provide the ability to input user-defined data for the IVIVE tool, and add a user guide for the “Search” tool.





## *Acute Systemic Toxicity*

### **In silico models**

The ICCVAM Acute Toxicity Workgroup organized a [global project in FY 2018](#) to develop in silico models of acute oral systemic toxicity that predict five specific endpoints needed by regulatory agencies. NICEATM invited scientists to develop in silico models that predict any or all of these endpoints. Models developed for the project that met review criteria were used by NICEATM to generate consensus predictions for the acute oral toxicity endpoints of interest and were combined using a weight-of-evidence (WOE) approach into a consensus model. The consensus predictions are available in the Collaborative Acute Toxicity Modeling Suite (CATMoS), a free resource for screening organic chemicals for acute oral toxicity. CATMoS is implemented in v2.0 of the Open Structure-Activity/Property Relationship App (OPERA), a free and open-source quantitative structure-activity relationship (QSAR) tool described in more detail below under “Computational Toxicology.” The consensus predictions generated for the project will also be available in ICE and the U.S. Environmental Protection Agency (EPA) CompTox Dashboard in addition to OPERA. The CATMoS project will be described in a manuscript to be submitted for publication in FY 2020.

### **Dermal absorption**

Dermal absorption is a key factor in the potential of substances to cause systemic toxicity upon skin exposure. EPA combines data from in vivo rat, in vitro rat, and in vitro human dermal absorption studies, commonly referred to as the “triple pack,” to calculate a chemical-specific dermal absorption factor. To assess the feasibility of using data from in vitro studies alone to estimate this value, NICEATM and EPA are conducting a retrospective analysis of triple pack reports completed between 2005 and 2015 and comparing in vivo to in vitro results. A stakeholder meeting was convened in May 2019 at EPA to discuss the preliminary findings from this review and to identify and discuss additional information needs for both U.S. and Canadian regulators to finalize the assessment. A paper detailing this work is planned for submission in FY 2020.

### **Additivity approaches to predicting toxicity of formulations**

The EPA Office of Pesticide Programs has been accepting submissions of oral and inhalation toxicity data for agrochemical formulations paired with toxicity calculations done in accordance with the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) additivity equation, which is based on the individual components of the formulation. NICEATM is evaluating the extent to which acute toxicity results predicted using the additivity equation compare with the in vivo results for the formulation. The evaluation will be finalized in FY 2020 along with a report describing the results.

### **Acute fish toxicity**

To assess potential hazards to wild fish species, EPA currently requires testing in each of three different fish types: warmwater, coldwater, and marine/estuarine. NICEATM and the EPA Office of Pollution Prevention and Toxics are extracting and evaluating acute fish toxicity data from pesticide safety data submitted to the EPA office. Data will be analyzed to determine whether one or more of the three fish types can be eliminated from testing requirements. Extracted data will be submitted to EPA’s Toxicological Reference Database (ToxRefDB).

## *Cardiotoxicity*

An initiative is underway to design, build, and test new nonanimal approaches to assess cardiotoxicity hazard. The goal is to identify and develop human cell- and protein-based assays, as well as in silico QSAR models, to more efficiently screen drugs and chemicals for their potential to be toxic to the heart or circulatory system. The initiative is supported jointly by NTP (NIEHS/NTP [Biomolecular Screening Branch](#) and NICEATM), the FDA Center for Drug Evaluation and Research, and the Health and Environment Sciences Institute (HESI). Two NTP projects within this initiative focus on in silico

screening approaches for assessing cardiovascular safety. The NICEATM project is mining public data sources to compile a reference cardiotoxicant tool compound list and identify human-relevant data, as well as analyzing the Tox21 data to prioritize chemicals on the basis of their activity against cardiotoxicity-related endpoints. This work is intended to help build and test the capabilities of models and integrated testing strategies for cardiovascular hazards. Work in FY 2019 identified molecular and cellular events potentially contributing to cardiovascular failure modes that may be measurable by assays as part of a translational toxicology pipeline.

### *Computational Toxicology*

NICEATM's computational toxicology group uses mathematics, informatics, data analytics, and computer models to better understand toxicity mechanisms and predict toxic effects.

### **OPERA**

QSAR models provide predictions of chemical activity that can augment nonanimal approaches for predicting toxicity. To provide robust QSAR models for chemical properties of environmental interest, the EPA National Center for Computational Toxicology created OPERA ([Mansouri et al. 2018](#)). OPERA, a free and open-source/open-data suite of QSAR models available on the [NIEHS GitHub repository](#), is an ongoing collaboration between NICEATM and EPA. OPERA predictions of toxicity endpoints and physicochemical properties are available through ICE and the EPA CompTox Chemicals Dashboard.

Several additions were made to OPERA in FY 2019:

- OPERA now includes consensus models for estrogenic and androgenic pathway activity and acute oral systemic toxicity. These models were the results of the international collaborative projects led by NICEATM and EPA to leverage the expertise of the worldwide modeling community to predict estrogenic activity (CERAPP; [Mansouri et al. 2016](#)), anti-androgenic activity (CoMPARA; Mansouri et al. submitted) and acute oral systemic toxicity ([CATMoS](#)). OPERA predictions of toxicity and physicochemical properties are available through ICE and the EPA CompTox Chemicals Dashboard.
- OPERA also includes new QSAR models to predict critical IVIVE parameters. To support an open-source workflow for IVIVE, NICEATM developed QSAR models to predict properties that affect how substances behave in biological systems, such as human plasma fraction unbound and hepatic clearance. The QSAR models are available via [OPERA](#), and the predictions are used in [ICE](#).
- Other improvements to OPERA in FY 2019 included the addition of a user-friendly graphical interface and improvements to speed. New features to support usability included different file type parsing options for chemical structures and identifiers such as CAS registry numbers.
- A JAVA library to be used for live predictions on tools such as ICE and the EPA Chemicals Dashboard will be added to OPERA in FY 2020.

### **Models to predict acid dissociation constant**

The acid dissociation constant pKa affects chemical absorption, distribution, metabolism, excretion, and toxicity properties. NICEATM used a freely available data set and three machine-learning approaches to develop open-source models for pKa prediction. Performance of these models, which are available as free and open-source software, compared favorably to commercial products. A paper describing the work ([Mansouri et al. 2019](#)) was published in September, and the models have been incorporated into OPERA.

## *Developmental Toxicity*

### **Embryonic vascular development**

Work to identify alternative methods for developmental toxicity testing has focused on understanding and predicting disruption of key mechanisms in embryonic and fetal development. Adverse outcome pathways (AOPs) provide a useful framework for integrating the evidence derived from in silico and in vitro systems to inform chemical hazard characterization. An ongoing collaboration between NICEATM and EPA has built and applied an AOP for developmental toxicity through a mode of action linked to embryonic vascular disruption. A publication in FY 2019 ([Saili et al. 2019](#)) reviewed the model for quantitative prediction of developmental vascular toxicity from ToxCast high-throughput screening (HTS) data and compared the HTS results to functional vascular development assays in complex cell systems, virtual tissues, and small model organisms. Results increased confidence in the capacity to predict adverse developmental outcomes from HTS in vitro data and model computational dynamics for in silico reconstruction of developmental systems biology.

### **Stem cell metabolomics**

A key issue with high-throughput in vitro testing methods is how to accurately relate concentrations of substances that induce in vitro responses to in vivo exposure levels that could result in corresponding adverse human or animal effects. This relationship is established through IVIVE. NICEATM scientists used IVIVE to evaluate the effect of pharmacokinetics and different modeling approaches on predicting relevant external exposures from in vitro developmental toxicity potential concentrations obtained from a stem cell-based in vitro metabolomics assay. Preliminary results showed close agreement between predictions derived from in vitro data and doses inducing responses in rat studies for two valproate analogues. This suggests that positive effects in the in vitro assay, when combined with IVIVE approaches, can quantitatively predict in vivo developmental toxicity potential. An abstract describing this work has been submitted for presentation at the 2020 Society of Toxicology (SOT) annual meeting.

### **SEAZIT**

The small size and rapid development of zebrafish make it a useful vertebrate model for assessing the potential effects of substances on growth and development using high-throughput screening methods. To enable the broader adoption of zebrafish for toxicological screening, NTP established the Systematic Evaluation of the Application of Zebrafish in Toxicology (SEAZIT) program, jointly led by NTP and NICEATM scientists. NICEATM presented an update on SEAZIT ([Hamm et al.](#)) at the 2019 annual meeting of the Society for Birth Defects Research and Prevention (formerly the Teratology Society), and supported SEAZIT in FY 2019 through the following activities.

- A summary of current zebrafish husbandry and toxicology study practices was published in January ([Hamm et al. 2019](#)).
- NICEATM is coordinating collaborative projects to establish ontologies (standardized nomenclature systems) for zebrafish screening. Three laboratories tested the same 90-chemical set using similar study designs and morphology assessments. Data from these studies are being curated and assessed to refine testing approaches and support development of informatics resources. A poster describing this project ([Ceger et al.](#)) was presented at the 2019 annual meeting of the Society for Birth Defects Research and Prevention.
- To further define ontologies for zebrafish screening, NICEATM coordinated an online evaluation of heterogeneity in terms used to describe zebrafish phenotypes following chemical exposure. Zebrafish images were posted online and researchers were asked to evaluate the phenotypes using their in-lab terminology. Results were collected and compiled, and terms were mapped to the Zebrafish Phenotype Ontology. A second online evaluation is scheduled for FY 2020 and will employ controlled vocabulary.



- An interlaboratory study to examine effects of key protocol elements was initiated in FY 2019. Participating laboratories are conducting dose range-finding experiments.

### *Endocrine Disruptors*

#### **Validation of androgen receptor activity assays**

NICEATM collaborated with test method developer CertiChem, Inc., to validate an in vitro test method that uses MDA-Kb2 human breast cancer cells to measure androgen receptor agonist and antagonist activity. Specifically, NICEATM provided guidance on incorporating a cytotoxicity assay into the test method protocol. Testing of 67 coded reference chemicals in agonist and antagonist modes to characterize method reliability and relevance is complete, and a report summarizing these results was prepared and provided to CertiChem in December 2018.

#### **In silico predictions of androgen receptor pathway activity**

NICEATM and EPA ran a global collaboration to leverage the expertise of the worldwide modeling community to predict androgenic activity in the Collaborative Modeling Project for Androgen Receptor Activity (CoMPARA). Predictions from these models are available through ICE and the EPA Chemistry Dashboard. The consensus models from CoMPARA were also added to the standalone OPERA application. A paper describing the CoMPARA project will be published in early FY 2020.

### *Skin Sensitization*

#### **Expanding the applicability domain of defined approaches to identifying skin sensitizers**

The DNTP Toxicology Branch is testing over 200 chemicals nominated by ICCVAM agencies using three in vitro test methods to assess and expand the applicability domain of defined approaches for identifying skin sensitizers. Mouse local lymph node assay data are available for the nominated chemicals, which include pesticides, formulations, industrial chemicals, and other chemicals of interest to ICCVAM agencies. The three in vitro test methods are the KeratinoSens method, the direct peptide reactivity assay (DPRA), and the human cell line activation test (h-CLAT). NICEATM is coordinating the testing, which began in 2017 and is scheduled for completion in early FY 2020. The study data will enable NICEATM and ICCVAM to evaluate the appropriateness of defined approaches using these three in vitro methods for various regulatory applications.

#### **Human reference data for skin sensitization**

To support the evaluation of nonanimal approaches for skin sensitization assessment, NICEATM, the German Federal Institute for Risk Assessment, and other collaborators collected data for over 2,500 human predictive patch tests from more than 1,500 publications. Results from 1,900 tests considered to be sufficiently reliable were classified using the GHS classification system. This database will be made publicly available for additional evaluation of alternative skin sensitization methods and development of new models. An abstract describing this work has been submitted for presentation at the 2020 SOT annual meeting, and a paper will be submitted for publication in FY 2020.

### *Eye Irritation*

#### **In vitro testing of agrochemical formulations**

NICEATM, the PETA International Science Consortium, EPA, and CropLife America member companies are collaborating to develop an in vitro defined approach for hazard classification of eye irritation potential of agrochemical formulations. A three-phased prospective evaluation was designed to (1) assess the applicability of seven in vitro eye irritation/corrosion protocols to agrochemical formulations and (2) develop a defined approach for agrochemical formulations testing for prediction of U.S. and international irritancy classifications. In Phase 1, completed in FY 2018, six formulations were tested in seven different eye irritation test protocols. Ten additional agrochemical formulations

with in vivo data representing a wider range of eye irritation classifications were evaluated in Phase 2 during FY 2019. Although none of the methods directly correlated with the in vivo results, several methods showed potential for use in a defined approach to assess agrochemical formulations. Phase 1 and 2 results will be used to identify which protocols will be evaluated in Phase 3 and can form the basis of a defined approach for testing of agrochemical formulations for eye irritation potential. An abstract describing this work has been submitted for presentation at the 2020 SOT annual meeting.

#### **Statistical models for eye irritant classification**

NICEATM developed statistical models to classify chemicals as eye irritants/corrosives or non-irritants/corrosives using an in silico approach. This approach was able to differentiate chemicals falling into EPA hazard categories I and II (which require eye protection when handling) from chemicals falling into hazard categories III and IV (which do not require eye protection). A paper describing the models is in preparation and will be submitted for publication in FY 2020.

#### *Skin Irritation*

The in vivo rabbit skin test is the benchmark against which nonanimal alternatives for skin irritation testing are compared. However, the variability inherent to the subjective scoring of erythema and edema responses in the rabbit test is a potential confounding factor for such comparisons. To better characterize the reproducibility of the in vivo assay, NICEATM compiled and curated a data set of over 3,000 in vivo assay test records to assess variability in results from chemicals tested multiple times. The analysis indicates that the level of variability present in the rabbit skin irritation test should be taken into consideration when evaluating the performance of nonanimal alternative methods. An abstract describing this work has been submitted for presentation at the 2020 SOT annual meeting. The data set is being expanded and further refined to facilitate a more detailed analysis of variability.

## **4.4. ICCVAM**

The [Interagency Coordinating Committee on the Validation of Alternative Methods](#) (ICCVAM) is a permanent interagency committee of NIEHS under NICEATM. Established by the ICCVAM Authorization Act of 2000 (42 U.S.C. 285l-3), its purpose is “to establish, wherever feasible, guidelines, recommendations, and regulations that promote the regulatory acceptance of new or revised scientifically valid toxicological tests that protect human and animal health and the environment while reducing, refining, or replacing animal tests and ensuring human safety and product effectiveness.”



ICCVAM is composed of representatives from 16 U.S. federal regulatory and research agencies that generate or use toxicological and safety testing information (see below). Warren Casey, Ph.D., serves as administrative director of ICCVAM.

- Agency for Toxic Substances and Disease Registry
- U.S. Consumer Product Safety Commission
- U.S. Department of Agriculture
- U.S. Department of Defense
- U.S. Department of Energy
- U.S. Department of the Interior
- U.S. Department of Transportation

- U.S. Environmental Protection Agency
- U.S. Food and Drug Administration
- National Cancer Institute
- National Institute of Environmental Health Sciences
- National Institutes of Health
- National Institute for Occupational Safety and Health
- National Institutes of Standards and Technology
- National Library of Medicine
- Occupational Safety and Health Administration

#### 4.4.1. Progress toward Strategic Roadmap Goals

ICCVAM coordinated the development of “[A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States](#),” published in January 2018 and available on the NTP website.

The roadmap created a framework to guide the development of enabling technologies and promote strategies to establish confidence in and ensure utilization of new approaches to toxicity testing that improve human health relevance and reduce or eliminate the need for testing in animals. The successful development and implementation of these new approaches will require coordinated efforts that address three strategic goals:

- (1) Connect users with developers of new approach methodologies.
- (2) Foster the use of efficient, flexible, and robust practices to establish confidence in new methods.
- (3) Encourage the adoption and use of new methods and approaches by federal agencies and regulated industries.

Pages on the NTP website describe activities underway to address the roadmap goals in the following areas:

- [Acute systemic toxicity](#)
- [Skin and eye irritation](#)
- [Skin sensitization](#)

A key objective for roadmap implementation is defining testing and information needs for U.S. regulatory agencies. Papers published in FY 2019 summarized those needs for skin and eye irritation ([Choksi et al. 2018](#)), skin sensitization ([Strickland et al. 2019](#)), and read-across approaches ([Patlewicz et al. 2019](#)). A paper summarizing U.S. regulatory agency testing needs for acute systemic toxicity was published in FY 2018 ([Strickland et al. 2018](#)).



#### 4.4.2. ICCVAM Meetings

NICEATM supported six teleconferences and one in-person meeting held by ICCVAM in FY 2019. NICEATM also supported eight ad hoc ICCVAM workgroups focused on acute systemic toxicity, ocular and dermal irritation, developmental and reproductive toxicity, skin sensitization, use of read-across in toxicity testing applications, in vitro-to-in vivo extrapolation, nanomaterials, and ecotoxicology.



The fifth ICCVAM Communities of Practice webinar was held on January 22, 2019. The webinar summarized ongoing collaborations to replace animal use for acute and subchronic inhalation toxicity testing. Amy Clippinger, Ph.D., PETA International Science Consortium, Ltd., provided an overview of regulatory agency information needs and nonanimal approaches to filling those needs. Paul Hinderliter, Ph.D., Syngenta Crop Protection, Inc., described a nonanimal approach to developing an inhalation risk assessment based on a specific case study. [Presentations from the webinar](#) are available on the NTP website.

ICCVAM, with NICEATM support, held its sixth public forum on May 23, 2019, at the National Institutes of Health in Bethesda, Maryland. Representatives from nine ICCVAM member agencies were joined by attendees representing stakeholder groups and over 200 webcast viewers.

Presenters at the public forum described ICCVAM agency activities to implement the [strategic roadmap](#) for establishing new approaches to evaluate the safety of chemicals and medical products in the United States. A key focus of the forum was the progress made toward reducing and replacing animal use for acute toxicity tests required by regulatory agencies: [acute systemic toxicity](#), [skin and eye irritation](#), and [skin sensitization testing](#). Public comments submitted to the meeting praised specific actions agencies took in the past year to advance the strategic roadmap goals and suggested additional activities that could support further progress. The meeting agenda and presentations are available on the [NTP website](#).

#### 4.4.3. ICCVAM Test Method Evaluation Activities

ICCVAM welcomes submissions of innovative test methods that might be acceptable for specific regulatory use and for which adequate validation studies have been completed. To maximize effective implementation of new test methods or approaches, however, ICCVAM evaluates and recommends only those test methods proposed for regulatory uses that align with ICCVAM member agencies' needs and priorities. More [information on ICCVAM test method submissions](#) is available.

##### *Test Method Evaluation Activities in FY 2019*

Test Method	ICCVAM Recommendations/Agency Status
<b>Electrophilic allergen screening assay</b>	<ul style="list-style-type: none"> <li>This test method, nominated by the National Institute for Occupational Safety and Health, is an in chemico assay intended to identify potential skin sensitizers.</li> <li>A validation study of the method began in FY 2017 with four ICCVAM agencies participating in the study. NICEATM is coordinating the study, and members of the ICCVAM Skin Sensitization Workgroup are serving on the study management team.</li> <li>Testing of 10 chemicals during FY 2018 showed that the method had sufficiently good reproducibility and accuracy rates to support further evaluation. In FY 2019, the Consumer Product Safety Commission and the National Institute of Standards and Technology modified the assay to a 96-well format to increase throughput and</li> </ul>

Test Method	ICCVAM Recommendations/Agency Status
	accessibility of the assay. Testing of 20 chemicals will be conducted using the 96-well assay during FY 2020.
<b>OptiSafe</b>	<ul style="list-style-type: none"> <li>▪ NICEATM coordinated a multi-laboratory validation study to determine the reliability and relevance of the OptiSafe test method. In this method, a test substance is applied to a semi-permeable membrane to assess the substance's potential to cause eye irritation.</li> <li>▪ The study was completed in FY 2018 and a report on the study has been submitted for publication. The study demonstrated that the OptiSafe test method is useful for identifying non-surfactant substances not requiring classification for ocular irritancy and thus can reduce the use of animals for this type of testing.</li> </ul>
<b>EpiAirway™</b>	<ul style="list-style-type: none"> <li>▪ A cooperative agreement under the NIEHS Phase IIb Small Business Innovation Research provides funding to MatTek Corporation to validate its EpiAirway™ in vitro human bronchial tissue model to predict the toxicity of inhaled chemicals. Testing of reference chemicals is ongoing to determine the usefulness and limitations of EpiAirway for this purpose.</li> <li>▪ Several ICCVAM agency representatives are members of the cooperative agreement steering committee. Testing is ongoing with chemicals selected by steering committee members.</li> <li>▪ NICEATM provides scientific oversight of this NIEHS small business program supporting validation of alternative test methods.</li> </ul>

The ICCVAM agencies are engaged in additional activities that support replacing, reducing, and refining animal use. Summaries of these [additional activities](#) are on the NTP website.

#### 4.4.4. ICCVAM International Validation Activities

NICEATM and ICCVAM participate in international test method validation activities through the Organisation for Economic Co-operation and Development (OECD). They also collaborate with countries and regions participating in the International Cooperation on Alternative Test Methods (ICATM), including the European Union, Japan, Korea, Canada, Brazil, and China.

Representatives from OECD and ICATM participating organizations in Canada, the European Union, Japan, and Korea attended the September 2019 meeting of the Scientific Advisory Committee on Alternative Toxicological Methods. In a separate ICATM meeting, attendees gave updates on their organizations' activities. Links to SACATM presentations are available on the [Past SACATM Meetings](#) page on the NTP website in the far-right column along with other materials for each meeting.

In FY 2019, ICCVAM agencies commented on draft OECD documents through the U.S. National Coordinator for the OECD Test Guidelines Programme, an ex officio ICCVAM member. Beginning in 2018, the U.S. National Coordinator has been joined at the annual meeting of the National Coordinators by one or more ICCVAM members who are subject matter experts in topics planned for discussion at the meeting. ICCVAM members and/or NICEATM staff supported the Test Guidelines Programme during FY 2019 by:

- Serving on an expert group developing a guideline for defined approaches for skin sensitization; the guideline will describe the adopted defined approaches with respect to their intended regulatory purposes of hazard identification or potency subcategorization



- Serving on expert groups for skin sensitization and eye irritation, which are tasked with reviewing various guidelines and guidance documents relevant to those endpoints
- Serving on a peer review panel for an OECD-coordinated study evaluating the use of the kinetic direct peptide reactivity assay for classifying substances for skin sensitization potency

ICATM collaborations address three critical areas of cooperation: test method validation studies, independent peer review of validation studies, and development of formal recommendations on alternative testing methods.

- In October 2018, representatives of NICEATM and ICCVAM attended an ICATM workshop titled “Validation and Establishing Scientific Confidence” and subsequent coordination meeting. Workshop participants discussed how to address the many challenges of evolving the process of validation, including demonstrating human health relevance without animal data and establishing more efficient validation procedures. A white paper outlining steps to be taken to establish new approaches to validation is being prepared by meeting participants.
- At the subsequent coordination meeting, attendees shared updates on their organizations’ activities and continued discussions from the preceding workshop. As a first step toward addressing the identified issues, ICATM representatives agreed that they would conduct a detailed review of [OECD Guidance Document 34](#), the current standard for validation of chemical safety test methods. The goal of this review is to identify more specific issues to be addressed in moving forward with new approaches to validation.

## 5. Literature Analysis

NTP analyzes scientific literature to evaluate the evidence of adverse noncancer health effects and carcinogenicity. The Report on Carcinogens is a congressionally mandated listing of substances that either are known to be human carcinogens or might reasonably be anticipated to be human carcinogens and to which a significant number of people residing in the United States are exposed.

### 5.1. Noncancer Research

NTP conducts literature-based evaluations using systematic review methods to assess the evidence that environmental substances may be associated with adverse, noncancer health effects. NTP also provides opinions on whether these substances might be of concern given what is known about current human exposure levels. The NIEHS/NTP [Office of Health Assessment and Translation \(OHAT\)](#) conducts health hazard assessments and other evidence evaluations—including scoping reviews, evidence maps, and state-of-the-science evaluations, which are published as NTP monographs, NTP research reports, and journal publications—and hosts workshops to address important issues in environmental health sciences. Andrew Rooney, Ph.D., served as acting director of OHAT in FY 2019.

In FY 2019, OHAT completed two health effects evaluations that resulted in a pair of NTP monographs: *NTP Monograph on the Systematic Review of Occupational Exposure to Cancer Chemotherapy Agents and Adverse Health Outcomes* and *NTP Monograph on the Systematic Review of Long-term Neurological Effects Following Acute Exposure to Sarin*. With these evaluations, and with other ongoing NTP literature-based evaluations, health effects assessment requires the identification, review, and integration of evidence from an increasing number of published studies. Given these challenges, NTP is exploring, developing, and implementing semi-automated approaches to the labor-intensive steps in the systematic review and evidence mapping processes to improve efficiency and to reduce the workload and resources required without comprising the rigor and transparency that is critical to the method.

#### *Ongoing Noncancer Health Effects Projects*

Project Study Scientist	Project Summary	Status
<b>Evaluation of long-term neurological effects of acute exposure to sarin</b>  Andrew Rooney	<ul style="list-style-type: none"> <li>Sarin is a highly toxic organophosphorus nerve agent developed for chemical warfare during World War II that continues to be used as a weapon today.</li> <li>In partnership with the NIH Countermeasures Against Chemical Threats (CounterACT) Program, NTP conducted a systematic review to evaluate the evidence for long-term neurological effects in humans following acute exposure to sarin.</li> <li>Conclusions were reached that are specific for three time periods following acute exposure to sarin based on evidence that sarin results in multiple neurological effects. NTP concludes that acute sarin exposure is known to be a neurological hazard to humans in the initial time period of greater than 24 hours to 7 days after exposure. NTP also concludes that acute sarin exposure is suspected of being a</li> </ul>	Report preparation

Project Study Scientist	Project Summary	Status
	neurological hazard to humans in both the intermediate and extended time periods from 8 days to years after exposure.	
<b>Evaluation of inflammation-based atherosclerosis associated with environmental exposures</b>  Brandiese Beverly and Andrew Rooney	<ul style="list-style-type: none"> <li>▪ This evaluation examines whether environmental substances contribute to inflammation, which ultimately leads to atherosclerosis, and identifies biomarkers of the inflammation involved.</li> <li>▪ Atherosclerosis was selected for investigation because of the significant public health impact of the disease, and the well-established role of inflammation in the disease process that leads to it.</li> </ul>	Evaluation ongoing
<b>NIEHS-EPA pilot study of exposure to chemicals in consumer products</b>  Kyla Taylor	<ul style="list-style-type: none"> <li>▪ NIEHS is collaborating with EPA to perform a small-scale, longitudinal pilot study to evaluate the performance of existing survey, measurement, and modeling methods for assessing exposures to chemicals in several consumer product categories, including personal and child care, household cleaning, lawn and garden, home improvement, and food packaging products.</li> <li>▪ The pilot study addresses several research needs related to the measurement and modeling of human exposures.</li> </ul>	Evaluation ongoing
<b>Respiratory effects associated with exposure to biocides</b>  Vickie Walker	<ul style="list-style-type: none"> <li>▪ Biocides are commercial products used to kill or control the spread of harmful microorganisms like bacteria and viruses. The EPA Office of Pesticide Programs nominated biocides to NTP for evaluation of the evidence for respiratory outcomes from occupational exposure to biocides.</li> <li>▪ The ongoing study includes developing a scoping review and evidence map on potential respiratory health effects of 10 major antimicrobial biocides commonly used for disinfection in hospitals.</li> </ul>	Evaluation ongoing
<b>Evaluation of children's health and traffic-related air pollution</b>  Brandiese Beverly and Kembra Howdeshell	<ul style="list-style-type: none"> <li>▪ Traffic-related air pollution (TRAP) is air pollution derived from fossil fuel combustion from vehicle emissions. These emissions contribute significantly to outdoor air pollution, particularly in urban settings, and is a major risk factor for cardiovascular disease, including hypertension.</li> <li>▪ Children are particularly sensitive to air pollution, and growing evidence suggest that TRAP may be associated with the development of hypertension during pregnancy, which is a leading cause of maternal and fetal morbidity and mortality.</li> <li>▪ NTP conducted a systematic review of published research to evaluate the evidence that TRAP is associated with hypertensive disorders in pregnant women. The systematic review evaluated the risk of hypertensive disorders by different pollutant measurements of TRAP, such as particulate matter, nitrogen oxides, carbon monoxide, and</li> </ul>	Report preparation

Project Study Scientist	Project Summary	Status
	<p>black and elemental carbon, along with parameters like traffic density and mother's proximity to main roads.</p> <ul style="list-style-type: none"> <li>NTP concluded that TRAP is a presumed hazard for hypertensive disorders in pregnant women.</li> </ul>	
<p><b>Evaluation of adverse health effects and occupational exposure to cancer chemotherapy agents</b></p> <p>Kembra Howdeshell</p>	<ul style="list-style-type: none"> <li>This evaluation examined the evidence that occupational exposure to cancer chemotherapy agents is associated with adverse health effects, including genetic toxicity, cancer, reproductive and developmental effects, and acute effects.</li> <li>NTP concluded that there is a moderate level of evidence that occupational exposure to chemotherapy agents is associated with increased incidence of spontaneous abortion and genetic toxicity. There is also evidence that chemotherapy drugs continue to be detected in workers' urine and blood samples and in the work environment as recently as 2016. However, some studies suggest that the levels of exposure to these drugs in the workplace have decreased over time as institutions provide exposure-protection equipment and employees follow safe handling guidelines.</li> </ul>	Report preparation
<p><b>Chemical factors affecting breast cancer risk: a state-of-the-science review</b></p> <p>Vickie Walker and Jason Stanko</p>	<ul style="list-style-type: none"> <li>This evaluation is examining the evidence that environmental substances or factors influence breast cancer risk.</li> <li>In collaboration with the DNTP NTP Laboratory, OHAT is conducting an evidence evaluation of chemicals, pharmaceuticals, and dietary components as well as other factors that are associated with adverse effects on the breast or mammary gland and could potentially influence breast cancer risk.</li> </ul>	Evaluation ongoing
<p><b>Neonicotinoid pesticides and adverse health outcomes</b></p> <p>Windy Boyd</p>	<ul style="list-style-type: none"> <li>Neonicotinoid pesticides are a class of chemicals that act as insecticides by exerting neurotoxic effects through irreversible binding to insect nicotinic acetylcholine receptors.</li> <li>This scoping review is identifying the extent of evidence available to understand human health effects of seven neonicotinoid pesticides (acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam).</li> <li>The draft neonicotinoid pesticides scoping review has been completed and is undergoing formatting for final publication as an NTP Research Report.</li> </ul>	Report preparation
<p><b>Parkinson's disease: associations with environmental exposures</b></p> <p>Windy Boyd</p>	<ul style="list-style-type: none"> <li>Although some Parkinson's disease cases can be attributed to genetic factors, the causes of many cases remain unknown.</li> <li>Many studies report associations between environmental exposures and Parkinson's disease or related symptoms. OHAT conducted two scoping reviews on this topic. The first</li> </ul>	Report preparation

Project Study Scientist	Project Summary	Status
	<p>systematically mapped the evidence of the associations between exposures to environmental chemicals considered broadly and Parkinson's disease.</p> <ul style="list-style-type: none"> <li>During scoping activities, hundreds of studies on the associations between exposure to the herbicide paraquat and Parkinson's disease were identified, making paraquat a candidate chemical for further systematic review. Therefore, a more detailed scoping review was developed to characterize reported associations between paraquat exposure and Parkinson's disease.</li> <li>The draft paraquat and Parkinson's disease scoping review has been completed and is undergoing formatting for final publication as an NTP Research Report.</li> </ul>	
<p><b>Systematic reviews on potential health effects of fluoride</b></p> <p>Kyla Taylor</p>	<ul style="list-style-type: none"> <li>This systematic review is evaluating potential neurobehavioral effects from exposure to fluoride during development that includes consideration of human epidemiological studies, experimental animal studies, and mechanistic data.</li> <li>The project includes an update to NTP's 2016 systematic review of published animal literature examining neurobehavioral effects of exposure to fluoride during development and adulthood in rodents. The <a href="#">2016 report</a> concluded that the level of evidence supporting adverse effects on learning and memory in animals exposed to fluoride in the diet or drinking water is low to moderate.</li> </ul>	Report Preparation
<p><b>Prenatal exposure to progestogens and adverse health outcomes</b></p> <p>Kembra Howdeshell</p>	<ul style="list-style-type: none"> <li>Progesterone (bioidentical, plant-based) and synthetic derivatives (synthetic progestogens) are administered to reproductive age women for a variety of health outcomes, including contraception, infertility, and treatment or prevention of miscarriage or preterm birth.</li> <li>Concern for possible adverse effects on the developing fetus stems from alterations in normal steroid hormone exposure during development that have been shown to cause adverse effects on offspring health and development.</li> <li>A scoping review is underway to identify and characterize the literature on the possible association between exposure to progestogens during pregnancy and adverse health outcomes in the offspring.</li> </ul>	Evaluation ongoing
<p><b>Evaluation of the findings from the consortium linking academic and regulatory insights on the toxicity of bisphenol A (CLARITY-BPA) program</b></p>	<ul style="list-style-type: none"> <li>Bisphenol A is used in the manufacture of plastics, among other products, and has been characterized by some as an endocrine disruptor. Its ubiquity in the environment has raised concerns about its potential health effects.</li> <li>Academic studies have reported several health-related effects of bisphenol A, whereas guideline-compliant studies have failed to detect effects, except at non human-relevant</li> </ul>	Evaluation ongoing



Project Study Scientist	Project Summary	Status
Kembra Howdeshell, Brandy Beverly, Retha Newbold, Andrew Rooney, and John Bucher	<p>high doses. Consequently, NTP and NIEHS designed the Consortium Linking Academic and Regulatory Insights on the Toxicity of Bisphenol A (CLARITY-BPA) program to enhance the links between academic and guideline-compliant research.</p> <ul style="list-style-type: none"> <li>▪ The published findings of the CLARITY-BPA program will be summarized in two reports. The first report, authored by the CLARITY-BPA participants with background and publication summaries written by NTP, is a collated summary of the published findings from the guideline-compliant and investigational research activities undertaken within the CLARITY-BPA program.</li> <li>▪ The second report, authored by NIEHS, will attempt to synthesize and compare the CLARITY-BPA findings with prior studies on BPA from CLARITY-BPA participants to assess technologies used in investigational studies of BPA for consideration as possible additions to guideline studies.</li> </ul>	

## 5.2. Report on Carcinogens

The Report on Carcinogens (RoC) is a congressionally mandated listing of substances that either are known to be human carcinogens or might reasonably be anticipated to be human carcinogens, and to which a significant number of people residing in the United States are exposed [Public Health Service Act, 42 U.S.C. 241(b)(4)]. Preparation of the RoC at NTP is under the direction of Ruth Lunn, Dr.P.H., director of the NIEHS/NTP Office of Report on Carcinogens (ORoC).

The RoC is cumulative, consisting of newly reviewed substances in addition to those substances listed in previous editions. NTP follows an established [four-part process](#) when preparing the report.

- (1) NTP selects nominations for evaluation.
- (2) ORoC conducts cancer hazard evaluations on the selected substances and prepares draft RoC monographs.
- (3) Draft RoC monographs are released for public comment and external peer review is conducted before finalization.
- (4) NTP submits the proposed listing of newly reviewed substances to the Secretary of Health and Human Services for review and approval, followed by transmittal to Congress and publication on the NTP website.



Each substance listed in the report has a profile, which contains the listing status determined by using established [listing criteria](#), a summary of the cancer studies supporting the listing status, information on human exposure, and federal regulations to reduce exposure.

### 5.2.1. Evaluating Cancer Hazards

In FY 2019, NTP expanded the scope of its activities to include other types of evaluations besides those for review for the RoC. On October 5th, 2018, NTP convened a peer review of a draft document assessing cancer hazards of two exposure scenarios: night shift work and light at night. NTP revised the report based on the peer-review comments. New systematic reviews to evaluate cancer hazards were initiated and informed by evidence mapping, scoping, and problem formulation activities. Also, NTP conducted preliminary scoping activities on several nominations to identify key questions related to public health or the need for a cancer hazard assessment. An analysis of red and processed meat consumption and the risk of colorectal cancer in women was completed.

#### *Cancer Hazard Evaluations or Studies*

Topic NTP Project Leader	Primary Uses/Exposures	Status
<b>Literature-based Cancer Hazard Evaluations</b>		
<a href="#">Antimony trioxide</a> Amy Wang RoC evaluation	<ul style="list-style-type: none"> <li>This compound is used mainly as a synergist for halogenated flame retardants in plastics, rubber, and textiles, which are used in a range of plastics and other products.</li> <li>It also can be used as a catalyst in polyethylene terephthalate production, as an additive in glass manufacturing, and in pigments or as an additive in paints and ceramics.</li> </ul>	Final monograph published October 2018  Potential submission for 15th RoC
<a href="#">Haloacetic acids found as water disinfection byproducts</a> Gloria Jahnke RoC evaluation	<ul style="list-style-type: none"> <li>This group of acids includes 13 individual haloacetic acids or a potential class or subclass of these haloacetic acids.</li> <li>People are exposed to these haloacetic acids by ingestion of chlorinated drinking water and by inhalation and dermal contact during bathing or showering or when using swimming pools and spas that use chlorine for disinfection.</li> </ul>	Potential submission for 15th RoC
<a href="#">Helicobacter pylori (H. pylori): Chronic infection</a> Ruth Lunn RoC evaluation	<ul style="list-style-type: none"> <li>A gram-negative, multiflagellated bacterium, <i>H. pylori</i> colonizes the stomach and causes peptic ulcer. Bacterium is spread by person-to-person contact, especially among family members.</li> <li>Routes of exposure include oral-oral, fecal-oral, and iatrogenic; exposure from contaminated water is also possible. Risk factors for infection include age, race, socioeconomic status such as crowded living conditions, and poor sanitation/hygiene.</li> </ul>	Final monograph published October 2018  Potential submission for 15th RoC
<a href="#">Light at night</a> Ruth Lunn NTP cancer hazard evaluation	<ul style="list-style-type: none"> <li>Unnatural (e.g., ill-timed) electrical light, especially light at night, might disrupt sleep and biological processes controlled by endogenous circadian clocks. People who by virtue of the nature of their work, lifestyle choices, or residence, can be subjected to unnatural light exposure.</li> </ul>	Peer review of draft monograph October 2018
<a href="#">Night shiftwork</a> Ruth Lunn	<ul style="list-style-type: none"> <li>Shift workers who work at night can experience an extreme type of exposure to light and changes in other activities, such as daily activities, eating, sleeping, lifestyle factors, and social behavior.</li> </ul>	Peer review of draft monograph October 2018

Topic NTP Project Leader	Primary Uses/Exposures	Status
NTP cancer hazard evaluation		
<a href="#">Nitropolycyclic aromatic hydrocarbon compounds (NPAHs)</a> Gloria Janke Cancer hazard assessment	<ul style="list-style-type: none"> <li>NPAHs are ubiquitous air pollutants produced as a result of incomplete combustion.</li> <li>People working in occupations with high levels of gasoline and diesel fuel combustion products, such as trucking industry workers, bus drivers, taxi drivers, and coal miners, have the highest exposure to NPAHs.</li> </ul>	Completed scoping and formulation activities  Initiated systematic review of cancer hazards
<a href="#">Polycyclic aromatic hydrocarbons (PAHs)</a> Amy Wang	<ul style="list-style-type: none"> <li>PAHs are common byproducts from the combustion of organic substances. They also are found in coal tar, coke, asphalt, and coal.</li> <li>People are mainly exposed to PAHs via inhalation of smoke (tobacco, wood, coal, etc.) and other air pollutants, and by ingesting food containing PAHs (e.g., grilled meat). Workers at workplaces and other facilities that use or produce petroleum or coal or burn organic material may be exposed to PAHs.</li> </ul>	Completed scoping and formulation activities  Initiated systematic review of cancer hazards
<a href="#">para-Chlorotrifluorotoluene</a> Gloria Janke Cancer hazard assessment	<ul style="list-style-type: none"> <li>This solvent is used in a variety of settings such as automotive body shops and is found in products such as paint thinners and cement sealants.</li> <li>People who use these products in the workplace or at home are exposed by inhalation or skin contact.</li> </ul>	Completed scoping and formulation activities  Initiated systematic review of cancer hazards
<b>Epidemiological Study</b>		
<a href="#">Red and processed meat and colorectal cancer risk</a> Suril Mehta Analysis of NIEHS study	<ul style="list-style-type: none"> <li><b>Red meat</b> refers to meat that has more red than white fibers such as beef, goat, lamb, and pork.</li> <li><b>Processed meat</b> is meat that is preserved by smoking, curing, salting, and adding chemical preservatives.</li> <li>In the United States, average daily total meat intake is 128 g, of which 55% is red meat and 23g is estimated to be processed meat.</li> </ul>	Analyses and report completed  Accepted for publication in September 2019; published October 1, 2019  DOI:  10.1158/1055-9965.EPI-19-0459

## 6. Partner Agency Research

NTP is a partnership of three federal agencies: National Institute for Occupational Safety and Health (NIOSH) of the Centers for Disease Control and Prevention (CDC); U.S. Food and Drug Administration (FDA), primarily through the National Center for Toxicological Research (NCTR); and National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health (NIH). This section describes research projects during FY 2019 at each of the three partner agencies.

### 6.1. NTP at NIEHS

Most NTP research testing and analysis activities are carried out at NIEHS. The following Division of NTP branches, offices, and center at NIEHS are actively involved in NTP research activities.

- [Biomolecular Screening Branch](#), Richard S. Paules, Ph.D., acting chief
- [Cellular and Molecular Pathology Branch](#), Robert Sills, D.V.M., Ph.D., chief
- [NTP Laboratory](#), Michael DeVito, Ph.D., acting chief
- [Program Operations Branch](#), Michelle Hooth, Ph.D., chief
- [Toxicology Branch](#), Nigel Walker, Ph.D., acting chief
- [NTP Interagency Center for the Evaluation of Alternative Methods](#), Warren Casey, Ph.D., director



NIEHS/NTP Staff

#### 6.1.1. Biomolecular Screening

The Biomolecular Screening Branch develops and implements programs in medium- and high-throughput screening of environmental substances for rapid detection of biological activities of significance to toxicology. Biomolecular screening projects for FY 2019 are listed below.



*Biomolecular Screening Projects in FY 2019*

Project Study Scientists	Project Summary
<b>Development of the S1500+ gene set</b> Richard Paules, Scott Auerbach, Steve Ferguson, Sreenivasa Ramaiahgari	<ul style="list-style-type: none"> <li>Developed human, mouse, rat, and zebrafish S1500+ gene set and evaluated its use in targeted sequencing approaches to provide a novel, cost-effective methodology for capturing broad biological responses to chemical exposures in a variety of in vitro and in vivo model systems, allowing for quantitative dose-response measurements and kinetic studies.</li> </ul>
<b>Development of human and rat liver novel organotypic cell culture model systems</b> Stephen Ferguson, Sreenivasa Ramaiahgari, Katelyn Lavrich	<ul style="list-style-type: none"> <li>Developed and characterized novel organotypic cell culture model systems (i.e., models that behave like living tissue) that allow for a rapid and deeper investigation of physiologically relevant biological responses to chemicals of concern and provide a better understanding of potential implications for human health; began with human and rat liver systems.</li> </ul>
<b>Genotoxicity testing projects</b> Kristine Witt, Stephanie Smith-Roe	<ul style="list-style-type: none"> <li>Evaluated the genotoxicity of test articles included in NTP's glyphosate toxicity study—including supplements of several members of the botanical cohosh family and of eight flame retardants of interest to NTP—using bacterial reverse mutation and in vitro mammalian cell micronucleus assays, in vitro comet assays, and the in vitro MultiFlow DNA Damage assay to provide information on mode of action for compounds shown to have genotoxic activity.</li> </ul>
<b>Development of more sophisticated genetic analyses to make better use of current animal models</b> Alison Harrill	<ul style="list-style-type: none"> <li>Developed sophisticated genetic analyses to make better use of current diversity animal models and, where possible, human diversity biological systems to provide more appropriate modeling of human population differences in toxicity and disease.</li> </ul>

**6.1.2. NTP Laboratory**

The mission of the [NTP Laboratory](#) is to provide research support and conduct investigative toxicology for NTP. Laboratory work is project-driven in collaboration with members of Health Effects Innovations (HEIs) and Program Management Teams (PMTs) within NIEHS/DNTP to advance the program mission. Investigative work uses NTP Laboratory's research capabilities to identify human health hazards from environmental and chemical exposures and further mechanistic understanding of environmental toxicity that contribute to human disease. The NTP Laboratory has several goals:

- To provide on-site, program-responsive laboratory support to HEIs and PMT project teams in NTP for screening, prioritization, and mechanistic understanding of chemical effects and environmental factors related to human exposure and health.
- To produce and deliver high-quality laboratory data in a timely manner.
- To develop novel in vitro systems, alternative animal models, and new cellular and molecular assays in a research setting to result in standardized and scalable tests for NTP's testing program and to translate to in vivo outcomes.
- To conduct bioanalytical disposition and metabolite analysis of environmental and test chemicals in experimental systems.
- To collaborate with researchers and environmental stakeholders for program-responsive outcomes to NTP.



Projects at the NTP Laboratory for FY 2019 are listed below.

*NTP Laboratory Projects in FY 2019*

Project Study Scientist	Project Objectives
<b>Development of in vitro models of chemical carcinogenesis</b> Erik Tokar	<ul style="list-style-type: none"> <li>Use in vitro cell transformation models (stem/progenitor and “mature” cells) of human target-relevant cells to elucidate carcinogenic mechanisms and modes of action of metals and other NTP chemicals. These models are used to examine chemical-induced effects on epigenetics (i.e., miRNAs) and microvesicles (quantity and cargo; i.e., cell-free circulating biomolecules).</li> </ul>
<b>In vitro evaluation of crumb rubber toxic effects</b> Erik Tokar	<ul style="list-style-type: none"> <li>Study the modes of action and metabolomics involved in the toxic effects of crumb rubber on various prospective human target tissues.</li> </ul>
<b>Stem cells in toxicology: Carcinogenesis, developmental toxicology, and developmental basis of adult disease</b> Erik Tokar	<ul style="list-style-type: none"> <li>Develop stem cell model systems (pluripotent, multipotent, progenitor) in carcinogenesis, developmental basis of adult disease, and assessing developmental toxicology.</li> <li>Test chemical effects on early differentiation, development, tissue specification, and organoid development to help predict or categorize teratogens and developmental toxicants.</li> </ul>
<b>Evaluation of the role of oxidative stress in biological effects of glyphosate and its formulations</b> Stephen Ferguson/Michael DeVito	<ul style="list-style-type: none"> <li>Compare effects of glyphosate to the effects of glyphosate formulations using measures of genotoxicity, oxidative stress, and cell viability.</li> <li>Compare the dose-response relationships among oxidative stress, genotoxicity, and cell viability.</li> </ul>
<b>Application of in vitro assays to evaluate botanicals</b> Stephen Ferguson	<ul style="list-style-type: none"> <li>Determine whether in vitro assays and chemical constituent analysis of botanicals can aid in selecting botanicals for in vivo testing and serve as mechanistic tools to link constituents to biological responses.</li> </ul>
<b>Chemical-induced transcriptomic and metabolomic changes in vitro</b> Stephen Ferguson	<ul style="list-style-type: none"> <li>Evaluate the transcriptomic changes in metabolically proficient cell culture models with 24 reference chemicals to qualify new approach methods.</li> </ul>
<b>Incorporation of metabolism into high-throughput screening assays</b> Stephen Ferguson	<ul style="list-style-type: none"> <li>Develop in vitro methods proficient for physiologically relevant xenobiotic metabolism.</li> </ul>
<b>Aqueous film-forming foams</b> Stephen Ferguson	<ul style="list-style-type: none"> <li>Conduct screening efforts to evaluate qualified aqueous film-forming foam products (AFFFs) and comparator per- and polyfluoroalkyl substances (PFAS) constituents to reference chemicals for binning and estimations of liver injury potential through benchmark concentration modeling.</li> </ul>
<b>Tox21 cross-partner project #5</b> Stephen Ferguson	<ul style="list-style-type: none"> <li>Create reference database of high-throughput transcriptomics with 3D HepaRG spheroids and a panel of ~315 reference chemicals with high affinities to specific molecular targets (e.g., nuclear receptors, kinases)</li> </ul>

Project Study Scientist	Project Objectives
<b>Rat versus human parallelogram with 20 chemicals evaluated with 5-day in vivo transcriptomics studies</b> Will Gwinn	<ul style="list-style-type: none"> <li>Evaluate reference chemicals alongside 20 historically evaluated NTP-studied chemicals in short-term animal (5-day), in vitro rat primary hepatocytes, and in vitro human hepatocytes to build translational context around these new approach methods (NAMs).</li> </ul>
<b>Metabolomics</b> David Crizer	<ul style="list-style-type: none"> <li>Explore mass spectrometry methods for metabolomic analysis of biofluids and a library of known analytes.</li> <li>Form and lead NIEHS interest group on current methods and applications of Metabolomics at NIEHS and the field.</li> </ul>
<b>In vitro clearance assays</b> David Crizer	<ul style="list-style-type: none"> <li>Use mass spectrometry for analysis of chemicals such as PFAS in hepatocyte suspensions to determine clearance and potential metabolism.</li> <li>Support in vitro-in vivo extrapolation (IVIVE) activities.</li> </ul>
<b>PCB 11: Screening for biological and toxicological activity</b> Michael DeVito	<ul style="list-style-type: none"> <li>Compare the biological and toxicological activity of polychlorinated biphenyl (PCB) 11 to prototype PCBs in response to a nomination from EPA.</li> </ul>
<b>Evaluation of 5-day in vivo rat high-throughput liver and kidney transcriptomics for estimating benchmark doses</b> William Gwinn/Michael DeVito	<ul style="list-style-type: none"> <li>Use high-throughput transcriptomics (HTT) in 5-day exposure rat model to estimate benchmark doses (BMDs) for transcriptional pathway changes in liver and kidney for traditional toxicological (apical endpoints) on 20 chemicals (18 2-year bioassays, two 90-day studies). Hypothesis was 5-day and bioassay BMDs will be comparable, so that 5-day studies might estimate chronic exposure BMDs.</li> </ul>
<b>Metalloestrogens and uterine/breast response</b> Darlene Dixon, Suzanne Fenton	<ul style="list-style-type: none"> <li>Test the ability of reported metalloestrogens such as cadmium and arsenic to cause nonclassical-estrogen receptor-mediated effects in the uterus as a mode of action for cancer development.</li> </ul>
<b>Membrane-associated estrogen receptors (ER-alpha36; GPER) as novel endocrine disruptors</b> Darlene Dixon	<ul style="list-style-type: none"> <li>Evaluate the role of novel receptors, estrogen receptor alpha36 (ER-alpha36) and G protein-coupled estrogen receptor (GPER) in mediating the endocrine-disrupting effects of environmental and industrial chemicals using human uterine fibroid 2D and 3D in vitro models.</li> </ul>
<b>Diagnostic criteria, classification, and appropriate terminology for histopathologic lesions found in NTP studies</b> Darlene Dixon	<ul style="list-style-type: none"> <li>Further define diagnostic criteria for classifying histopathologic lesions found in the female reproductive tract by incorporating IHC and other technologies to help understand the biological significance and human relevance of lesions.</li> </ul>
<b>Techniques for histologic evaluation of 3D spheroids for in vitro assessment</b> Darlene Dixon	<ul style="list-style-type: none"> <li>Develop techniques for moving 3D spheroid or embryoid cultures from 96 well plates into paraffin blocks for histochemical and immunohistochemical staining.</li> </ul>
<b>Literature scoping review on interplay between stressors and environment on cardiovascular health in U3 women</b>	<ul style="list-style-type: none"> <li>Review and summarize literature on the effect of psychosocial stressors related to health disparities and environmental exposures on</li> </ul>

Project Study Scientist	Project Objectives
Ruth Lunn, Darlene Dixon	<p>cardiovascular health in women of understudied, underrepresented, and under reported (U3) populations.</p> <ul style="list-style-type: none"> <li>Inform Cardiovascular HEIs team on environmental-chemical interactions in U3 populations.</li> </ul>
<b>Effects of TBBPA on developmental and reproductive endpoints in rats</b> Suzanne Fenton, Linda Birnbaum	<ul style="list-style-type: none"> <li>Evaluate the effects of tetrabromobisphenol A (TBBPA) following prenatal and early life exposure and determine the transcriptomic/metabolomic pathways involved in low-dose, hormone-driven responses.</li> </ul>
<b>Effects of PFOA and GenX on developmental and reproductive endpoints in mice</b> Suzanne Fenton	<ul style="list-style-type: none"> <li>Evaluate developmental effects of perfluorooctanoic acid (PFOA) and GenX in placental toxicity, metabolism, liver toxicity, and mammary gland proliferation in adult CD-1 mice and their offspring.</li> </ul>
<b>Screening perfluorinated compounds for effects in human, mouse cell-based assays</b> Suzanne Fenton	<ul style="list-style-type: none"> <li>Compare the potencies and effect profiles of 40–45 perfluorinated compounds in cells as known targets of PFOA and perfluorooctane sulfonic acid (PFOS).</li> </ul>
<b>Toxicants and mammary gland development</b> Suzanne Fenton	<ul style="list-style-type: none"> <li>Determine the effects of and mechanisms of action of different toxicants, including PFAS, flame retardants, and bisphenols, on mammary gland development in rats or mice.</li> </ul>
<b>Use of in vitro screens to evaluate potential obesogens</b> Suzanne Fenton	<ul style="list-style-type: none"> <li>Develop orthogonal assays to evaluate findings from Tox21 that identified potential obesogens.</li> <li>Investigate mechanisms of action for select chemicals.</li> </ul>
<b>Refinement of developmental neurotoxicology methods</b> G. Jean Harry	<ul style="list-style-type: none"> <li>Improve methods for assessing differential changes as a function of developmental exposures, including in vivo molecular phenotypes, cellular phenotypes, maternal/developmental inflammation, and behavioral assessments.</li> </ul>
<b>Method development to assess neuroinflammation</b> G. Jean Harry	<ul style="list-style-type: none"> <li>Examine methods (from screening to mechanisms) for assessing in vitro and in vivo induction of inflammation in the nervous system following chemical exposures.</li> <li>Continue development of a neurodevelopmental chemical library of stroubins.</li> </ul>
<b>Evaluation of mass cytometry to assess multi-protein expression in complex systems</b> G. Jean Harry, Eric Tokar	<ul style="list-style-type: none"> <li>Develop and evaluate the utility of mass cytometry, or CyTOF (Fluidigm), for assessing developmental or cellular shifts in targeted protein expression in complex cultures and in vivo models.</li> </ul>

## 6.2. NTP at NCTR

### *Research in Partnership with NCTR*

The National Center for Toxicological Research (NCTR) collaborates with researchers from elsewhere in the U.S. Food and Drug Administration (FDA), other government agencies, academia, and industry to provide innovative technology, methods development, vital scientific training, and technical expertise. The unique scientific expertise of NCTR is critical in supporting FDA product centers and their regulatory roles. NCTR research for NTP is funded by both voluntary allocations and an interagency agreement.



NCTR/NTP Staff

NCTR studies funded by voluntary allocations in FY 2019 are listed below.

### *Biochemical and Molecular Basis of Toxicology in FY 2019*

Project Study Scientist	Project Objectives
<b>Development of a next-generation sequencing method for the quantification of low-frequency somatic mutations in oncogenes</b> Page Mckinzie	<ul style="list-style-type: none"> <li>Develop a next-generation sequencing method for quantifying somatic mutations in oncogenes at fractions in the range of <math>10^{-5}</math> to <math>10^{-2}</math>.</li> </ul>
<b>Detection of rare genomic mutations induced by genotoxic carcinogens using next-generation sequencing</b> Tao Chen	<ul style="list-style-type: none"> <li>Establish tagging and duplex sequencing methods to detect rare mutations using synthesized DNA fragments containing known mutation fractions and types.</li> </ul>

Project Study Scientist	Project Objectives
<b>Tumor mutational signatures of acrylamide and glycidamide</b> Fred Beland	<ul style="list-style-type: none"> <li>Determine the mutational signatures of tumors induced in experimental animals by acrylamide and glycidamide.</li> <li>Compare the mutational signatures obtained from acrylamide and glycidamide in experimental animals with mutational signatures obtained from acrylamide and glycidamide in experimental animals with mutational signatures of human tumors in published databases.</li> </ul>
<b>In vitro and in vivo methods for functional evaluation of genomic alterations induced by genome editing</b> Javier Revollo	<ul style="list-style-type: none"> <li>Generate human cell lines with defined genetic alterations. To establish an iPSC-based functional evaluation platform, defined chromosomal translocations observed in preclinical studies of genome-edited CAR-T cells will be created in a panel of isogenic iPSCs.</li> <li>Determine effects of the genetic alteration on clonal expansion and global gene expression. The iPSCs will be differentiated toward hematopoietic stem/progenitors.</li> <li>Perform comparative colony forming, cell proliferation, and transcriptome analyses.</li> <li>Establish an enhanced transplantation mouse model to evaluate the transformation potential of the genetic alterations.</li> </ul>
<b>Thermal inactivation of staphylococcal enterotoxins in milk</b> Woody Tolleson	<ul style="list-style-type: none"> <li>Use staphylococcal enterotoxin (SE) types H, K, and the superantigen TSST-1 to: (1) evaluate SE thermal stability via differential scanning calorimetry (DSC); (2) measure kinetics of SE thermal inactivation via fluorescence quenching; (3) develop in vitro methods to evaluate effects of heat treatments on superantigenic and emetic properties; (4) determine z-values for thermal inactivation of toxic activity in milk; and (5) evaluate the potential for reversible heat denaturation of toxins using DSC and biological assays.</li> </ul>
<b>Investigation of the mechanistic aspects of sex-based differences in susceptibility to doxorubicin-induced cardiac toxicity in mice</b> Varsha Desai	<ul style="list-style-type: none"> <li>Understand the molecular basis associated with differential susceptibility to doxorubicin (DOX) toxicity between sexes in a newly established mouse model exhibiting sex-related differences in DOX cardiotoxicity.</li> </ul>
<b>Establish genotoxicity assessment approaches in male germline cells at FDA</b> Dayton Petibone	<ul style="list-style-type: none"> <li>Develop a sperm Pig-a mutation assay.</li> <li>Develop in vitro testis organoids for evaluating mutation.</li> </ul>
<b>Fetal and neonatal toxicokinetics of the C6-Fluorotelomer alcohol</b> Dan Doerge	<ul style="list-style-type: none"> <li>Examine the toxicokinetics of 6:2FTOH and metabolites in pregnant and lactating rats and their fetuses and neonates. In vivo toxicokinetic studies in pregnant and lactating rats will be conducted to quantify basic toxicokinetic parameters, such as clearance and systemic half-life, of 6:2FTOH and major metabolites.</li> <li>Evaluate the role of renal transport mechanisms in the toxicokinetics of 6:2FTOH and metabolites. In vitro studies using rat and human kidney transporter over-expression cell models will be conducted to examine</li> </ul>



Project Study Scientist	Project Objectives
	<p>the role of renal transport mechanisms in renal reabsorption and/or tubular secretion of 6:2FTOH and metabolites and to identify and quantify species-based differences.</p> <ul style="list-style-type: none"> <li>▪ Toxicokinetic analysis and biologically based dose response (BBDR) modeling of 6:2FTOH metabolism and disposition to predict exposure and effects of 6:2FTOH metabolites in the mother and fetus/newborn during pregnancy and lactation, respectively.</li> </ul>
<p><b>Identification of mechanistic biomarkers of pyrrolizidine alkaloid (PA)-induced hepatocarcinogenesis</b></p> <p>William Tolleson</p>	<ul style="list-style-type: none"> <li>▪ Use high-throughput profiling approaches to identify microRNAs that regulate genes involved in PA carcinogenicity in hepatic cell systems and investigation of the functions of microRNAs by bioinformatics tools and in vitro functional assays.</li> </ul>

### Neurotoxicology in FY 2019

Project Study Scientist	Project Objectives
<p><b>Effects of developmental sevoflurane exposure and pretreatment with acetyl-L-carnitine on complex brain function in rats</b></p> <p>John Talpos</p>	<ul style="list-style-type: none"> <li>▪ Examine the effects of early developmental sevoflurane exposure on neurodegeneration and complex operant learning.</li> <li>▪ Determine whether impairments in these measures can be attenuated by pretreatment with acetyl-L-carnitine.</li> <li>▪ Examine the time course of acetyl-L-carnitine pretreatment on sevoflurane-induced neuroapoptosis.</li> </ul>
<p><b>Rat blood-brain-barrier-on-a-chip model to study traumatic brain injury</b></p> <p>Syed Ali</p>	<ul style="list-style-type: none"> <li>▪ Use soft lithograph and microfabrication techniques to engineer a multilayered blood-brain-barrier-on-a-chip model that can be subjected to different magnitudes and durations of mechanical stress that mimic mild and repetitive traumatic brain injury.</li> <li>▪ Characterize the effects of traumatic brain injury on blood-brain-barrier integrity using the chip model.</li> </ul>
<p><b>Development of MRI imaging and informatics techniques for tissue sampling to guide and confirm classical neuropathology</b></p> <p>Serguei Liachenko</p>	<ul style="list-style-type: none"> <li>▪ Build dose-response and time-course curves of trimethyltin (TMT) and hexachlorophene (HC) neurotoxicity using MRI T2 mapping.</li> <li>▪ Assess sensitivity and specificity of T2 mapping in relation to histopathology using the receiver operating characteristic curves approach.</li> <li>▪ Assess neurotoxicological effect of mefloquine.</li> </ul>
<p><b>Developing animal models of a weakened blood-brain barrier for testing the neurotoxicity of drugs that do not normally enter the CNS</b></p> <p>John Bowyer</p>	<ul style="list-style-type: none"> <li>▪ Develop an acute and a more prolonged model of BBB disruption.</li> </ul>

Project Study Scientist	Project Objectives
<b>Acute and cumulative effects of isoflurane anesthesia on neurobehavioral functions in adult male and female rats</b> Sherry Ferguson	<ul style="list-style-type: none"> <li>Use a single lipopolysaccharide injection in each of three adult male Sprague Dawley rats to serve as a positive control group for the analysis of cytokine/chemokine levels in the brain.</li> </ul>
<b>Validation of the blood-brain barrier-on-a-chip technology as a tool for toxicological screening of FDA-regulated products</b> Syed Ali	<ul style="list-style-type: none"> <li>Validate blood-brain barrier-on-a-chip technology that can be used as a novel tool to evaluate the effects of toxic agents and drugs in the neurovascular unit in vitro.</li> </ul>
<b>High-throughput neurotoxicity screening of metallic nanoparticles: In vitro and in vivo imaging</b> Syed Imam	<ul style="list-style-type: none"> <li>Assess the utility of high-throughput neurotoxicity screening of metallic nanoparticles for detecting neurochemical and neurophysiological alterations in vitro for use in developing reference standards for metallic nanoparticles. Once developed and validated, these techniques can be optimized for analyzing other FDA-regulated nanomaterials.</li> </ul>

### Nanotoxicology in FY 2019

Project Study Scientist	Project Objectives
<b>Complement assays for the detection of immune-sensitizing activity of nanomaterials</b> Julian Leakey	<ul style="list-style-type: none"> <li>Establish two complement assays for routine evaluation of immune-sensitizing activity of nanomaterials.</li> <li>Validate the assays using nanoparticles with known immunoreactivity.</li> <li>Determine the immune-sensitizing activity of novel nanomaterials.</li> </ul>
<b>Nonclinical modeling and risk assessment of FDA-regulated drug nanocrystals</b> Kuppan Gokulan	<ul style="list-style-type: none"> <li>Investigate various media milling/high pressure homogenization and spray/freeze drying process parameters and formulation parameters to determine the critical parameters that affect particle size and drug polymorphic form.</li> <li>Evaluate drug-nanocrystal effects on epithelial cell permeability and mucoadherence using in vitro and ex vivo culture models and drug permeability and stability using in vitro intestinal epithelial cells.</li> <li>Determine macrophage cell viability and proliferation during the treatment with different sizes of drug nanocrystals; evaluate the immunotoxic effects of drug nanocrystals on intestinal tissue by measuring pro-inflammatory cytokines; and determine the effect of drug nanocrystals on the intestinal commensal microbiota using in vivo model.</li> </ul>
<b>Evaluation of cadmium oxide nanoparticle as a nanoparticle-type positive control for toxicity assays</b> Tao Chen	<ul style="list-style-type: none"> <li>Characterize cadmium oxide nanoparticles and determination of their toxicity using in vitro toxicity and genotoxicity assays.</li> <li>Explore the possible mechanisms of cadmium oxide nanoparticle toxicity.</li> </ul>

Project Study Scientist	Project Objectives
	<ul style="list-style-type: none"> <li>Evaluate where cadmium oxide nanoparticles are suitable for use as a positive control according to their toxicity and genotoxicity responses.</li> </ul>
<b>In vitro genotoxicity of graphene-family nanomaterials using FDA-recommended short-term genetic toxicity test battery</b> Nan Mei	<ul style="list-style-type: none"> <li>Determine the genotoxicity of graphene and derivatives in standard regulatory test battery assays.</li> <li>Determine whether any mutagenicity in mouse lymphoma cells is due to loss of heterozygosity in chromosome 11.</li> <li>Investigate whether genotoxic and mutagenic responses are mediated through oxidative pathways.</li> <li>Establish the genotoxic and mutagenic mode of action using gene expression arrays.</li> </ul>
<b>Evaluation the migration and toxic potential of silver nanoparticles in feminine hygiene products to vaginal tissue: In vivo rodent and in vitro 3D mucosal models</b> Mugimane Manjanatha	<ul style="list-style-type: none"> <li>To assess the immunotoxicity of different categories of nanoparticles utilizing biomarkers of innate immunity measured in vitro in human immune cell(s) (monocytes, human peripheral blood mononuclear cells [Fu et al.]).</li> </ul>
<b>Determination of cytotoxicity and genotoxicity of nanomaterials of interest to the FDA and their mechanism of action</b> Peter Fu	<ul style="list-style-type: none"> <li>Develop a set of cell-free and cell-based in vitro tests that can be used to rapidly identify nanomaterials of interest to the FDA that elicit oxidative damage.</li> <li>Determine whether, in the presence of nano-metal materials, endogenous and dietary antioxidants can display pro-oxidative activity.</li> </ul>
<b>Assessing epigenetic effects of nanoparticles in human cells</b> George Hammons	<ul style="list-style-type: none"> <li>Determine the effect of two types of nanoparticles, silver and titanium dioxide, at various particle sizes, surface coatings, dosages, and durations of exposure on global methylation and genome-wide DNA methylation using array profiling in four types of human cells (liver, lung, skin, and colorectal).</li> <li>Determine the effect of these nanoparticles on the pattern of global histone modifications and on genome-wide profiles of histone modifications in the four types of human cells. The analysis includes comparisons with disease-associated histone modifications.</li> <li>Correlate the nanoparticle effect on DNA methylation with its effect on DNA methyltransferase expression.</li> <li>Correlate the nanoparticle effect on global histone modifications with its effect on expression of histone-modifying enzymes as potential underlying mechanisms of the alteration in DNA methylation or histone modification patterns.</li> </ul>
<b>An assessment of the interactions of nanoscale (TiO<sub>2</sub> and zinc oxide) materials used in sunscreens on the skin microbiome</b> Huizhong Chen	<ul style="list-style-type: none"> <li>Examine human skin microbiota cell viability in the presence of nanoscale materials in cosmetics.</li> <li>Determine the effect of nanomaterials in cosmetics on human skin microbial ecology.</li> </ul>

Project Study Scientist	Project Objectives
	<ul style="list-style-type: none"> <li>▪ Demonstrate the mechanisms of toxicity of nanoscale materials in cosmetics to skin microbiota using the human skin tissue model EpiDerm and reverse transcription polymerase chain reaction (RT-PCR) and whole-genome microarray technologies.</li> <li>▪ Elucidate the dose-response relationship of nanoscale materials in cosmetics on skin bacterial cell toxicity.</li> <li>▪ Assess the potential health risk of human skin exposure to nanomaterials in cosmetics.</li> </ul>
<b>Evaluation of the migration and toxic potential of Ag nanoparticles in feminine hygiene products to vaginal tissue: In vivo rodent and in vitro 3D mucosal models</b> Anil Patri	<ul style="list-style-type: none"> <li>▪ Use established qualitative methods to characterize different species of nanoscale silver (Ag) contained in five types of dry and five types of liquid feminine hygiene products.</li> <li>▪ Evaluate the migration/uptake and toxicity of Ag nanoparticles and ions used in feminine hygiene products using a human cell-based in vitro three-dimensional culture model that has many of the structural and functional features of the human vaginal mucosal layer.</li> <li>▪ Evaluate the effects of Ag nanoparticles and ions contained in feminine hygiene products on human vaginal microbiota using culture techniques and semiquantitative molecular methods.</li> </ul>
<b>Interaction of nanoparticles with gastrointestinal tract</b> Sangeeta Khare	<ul style="list-style-type: none"> <li>▪ Determine the effect of nanomaterials on the permeability of epithelial cells and establishment of immune correlates.</li> <li>▪ Delineate the interaction of nanomaterials with gastrointestinal tract and gut-associated microbiota using an ex vivo model (intestinal explants).</li> <li>▪ Establish the effect of nanoparticles on the developmental stage of the intestine and assessment of the biodistribution of nanoparticles using the zebrafish model.</li> </ul>

### *Bioassay and Biomarker Development and Evaluation in FY 2019*

Project Study Scientist	Project Objectives
<b>Study of translational biomarkers for drug-induced liver injury with next-generation sequencing</b> Baitang Ning	<ul style="list-style-type: none"> <li>▪ Conduct a comprehensive survey of microRNAs using the next-generation sequencing technology.</li> <li>▪ Elucidate the molecular pathways and processes modulated by RNAs (including messenger RNAs, microRNAs, and other noncoding RNAs) and their importance in drug-induced liver injury risk and phenotypes.</li> </ul>
<b>A comprehensive characterization of induced pluripotent stem cell-derived cardiomyocyte (iPSC-CM) models for drug-induced arrhythmia using high-throughput screening assays</b> Li Pang	<ul style="list-style-type: none"> <li>▪ Develop standard baseline criteria for high-throughput readouts of drug-induced arrhythmia in human iPSC-CMs from different suppliers.</li> <li>▪ Assess individual variance and possible sex differences in drug-induced cardiotoxic responses across a panel of nongenetically modified iPSC lines.</li> </ul>

Project Study Scientist	Project Objectives
<b>Validating the rat Pig-a assay for regulatory use: Determining the molecular basis of mutants detected in the rat Pig-a gene mutation assay</b> Vasily Dobrovolsky	<ul style="list-style-type: none"> <li>Develop a method that could routinely identify Pig-a mutations in individual Pig-a mutant phenotype cells.</li> </ul>
<b>Developing in vitro approaches to assess drug-induced liver toxicity</b> Lei Guo	<ul style="list-style-type: none"> <li>Develop and use in vitro assays for assessing drug-induced liver toxicity by evaluating cytotoxicity and quantifying representative endpoints for assessing clinical-related outcomes such as apoptosis/necrosis, steatosis, and cholestasis.</li> </ul>
<b>Development and characterization of a diet-induced obesity model using B6C3F1 mouse for evaluation of drug toxicity in obesity</b> Vijayalakshmi Varma	<ul style="list-style-type: none"> <li>Develop a B6C3F1 mouse model of obesity to investigate the effect of obesity on anthracycline-induced cardiotoxicity and the model's suitability to investigate other potential drug-induced toxicities under conditions of obesity.</li> </ul>
<b>Development of advanced safety assessments of FDA-regulated products using high-throughput and high-content quantitative approaches in cultured human cells to evaluate genotoxicity</b> Carol Guo	<ul style="list-style-type: none"> <li>Establish and demonstrate the feasibility of novel high-throughput and high-content in vitro genotoxicity assays conducted using human liver cells in conjunction with quantitative dose-response approaches for assessing and distinguishing the genotoxicity of FDA-regulated products.</li> </ul>
<b>Evaluation of an in vitro testis organ system as an alternative model for male reproductive toxicology</b> Noriko Nakamura	<ul style="list-style-type: none"> <li>Evaluate the in vitro testis organ system as an alternative model to assess male reproductive toxicology and establishment of a standardized protocol for the assay.</li> </ul>
<b>Using metabolically competent human cell lines to perform high-throughput genotoxicity testing</b> Nan Mei	<ul style="list-style-type: none"> <li>Develop HepG2-derived cell lines that simultaneously express 3–5 Phase I cytochrome P450s (CYPs).</li> <li>Develop HepG2-derived cell lines co-expressing multiple CYPs and Phase II UDP-glucuronosyltransferase (UGT) enzymes.</li> <li>Develop TK6-derived cell lines that express 14 CYP genes individually and TK6-derived cell lines simultaneously expressing 3–5 CYPs.</li> <li>Develop TK6 cells that co-express CYPs and Phase II UGT or sulfotransferase (SULT) enzymes.</li> <li>Assess the utility and feasibility of newly developed cell lines for toxicity studies using a small set of chemicals with known or postulated metabolism-related toxicity.</li> <li>Perform a pilot high-throughput genotoxicity study (HT-micronucleus assay, HT-Comet assay, and HT-γH2AX detection) using the established cell lines.</li> </ul>



Project Study Scientist	Project Objectives
<b>Predictive clinical biomarkers for chemotherapy-induced cardiotoxicity</b> Li-Rong Yu	<ul style="list-style-type: none"> <li>Discover novel omics predictive biomarkers of cardiotoxicity and diagnostic biomarkers of cardiac injury from Dox-treated breast cancer patients.</li> <li>Verify and translate predictive and diagnostic preclinical miRNA and metabolomics biomarkers of cardiotoxicity in clinical plasma samples.</li> <li>Develop mass spectrometry-based multiplex assays and verification of proteomic biomarker candidates in plasma.</li> </ul>
<b>Prediction of tyrosine kinase inhibitor (TKI) induced cardiotoxicity using induced pluripotent stem cell-derived cardiomyocytes</b> Li Pang	<ul style="list-style-type: none"> <li>Present in vitro mechanistic analysis beyond proarrhythmic toxicity.</li> <li>Identify noninvasive biomarkers that detect and predict the severity of structural cardiotoxicity.</li> <li>Develop a systems-based database to capture the characteristics of TKI-induced cardiotoxicity.</li> </ul>
<b>Somatic oncomutations as biomarkers for translating preclinical safety data to human cancer risk</b> Barbara Parsons	<ul style="list-style-type: none"> <li>Identify the most promising human oncomutation biomarkers by next-generation sequencing (NGS).</li> <li>Analyze batteries of rat and mouse amplicons for hotspot oncomutations by NGS.</li> <li>Validate rodent oncomutations as biomarkers of carcinogenic effect.</li> </ul>
<b>Genetic and epigenetic mechanisms of sex differences in the kidney of a rat model system: Developing safety biomarkers for FDA-regulated products</b> James Fuscoe	<ul style="list-style-type: none"> <li>Conduct whole-genome expression profiling on ten rat tissues of both sexes at nine ages.</li> <li>Conduct miRNA profiling of selected tissues, including liver.</li> <li>Conduct DNA methylation profiling of selected tissues, including liver.</li> <li>Use bioinformatics and statistical approaches to understand the genetic machinery operational at each developmental stage in each sex and relate the findings to potential susceptibility to adverse drug reactions and disease.</li> <li>Use bioinformatics approaches to extrapolate these findings of potential age- and sex-associated susceptibility in an animal model system to humans.</li> </ul>
<b>Development of a simple in vitro approach for the rapid detection of neurotoxicity</b> Qiang Gu	<ul style="list-style-type: none"> <li>Characterize FluoroJade-C (FJ-C) labeling in vitro.</li> <li>Validate FJ-C labeling in vitro.</li> <li>Develop an FJ-C based in vitro approach for high-throughput assessment of neurotoxicity.</li> <li>Explore the mechanism underlying FJ-C labeling.</li> </ul>
<b>Validating the rodent Pig-a gene mutation assay: Development of a human reticulocyte Pig-a assay to evaluate the ability of the rodent Pig-a assay to predict the genotoxicity of FDA-regulated products in humans</b>	<ul style="list-style-type: none"> <li>Develop a sensitive high-throughput protocol for performing the flow cytometry and magnetic enrichment-based human RBC PIG-A assay that is capable of detecting GPI-anchored marker-deficient mutants among at least several million polychromatic RBCs and at least 100 million normochromatic RBCs from human peripheral blood samples.</li> </ul>

Project Study Scientist	Project Objectives
Vasily Dobrovolsky	<ul style="list-style-type: none"> <li>Determine some basic properties of the high-throughput PIG-A assay that is developed and measurement of background PIG-A mutant frequency in the general population using blood samples from self-identified healthy human volunteers.</li> <li>Test the ability of the high-throughput RBC PIG-A assay that is developed to detect PIG-A mutation in human cancer patients receiving Pt-based drugs as part of their antineoplastic chemotherapy.</li> <li>Perform a comparative study in rats employing a chemotherapeutic treatment regimen similar to human cancer patient Pt-based therapy protocol using the most sensitive version of the rat PIG-A assay for detection of somatic mutation.</li> </ul>
<b>Quantification of in vivo genomic damage by whole-genome clone analysis and high-fidelity next-generation sequencing</b> Javier Revollo	<ul style="list-style-type: none"> <li>Develop whole-genome clone analysis, a method that detects somatic mutations in clones derived from individual cells, to quantify basal and xenotoxin-induced whole-genome somatic mutations.</li> <li>Develop an efficient ultra-high-fidelity next-generation sequencing (NGS) method capable of detecting somatic mutations in genome pools.</li> <li>Direct identify and quantify by ultra-high-fidelity NGS the frequency of somatic mutations in tissues derived from laboratory animals treated with known mutagens.</li> </ul>

### Computational Toxicology in FY 2019

Project Study Scientist	Project Objectives
<b>Sequencing Quality Control Phase 2 (SEQC2): A consortium effort to assess next-generation sequencing for enhanced regulatory science research and precision medicine</b> Weida Tong	<ul style="list-style-type: none"> <li>Engage the stakeholders and research community for consensus building with respect to the reliable use of next-generation sequencing (NGS) data with standard analysis protocols toward regulatory application.</li> <li>Develop quality metrics for reproducible NGS results for both whole-genome sequencing (WGS) and targeted genome sequencing (TGS), which involve the quality control establishment and validation processes.</li> <li>Benchmark bioinformatics methods for whole-genome sequencing (WGS) and targeted genome sequencing (TGS) toward the development of standard data analysis protocols.</li> </ul>
<b>Genome-wide analysis of in vitro to in vivo extrapolation (IVIVE) for drug safety</b> Zhichao Liu	<ul style="list-style-type: none"> <li>Investigate the correlation of IVIVE potential for known drugs across different relevant drug-induced liver injury (DILI) endpoints.</li> <li>Develop predictive models for assessing IVIVE potential of untested compounds with quantitative structure-activity relationships.</li> </ul>

Project Study Scientist	Project Objectives
<b>Enhance prediction of potential endocrine activity of chemicals by integrating multiple endpoints data</b> Huixiao Hong	<ul style="list-style-type: none"> <li>▪ Augment different endocrine-related endpoints data and develop prediction models for screening chemicals with endocrine activity potential by integration of the augmented multiple types of endocrine-related endpoints data.</li> </ul>
<b>Development of a reproducible workflow to analyze real-world incomplete or uncertain qPCR data</b> Vivian Zhuang	<ul style="list-style-type: none"> <li>▪ Design a new scientifically and statistically sound method to analyze the incomplete qPCR data in real-life experiments and studies with qPCR technology.</li> <li>▪ Automate the selection of an appropriate method or strategy to account for incomplete data in a real-life qPCR experiment or study.</li> <li>▪ Create a reproducible workflow for qPCR data analysis in general.</li> </ul>
<b>Develop a database of herbal/dietary supplement (HDS) hepatotoxicity to support the Agency's new efforts to strengthen regulation of HDS products</b> Minjun Chen	<ul style="list-style-type: none"> <li>▪ Develop an evidence-based assessment of HDS-induced liver injury.</li> <li>▪ Conduct an assessment of the severity of HDS-induced liver injury.</li> <li>▪ Evaluate histological phenotypes of herb-induced liver injury (HILI).</li> </ul>
<b>Evaluation of transcriptomics-based predictions of sex and age-related susceptibilities to treatment-induced adverse effects in F344 rats</b> James Fuscoe	<ul style="list-style-type: none"> <li>▪ Integrate hepatic life cycle basal gene expression data in male and female rats into a toxicogenomics model and enable the prediction of age- and sex-related differences in drug or chemical disposition and downstream adverse events in primary rat hepatocytes.</li> </ul>
<b>Statistical methods for whole transcriptome sequencing data analysis</b> Vivian Zhuang	<ul style="list-style-type: none"> <li>▪ Develop and evaluate one-sided and two-sided gene set analysis methods for RNA sequencing (RNA-seq) data with discrete counts.</li> <li>▪ Develop and evaluate methods for combining correlation estimates and sequencing depth to determine sample size calculations in RNA-seq experimental design.</li> <li>▪ Develop and evaluate methods for identifying outlier samples in RNA-seq data.</li> </ul>
<b>The design and development of machine-learning algorithms to assist with automated pattern recognition of persistent organic pollutants (POPs) in foods and feeds</b> Huixiao Hong	<ul style="list-style-type: none"> <li>▪ Identify trends and potential risks using historical results.</li> <li>▪ Reduce analyst hours spent reviewing data to determine quality and data usability.</li> <li>▪ Expand automated algorithms outside of the POPs field.</li> </ul>

### 6.2.1. NTP at NCTR: Interagency Agreement Projects

NCTR research for NTP is funded by [voluntary allocations](#) and an interagency agreement. Below are FY 2019 projects funded through an NIEHS/NTP interagency agreement with FDA.

*Food Additives and Contaminants in FY 2019*

Project Study Scientist	Project Objectives
<b>Role of perinatal development on toxicokinetics of inorganic arsenic</b> Daniel Doerge	<ul style="list-style-type: none"> <li>Determine serum pharmacokinetics and metabolism of low-dose inorganic arsenic in adult female CD-1 mice, Sprague Dawley rats, and rhesus monkeys.</li> </ul>
<b>Evaluation of brominated vegetable oil in rats</b> Gonçalo Gamboa da Costa	<ul style="list-style-type: none"> <li>Assess the dose-response relationships of a 90-day dietary exposure to brominated vegetable oil in Sprague Dawley rats.</li> <li>Evaluate the bioaccumulation and clearance of inorganic and organic bromine in organs and tissues of Sprague Dawley rats upon dietary exposure to brominated vegetable oil.</li> </ul>
<b>Long-term evaluation of cognitive, neurochemical, and histopathological effects of developmental inorganic arsenic (iAs) exposure in Sprague Dawley rats</b> Sherry Ferguson	<ul style="list-style-type: none"> <li>Determine the effects of developmental iAs exposure on cognitive behaviors, neurochemistry, and histopathology in Sprague Dawley rats.</li> </ul>

*Dietary Supplement Program in FY 2019*

Project Study Scientist	Project Objectives
<b>Effects of fibrinolytic enzymes nattokinase and lumbrokinase alone or in combination with aspirin in blood parameters</b> Luisa Camacho	<ul style="list-style-type: none"> <li>Evaluate an animal model of the effects of nattokinase and lumbrokinase on blood parameters and an assessment of their effects in combination with pharmacological doses of aspirin.</li> </ul>

*Drugs Program in FY 2019*

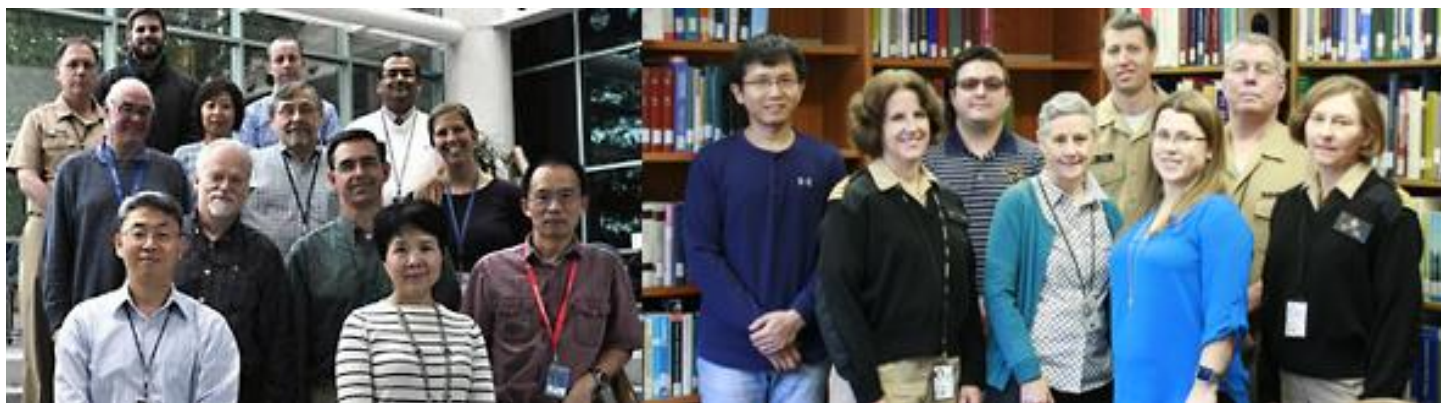
Project Study Scientist	Project Objectives
<b>Toxicokinetic profile and toxicity of high-molecular-weight polyethylene glycols in rats</b> Jia-Long Fang	<ul style="list-style-type: none"> <li>Evaluate the toxicokinetic profile of high-molecular-weight polyethylene glycols in Sprague Dawley rats given a single dose of the substances via subcutaneous injection.</li> <li>Evaluate the bioaccumulation of high-molecular-weight polyethylene glycols in organs/tissues of rats upon repeated subcutaneous injection for 24 weeks.</li> <li>Assess the toxicities resulting from the bioaccumulation of the substances.</li> </ul>

*Enhancing Toxicology Program in FY 2019*

Project Study Scientist	Project Objectives
<b>NTP capability building for microbiome assessment on toxicology studies: Assessing the role that the microbiome might play in the toxicity of xenobiotics</b> Carl Cerniglia	<ul style="list-style-type: none"> <li>Address critical knowledge gaps in the microbiome field using the latest advances in microbiome analysis through in vitro, in vivo, and ex vivo models in toxicity testing risk assessments.</li> </ul>
<b>Developing an in vitro system to evaluate the disease-related toxic effects of inhaled test agents in human airway tissue models</b> Xuefei Cao	<ul style="list-style-type: none"> <li>Develop exposure and dosimetry methods for exposing human air-lung interface airway cultures to aerosolized test agents.</li> <li>Use previously developed disease-related endpoints and air-lung interface culture exposure methods to evaluate the respiratory toxicity of two known airway toxicants, two presumed non-toxicants, and one compound of current interest.</li> </ul>

### 6.3. NTP at NIOSH

In accordance with its mandate to protect worker health and safety, the National Institute for Occupational Safety and Health (NIOSH) carries out research projects with NTP funded through an [interagency agreement](#) and voluntary allocations. These projects focus on [comprehensive assessment of occupationally relevant exposures](#) and [immunotoxicology](#) research. Setting priorities in occupational toxicological research is informed by several sources of information NIOSH develops and maintains. Sources include health hazard evaluations, industry-wide studies, gaps in knowledge identified while developing criteria for recommended standards or criteria documents, current intelligence bulletins, hazard reviews and alerts, other technical reports, and information profiles on chemical hazards.



NIOSH Staff: Health Effects Laboratory Division (left) and Division of Field Studies and Engineering (right)



NIOSH/NTP projects in FY 2019 funded through voluntary allocations are listed below.

*Biomonitoring, Biomarker Development, and Health Assessment in FY 2019*

Project Study Scientist	Project Objectives
<b>Exposure assessment research and support</b> John Snawder	<ul style="list-style-type: none"> <li>Provide support to multiple branch and interdivisional projects, which includes (1) managing and planning field sample collection, (2) developing new analytical and immunochemical biomonitoring methods, and (3) validating and adapting existing methods.</li> </ul>
<b>Evaluation of welding fumes as a lung carcinogen in mice exposed by inhalation</b> Patti Erdely	<ul style="list-style-type: none"> <li>Examine different types of welding processes and generate data pertinent to the carcinogenic potential of fumes.</li> <li>Determine which metal oxide components of welding fumes have the greatest carcinogenic potency.</li> <li>Use findings to establish if welding fume inhalation at relevant occupational exposure levels increases lung tumorigenesis.</li> </ul>
<b>Mortality, cancer incidence, and biomarker studies</b> James Yiin	<ul style="list-style-type: none"> <li>Clarify exposure-outcome associations, especially dose-response relationships, for risk assessment.</li> <li>Examine relationships between biomarkers of exposure, susceptibility, and oncogene expression; and determine health effects.</li> </ul>
<b>Toxicity assessment of carbon nanotubes and carbon nanofibers from U.S. facilities</b> Aaron Erdely	<ul style="list-style-type: none"> <li>Assess general pulmonary and systemic toxicity, pathology, biodistribution, and genotoxicity of carbon nanotubes and carbon nanofibers obtained from U.S. facilities.</li> <li>Examine the toxicity of such a broad range of materials collected from U.S. manufacturing facilities with direct relevance to U.S. worker health.</li> </ul>
<b>Toxicity along the life cycle of a MWCNT reinforced construction composite</b> Vamsi Kodali	<ul style="list-style-type: none"> <li>Evaluate occupational pulmonary toxicity arising during occupational handling of carbon nanotube (MWCNT)-enabled construction composites as it undergoes product handling or manipulation, a stage in the product's occupational life cycle.</li> <li>Develop a simulated environment for product handling to collect/measure the particulate released.</li> <li>Perform acellular, in vitro, and in vivo toxicity assessments to evaluate the pulmonary toxicity and determine the mechanism of toxicity.</li> </ul>

*Environmental Monitoring in FY 2019*

Project Study Scientist	Project Objectives
<b>Analytical methods research and development infrastructure</b> Robert Streicher	<ul style="list-style-type: none"> <li>Conduct research and develop sampling and analytical methods to enable assessment of exposure to workplace chemicals including volatile organic compounds, peracetic acid, hazardous drugs, per- and polyfluorinated substances, and pesticides.</li> </ul>

Project Study Scientist	Project Objectives
<b>Method development for crystalline silica</b> Pramod Kulkarni	<ul style="list-style-type: none"> <li>Develop laboratory and direct reading methods for the measurement of air concentration of particulate crystalline silica in workplace atmospheres.</li> </ul>
<b>Chemical and imaging methods for nanomaterials</b> Pramod Kulkarni	<ul style="list-style-type: none"> <li>Develop and evaluate physical and chemical imaging methods for measurement of airborne nanomaterials using electron microscopy, AFM-Raman, and infrared microscopy.</li> </ul>
<b>Release of nanoparticles during the life cycle of treated wood</b> Chen Wang	<ul style="list-style-type: none"> <li>Develop sampling and analytical methods for better assessment of the risks of exposure to nanoparticles released during sanding of treated wood products at different stages of their life cycle.</li> </ul>
<b>Automated nanoscale imaging and analysis using machine learning</b> Chen Wang	<ul style="list-style-type: none"> <li>Develop supervised machine-learning algorithms for automated structure classification and counting of carbon nanotube/nanofiber (CNT/CNF) materials.</li> </ul>
<b>Method development for sampling and exposure measurement for cellulose nanomaterials</b> Bon-Ki Ku	<ul style="list-style-type: none"> <li>Develop an approach to evaluate generation, sampling, and characterizing methods for airborne cellulose nanomaterial particles.</li> </ul>
<b>Biomarker detection device for early effect of exposure to respirable crystalline silica</b> Bon-Ki Ku	<ul style="list-style-type: none"> <li>Develop and evaluate field-portable, easy-to-use, cost-effective sensors (lab-on-a-chip devices) for early detection of biomarker levels in workers exposed to respirable crystalline silica.</li> </ul>
<b>NIOSH Center for Direct Reading and Sensor Technologies</b> John Snawder	<ul style="list-style-type: none"> <li>Coordinate research and develop recommendations on the use of direct reading instruments and sensor technologies to allow for rapid interventions and lead to reduced worker exposures and prevention of occupational injury, illness, and disease.</li> </ul>

### Exposure Assessment in FY 2019

Project Study Scientist	Project Summary
<b>Exposure assessment for toxicologically important chemicals</b> Brian Curwin	<ul style="list-style-type: none"> <li>Characterized workplace exposures to chemicals of toxicological concern as identified by NTP and NIOSH.</li> <li>Evaluated occupational exposure to per- and polyfluoroalkyl substances, carbon nanotubes and nanofibers, flame retardants, and polycyclic aromatic hydrocarbons in coal tar sealants.</li> <li>Goals included: (1) identifying industries, workplaces, uses, and users; (2) determining occupational health relevance; (3) estimating the number of workers exposed; and (4) conducting exposure sampling.</li> </ul>

Project Study Scientist	Project Summary
<b>Industry-wide studies, branch research, development, and planning</b> Elizabeth Whelan	<ul style="list-style-type: none"> <li>Supported strategic planning and feasibility studies of high-priority issues and emerging problems in occupational health.</li> </ul>
<b>Nanotechnology field evaluations</b> Charles Geraci	<ul style="list-style-type: none"> <li>Collected information from as many different facilities in the field as possible about the (1) nature of engineered nanomaterials, (2) processes involved in the manufacture and use of nanomaterials, (3) potential worker exposures to nanomaterials, and (4) practices and control procedures in the workplace where nanomaterials are produced or used.</li> </ul>
<b>Next-phase epidemiology study of U.S. carbon nanotube and nanofiber workers</b> Matt Dahm	<ul style="list-style-type: none"> <li>Conducted the next phases of an ongoing epidemiological and exposure assessment study of workers exposed to carbon nanotubes (CNT) and nanofibers (CNF).</li> <li>Study aims were threefold: (1) identify new CNT/F companies operating within the U.S. that have not been previously contacted by the NTRC; (2) expand the existing exposure registry of workers by recruiting additional CNT/F companies to participate; and (3) assess exposures at CNT/F facilities, not previously visited by the NTRC, to add additional data to strengthen a previously developed statistical model that uses various workplace determinants to predict CNT/F exposures and to further develop a job-exposure matrix (JEM).</li> </ul>
<b>Feasibility of characterizing workforces exposed to two-dimensional nanomaterials in the United States</b> Matt Dahm	<ul style="list-style-type: none"> <li>Strategically and systematically collected research data into a single database for 2-D nanomaterials that can be used to prioritize and recruit companies for future exposure assessment studies.</li> <li>Study aims were twofold: (1) compile information from individual workplaces and industries handling 2-D nanomaterials from existing contacts and from comprehensive industry market characterization reports into a comprehensive electronic database, and (2) contact the identified company liaison to collect additional information on types of 2-D nanomaterials produced or used, the size of the working population, and the quantity of material used daily.</li> <li>Record the collected information in the electronic database as part of an ongoing exposure assessment study on 2-D nanomaterials.</li> <li>After the standardized questions have been answered by all participating companies or workforce, the information will be organized by material and analyzed in FY 2021 for trends in quantities produced or used as well as work force size.</li> </ul>

### *Immunotoxicity and Immunology in FY 2019*

Project Study Scientist	Project Objectives
<b>Immunotoxicological evaluation of occupational chemicals</b>	<ul style="list-style-type: none"> <li>Identify occupational and environmental-chemical immune system hazards.</li> </ul>

Project Study Scientist	Project Objectives
Stacey Anderson	<ul style="list-style-type: none"> <li>Evaluate immune function and mechanisms associated with exposure.</li> <li>Contribute to better risk assessment and increased identification of immunological hazards encountered in the workplace, which ultimately will establish occupational exposure limits.</li> </ul>
<b>Identification of occupational allergens</b> John Noti	<ul style="list-style-type: none"> <li>Identify exposures to substances that can cause inflammatory or immune hypersensitivity reactions in certain work environments (these exposures can cause occupational lung diseases, such as asthma and allergic alveolitis).</li> <li>Develop improved techniques for detecting such immune reactions before adverse clinical outcomes occur.</li> <li>Develop improved techniques for detecting and identifying occupational agents that incite inflammation.</li> </ul>
<b>Pathogenesis of allergic disease following exposures to MDI</b> Justin Hettick	<ul style="list-style-type: none"> <li>Study the molecular basis for development of occupational asthma following exposures to methylene diphenyldiisocyanate (MDI) using proteomic, metabolomic, and transcriptomic approaches.</li> </ul>
<b>Immunotoxicity of subchronic fungal exposures</b> Brett Green	<ul style="list-style-type: none"> <li>Determine pulmonary immunopathological outcomes of subchronic exposures to fungi nominated to NTP and fungal contaminants encountered in the workplace.</li> <li>Focus subchronic exposure studies on <i>Aspergillus fumigatus</i>, mycotoxin-producing strains of <i>Stachybotrys chartarum</i>, <i>Aspergillus versicolor</i>, <i>Alternaria alternata</i>, as well as fungi identified in NIOSH Health Hazard Evaluations and collaborative exposure assessment studies.</li> </ul>

### Genetics in FY 2019

Project Study Scientist	Project Objectives
<b>Highly sensitive and practical biomarkers for nanotoxicity</b> Pius Joseph	<ul style="list-style-type: none"> <li>Develop, validate, and test highly sensitive and minimally invasive biomarkers for early detection of pulmonary toxicity resulting from exposure to nanomaterials.</li> <li>Conduct bioinformatic analyses of the global transcriptomics data to gain insights into the molecular mechanisms underlying the pulmonary toxicity of nanomaterials.</li> </ul>
<b>Neurological risks associated with workplace chemicals and nanomaterials</b> Krishnan Sriram	<ul style="list-style-type: none"> <li>Evaluate the potential neurotoxicological effects associated with exposure to chemical agents, incidental nanoparticles, and engineered nanomaterials in experimental models.</li> <li>Identify hazards, evaluate molecular mechanisms of neurotoxicity, and identify potential biomarkers of neurotoxicity.</li> <li>Develop novel biomarkers for monitoring exposures and health effects that will result in improved pre-job planning protocols, hazard and risk</li> </ul>

Project Study Scientist	Project Objectives
	assessment paradigms, and occupational safety standards for neurotoxic exposures.
<b>Occupational heat stress, toxicant exposure, and neurological health risks</b> Krishnan Sriram	<ul style="list-style-type: none"> <li>Conduct laboratory-based studies to model relevant worker exposures to mild or moderate heat, as well as co-exposure, to select chemical and particulate agents.</li> <li>Determine the toxicological synergy between a high-temperature work environment and toxicant exposure.</li> <li>Conduct high-throughput analysis of the brain genome and proteome to determine the underlying mechanisms these effects of and identify biomarkers that might serve as reliable predictors of neural injury.</li> </ul>
<b>Welding-related neurological risks: Influence of shielding gases</b> Krishnan Sriram	<ul style="list-style-type: none"> <li>Evaluate the neurotoxic potential of welding fumes generated by Flux Core Arc (FCA) welding.</li> <li>Determine the influence of shielding gases in modulating the neurotoxicity of welding fumes.</li> <li>Identify protein, lipid, and metabolite changes associated with welding fume-related neural injury.</li> </ul>
<b>3D air-liquid interface as a relevant in vitro lung model to evaluate specific nanotoxicity</b> Liyang Rojanasakul	<ul style="list-style-type: none"> <li>Develop and test physiologically relevant in vitro models to assess cytotoxicity on target lung cells, predict the particles' toxic potential in vivo, and to screen priority particles for in vivo studies.</li> <li>Evaluate long-term/low-dose exposure of iron oxide nanoparticle (ION)-induced cell transformation and determine property modifications (i.e., nano-SiO<sub>2</sub> coating) that could prevent toxic effects of uncoated IONs toward supporting the "safe-by-design" strategy.</li> <li>Characterize the cytotoxicity of incinerated virgin thermoplastics versus incinerated carbon nanotube-enabled thermoplastic composites on two in vitro pulmonary models using multiple methods to confirm particle-specific toxic effects.</li> </ul>
<b>Hydraulic fracturing: Toxicological effects of silica and diesel exhaust exposure</b> Jeffrey Fedan	<ul style="list-style-type: none"> <li>Investigate toxicities of inhaled hydraulic fracturing sand dust (silica) alone and in combination with inhaled diesel exhaust to mimic worker exposures during hydraulic fracturing operations using an array of in vivo and in vitro models to examine the effects of exposure on the lungs, cardiovascular system, immune system, brain, skin, and blood.</li> <li>Design and build exposure systems for fracking sand dust and diesel exhaust (exposures of animals to fracking sand dust alone and diesel exhaust alone are completed).</li> <li>Assess inhalation exposures to silica in combination with diesel exhaust (the last phase of this study).</li> </ul>
<b>Health effects of inhaled crude oil</b> Jeffrey Fedan	<ul style="list-style-type: none"> <li>Design and build a crude oil vapor inhalation exposure system.</li> <li>Investigate effects of inhaled crude oil vapor on the lungs, cardiovascular system, immune system, brain, and skin.</li> </ul>



### 6.3.1. NTP at NIOSH: Immunotoxicology Research

#### *Interagency Agreement on Immunotoxicology Research*

The NIEHS and NIOSH [interagency agreement](#) provides for support of NTP hazard identification activities aimed at preventing diseases or adverse effects caused by environmental exposure to chemical or physical agents. These cooperative studies continue to improve risk assessment by measuring what constitutes an adverse health effect on the immune system in humans. The FY 2019 studies listed below evaluate unique cohorts of individuals from professions associated with immune-mediated occupational diseases.

#### *Immunotoxicology Studies in FY 2019*

Project Study Scientist	Project Objectives
<b>Identification and characterization of fungal exposures</b> Brett Green	<ul style="list-style-type: none"> <li>Investigate and characterize the diversity of fungi in indoor and occupational environments using internal transcribed spacer region sequencing.</li> <li>Develop monoclonal and polyclonal antibodies to recombinant fungal biomarker antigens.</li> </ul>
<b>Toxicity of subchronic fungal exposures</b> Brett Green	<ul style="list-style-type: none"> <li>Characterize the toxicological and pulmonary immune responses associated with subchronic fungal exposures utilizing an acoustical generator system and nose-only exposure chamber (NTP subchronic exposure studies examining <i>Aspergillus fumigatus</i> and <i>Stachybotrys chartarum</i> are complete).</li> </ul>
<b>Analysis of mycotoxins in dust samples from water-damaged buildings</b> Ju-Hyeong Park	<ul style="list-style-type: none"> <li>Develop and refine cost-effective methods to simultaneously analyze multiple fungal secondary metabolites, including mycotoxins, in the environmental samples (isotopically labeled internal standard and standard addition method have been examined).</li> <li>Apply the developed method to analyze floor dust samples collected from epidemiological studies of moisture-infiltrated buildings for the fungal metabolites.</li> <li>Examine associations of exposures to the fungal secondary metabolites with health using complex statistical models in an epidemiological study of schoolteachers.</li> </ul>

### 6.3.2. NTP at NIOSH: Occupationally Relevant Exposures

#### *Comprehensive Assessment of Occupationally Relevant Exposures*

NIEHS is coordinating an NTP effort with NIOSH to better understand worker exposures, identify occupational health research gaps, and educate workers. The [NIEHS and NIOSH interagency agreement](#) supports these projects. The FY 2019 efforts listed below address worker exposures to welding fumes, nanosized materials, food flavorings, and other industrial chemicals.



NIOSH mobile lab for field studies

### *Occupationally Relevant Exposures in FY 2019*

Project Study Scientist	Project Summary
<b>Administrative support</b> Elizabeth Whelan	<ul style="list-style-type: none"> <li>Provided support to NIOSH scientists for (1) participating in review and oversight of NTP activities and (2) attending NTP-related meetings at NIEHS in Research Triangle Park, NC, and Washington, DC.</li> </ul>
<b>Occupational exposure assessment of welding fumes with emphasis on manganese compounds</b> Kevin Hanley	<ul style="list-style-type: none"> <li>Evaluated welders' exposures to total and respirable manganese using a novel sequential chemical extraction method to: (1) identify industries, such as construction, shipbuilding, manufacturing companies, and unions, involved in welding operations for which the potential for substantial manganese exposure exists; (2) develop methods to identify manganese compounds and different oxidation states informed by selective solubility with various welding fume matrices; and (3) characterize welding fume exposures on the basis of welding-associated jobs, tasks, and processes.</li> <li>As of the end of FY 2019, three manuscripts have been published (and a fourth has been submitted to a journal) that demonstrated excessive manganese exposures associated with welding fumes, often exceeding Threshold Limit Values of the American Conference of Governmental Industrial Hygienists (ACGIH TLVs) by an order of magnitude.</li> </ul>
<b>Exposure assessment of engineered nanoparticles</b> Charles Geraci	<ul style="list-style-type: none"> <li>Identified workplaces and engaged in the synthesis, manufacture, and use of engineered nanomaterials in characterizing workplace exposures to selected engineered nanoparticles.</li> </ul>

Project Study Scientist	Project Summary
<b>Durability of nanoscale cellulose fibers in artificial human lung fluids</b> Aleksandr Stefaniak	<ul style="list-style-type: none"> <li>Investigated the in vitro durability of nanocellulose materials in artificial lung fluids.</li> <li>Data generated from this study will be used to inform larger in vivo inhalation studies.</li> </ul>
<b>Assessment of occupational exposures to flame retardants</b> Cheryl Estill	<ul style="list-style-type: none"> <li>Compared exposures among industries, processes, and tasks; determined the routes of exposure; and made recommendations to reduce exposures.</li> <li>Assessed exposure at 19 facilities involved in the manufacture, installation, or use of goods containing these flame retardants. Worksite categories included are manufacture of products that use flexible polyurethane foams; fabrication and manufacture of rigid polystyrene foam; cutting, installing, or spraying polyurethane foam insulation at construction sites; gymnasiums; nail salons; and the fire service industry.</li> <li>These data will be used to determine exposure levels of workers in different occupations and how they relate to the general population by comparison to the National Health and Nutrition Examination Survey data. The results will aid in the design, understanding, and use of toxicological studies and risk assessment.</li> </ul>
<b>Assessment of occupational exposure to polycyclic aromatic hydrocarbons in coal tar sealant applications</b> Kevin Hanley	<ul style="list-style-type: none"> <li>Assessed occupational exposure to polycyclic aromatic hydrocarbons (PAHs) among coal tar sealant workers. (Currently, no data are available in the scientific literature on exposure to PAHs and their metabolites for workers applying coal tar sealant-based coatings on pavements.)</li> <li>Provide data on levels of exposure to airborne chemicals for comparison to current NIOSH-recommended exposure limits, if available.</li> <li>Reported results for specific PAH chemicals using NIOSH analytical methods. PAHs were measured in skin wipe samples, and PAH metabolites were measured in biological samples collected from workers to characterize levels present in this workforce.</li> <li>A total of 264 air samples, 396 skin wipe samples, and 252 urine samples were collected and analyzed for PAHs or PAH metabolites.</li> <li>Wrote draft manuscript, which is expected to be published in FY 2020.</li> </ul>
<b>Occupational exposure assessment of emerging per- and polyfluoroalkyl substances</b> Miriam Calkins	<ul style="list-style-type: none"> <li>Identified per- and polyfluoroalkyl substance (PFAS) compounds currently in use in commerce or industry and the companies, industries, and worker populations that are manufacturing, processing, or using PFAS products.</li> <li>Conducted targeted occupational exposure assessments in high- and moderate-PFAS volume industries.</li> <li>Assessed health effects associated with PFAS exposure. This study will:</li> </ul>

Project Study Scientist	Project Summary
	<ul style="list-style-type: none"> <li>– <b>Aim 1.</b> Characterize the presence of PFAS compounds across U.S. industries through review of literature and regulatory documents as well as direct communications with industry and worker representatives.</li> <li>– <b>Aim 2.</b> Develop and validate air monitoring methods for short-chain PFAS using existing sampling and analytical methods for longer-chain PFAS in preparation for an occupational exposure assessment.</li> <li>– <b>Aim 3.</b> Assess exposure to PFAS in a sample of occupational environments and worker populations from high- and moderate-volume PFAS industries.</li> <li>– <b>Aim 4.</b> Evaluate the association between PFAS exposure and health indicators, including thyroid function and cholesterol.</li> <li>– Study development will continue into FY 2020. Participant recruitment is expected to begin in the fourth quarter of FY 2020.</li> </ul>

## **Appendix I: NTP Publications in FY 2019**

All NTP publications published in fiscal year 2019 can be found in Appendix I here: [https://ntp.niehs.nih.gov/go/2019AR\\_Appendix1\\_Publications](https://ntp.niehs.nih.gov/go/2019AR_Appendix1_Publications).

## **Appendix II: Testing and Toxicology Studies in FY 2019**

All testing and toxicology studies produced in fiscal year 2019 by NTP can be found in Appendix II here: [https://ntp.niehs.nih.gov/go/2019AR\\_Appendix2\\_Testing](https://ntp.niehs.nih.gov/go/2019AR_Appendix2_Testing).