

2020

The Science of Substance

ANNUAL REPORT FOR
FISCAL YEAR 2020

NTP
National Toxicology Program

DEPARTMENT OF HEALTH & HUMAN SERVICES · USA

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1.0

FY 2020
in Review

1.1

Letter from the Director



Rick Woychik, Ph.D., was appointed Director of the National Institute of Environmental Health Sciences and the National Toxicology Program in June 2020

The National Toxicology Program (NTP) annual report for fiscal year (FY) 2020 is titled *The Science of Substance*. This title carries two meanings about the vital work we perform. First, it represents the substances we study to provide the scientific knowledge necessary to inform public health policy decision makers. Second, it reflects the substantive way the science we conduct on emerging public health concerns touches and improves lives every day.

This annual report spotlights our many accomplishments, including:

- Publication of 260 peer-reviewed research articles and reports on substances of concern, including perfluorooctanoic acid, 2-hydroxy-4-methoxybenzophenone (used in sunscreens), and traffic-related air pollution.
- Release of scoping reviews for neonicotinoid pesticides, Parkinson's disease, and progestogens.
- Reviews of eight substances for potential listing in the 15th Report on Carcinogens, a congressionally mandated, science-based, public health document that NTP prepares for the Health and Human Services Secretary.

In addition to evaluating substances and reviewing literature, NTP develops new technologies and research methods to stay on the cutting edge of toxicological science. In FY 2020, this work included several activities to advance alternatives to animal testing, such as a workshop to discuss acute systemic toxicity testing, a series of webinars on the application and benefits of recombinant antibodies, and development of human-based in vitro systems and computational approach methodologies.

In FY 2021, we will continue NTP's critical role in the generation, interpretation, and communication of toxicological information that strengthens science and informs decisions that protect public health. Communication and collaboration among stakeholders is key. We should advocate to ensure that toxicologists around the world establish common approaches for collecting, sharing, and archiving their research information.

My goal is to enhance our program's effectiveness and stature as a global leader in toxicology research and to develop our role as a valuable resource that informs decisions for a healthier tomorrow.

Rick Woychik, Ph.D.
Director, National Toxicology Program
National Institute of Environmental Health Sciences

1.2

Timeline

December 12

2019

Peer-review Meeting: Draft NTP Technical Reports on the Toxicology and Carcinogenesis Studies of 2-Hydroxy-4-methoxybenzophenone and Perfluorooctanoic Acid

During the meeting, panelists discussed draft NTP technical reports on the studies of 2-hydroxy-4-methoxybenzophenone (TR 597) and perfluorooctanoic acid (TR 598).

February 21

2020

NTP Board of Scientific Counselors Meeting

During the meeting, members discussed recent activities related to exposures (per- and polyfluoroalkyl substances (PFAS), nanoplastics, and microplastics), disease (hypertensive disorders of pregnancy), and new hazard-assessment approaches.

May 21

2020

ICCVAM Public Forum

During the forum, the International Coordinating Committee on the Validation of Alternative Methods (ICCVAM) members presented on their activities to advance new approaches to testing chemicals and medical products and reducing the amount of testing required.

October 30–31

2019

Mind the Gaps: Prioritizing Activities to Meet Regulatory Needs for Acute Systemic Toxicity

During the workshop, organized by NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Physicians Committee for Responsible Medicine, attendees discussed the potential for assessing acute systemic toxicity without animal testing.

January 21

2020

ICCVAM Communities of Practice Webinar: Use of Animal-free Affinity Reagents

The webinar presented an overview of monoclonal and polyclonal antibodies and examined animal-free technologies to produce affinity reagents.

April 6

2020

NIEHS Science Symposium: Honoring Former NTP Director Dr. Linda Birnbaum

The National Institute of Environmental Health Sciences (NIEHS) hosted the 2020 Birnbaum Research Symposium to honor the contributions of former NIEHS and NTP Director Dr. Linda Birnbaum, who retired in October 2019.

September 2–3, 2020

2020

Scientific Advisory Committee on Alternative Toxicological Methods Meeting

During the meeting, the committee heard presentations on synthetic antibody technology, computational toxicology tools, and approaches to curate and characterize data for alternative methods use.

1.3

NTP Public Health Impact

NTP contributes study data and recommendations used by federal and state health regulatory and research agencies to help protect public health. The following regulations passed in FY 2020 were informed by NTP research.

Federal Regulatory Updates

Dust-lead Clearance Levels

- The Environmental Protection Agency (EPA) issued a final rule (86 FR 983) lowering the dust-lead clearance levels for floors and window sills after completing an abatement activity.
- EPA relied on NTP research on the health effects of low-level lead exposure.

n-Propyl Bromide

- EPA is granting petitions (85 FR 36851) to add n-propyl bromide (also known as 1-BP) to the list of hazardous air pollutants contained in the Clean Air Act.
- The 14th Report on Carcinogens identifies 1-BP as "reasonably anticipated to be a human carcinogen" based on evidence of carcinogenicity from studies in experimental animals.

Vehicle Fuel Emissions

- EPA and the National Highway Traffic Safety Administration, on behalf of the U.S. Department of Transportation, issued final rules (85 FR 24174) to amend and establish carbon dioxide and fuel economy standards for model year 2021–2026 passenger cars and light trucks.
- The 14th Report on Carcinogens informed the rule.

Per- and Polyfluoroalkyl Substances

- EPA is soliciting information from the public (84 FR 66369) as it considers proposing a future rule on adding certain PFAS to the list of toxic chemicals subject to reporting under Section 313 of the Emergency Planning and Community Right-to-Know Act and Section 6607 of the Pollution Prevention Act.
- EPA is collaborating with NTP to study the toxicology of PFAS.

Toxics Release Inventory Program

- EPA is proposing corrections to existing regulatory language for the Toxics Release Inventory (TRI) Program (84 FR 65739), including updated identifiers, formulas, and names for certain TRI-listed chemicals.
- These proposed corrections maintain previous regulatory actions and do not alter existing reporting requirements or impact compliance burdens or costs.
- The 14th Report on Carcinogens informs the TRI.

Federal Regulatory Updates

Lead and Copper in Drinking Water

- EPA is proposing regulatory revisions (84 FR 61684) to the National Primary Drinking Water Regulation for lead and copper under the authority of the Safe Drinking Water Act.
- EPA has also finalized changes (85 FR 54235) to existing regulations to require use of lead-free plumbing materials in public water systems and residential and nonresidential facilities.
- NTP research found sufficient evidence that blood lead concentrations are associated with adverse health effects in adults and children. EPA used this research and its findings as one of two main sources to assess the health impacts associated with reductions in lead ingestion.

California Regulatory Updates

Toluene Solvents

- The California Office of Environmental Health Hazard Assessment (OEHHA) has adopted new reference exposure concentrations for the chemical solvent toluene for use in the Air Toxics Hot Spots Program.
- NTP published research on the effects of toluene in rodent studies, where necrosis of the brain was observed.
- OEHHA is also adopting a new cancer inhalation unit risk and slope factor for p-chloro-α,α,α-trifluorotoluene for use in the Air Toxics Hot Spots Program.
- OEHHA also proposed adopting a Proposition 65 No Significant Risk Level of 23 micrograms per day for p-chloro-α,α,α-trifluorotoluene.
- NTP summarized carcinogenicity data for p-chloro-α,α,α-trifluorotoluene in research published in 1992, 2009, and 2018.

Halogenated Acetic Acids

- OEHHA proposed adopting Proposition 65 No Significant Risk Levels for trichloroacetic acid, dibromoacetic acid, and dichloroacetic acid, which are all used in cosmetic treatments (such as chemical peels and tattoo removal) and as topical medication.
- This action was supported by NTP research on trichloroacetic acid and dichloroacetic acid and its review of carcinogenicity studies on dibromoacetic acid.

1,2-Dibromo-3-Chloropropane and Trihalomethanes

- OEHHA updated its public health goal (PHG) for 1,2-dibromo-3-chloropropane, a soil fumigant formerly used in agriculture that causes adverse health effects.
- A PHG is the concentration of a drinking water contaminant at which adverse health effects are not expected to occur from a lifetime of exposure.
- OEHHA relied on NTP data from inhalation studies in rodents.
- OEHHA also updated its PHG for four trihalomethanes found in drinking water as a result of disinfection methods: chloroform, bromodichloromethane, dibromochloromethane, and bromoform.
- In its guidance, OEHHA cites numerous NTP studies, including the 14th Report on Carcinogens.

California Regulatory Updates

Bisphenol A

- OEHHA determined that exposures to bisphenol A (BPA) from certain polycarbonate eyewear products did not exceed the maximum allowable dose concentration needed to require a warning under Proposition 65.
- The California issuance cited NTP research on BPA, which has possible health effects on the brain and prostate gland of fetuses, infants, and children.

Cannabis Smoke and Δ9-THC

- OEHHA added cannabis (marijuana) smoke and Δ9-tetrahydrocannabinol (Δ9-THC) to the list of chemicals known by the state to cause reproductive toxicity for purposes of Proposition 65.
- NTP toxicological and carcinogenic rodent studies helped inform the addition.



2.0

About NTP

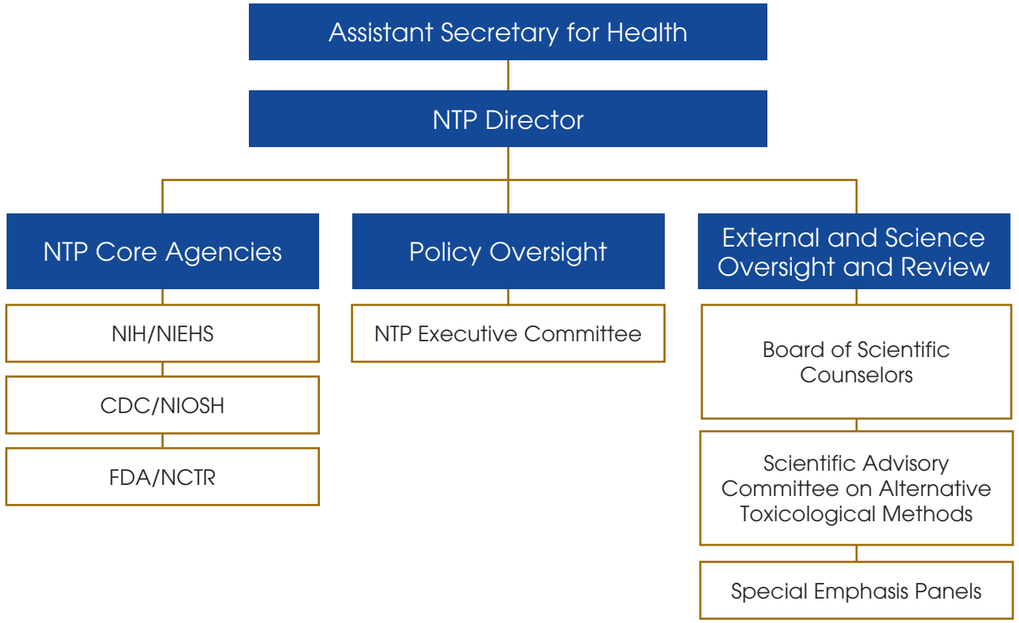
The National Toxicology Program (NTP) provides the scientific basis for programs, activities, and policies that promote health or lead to the prevention of disease. Founded in 1978, NTP plays a critical role in generating, interpreting, and sharing toxicological information about hazardous substances in our environment.

2.1

Organization and Structure

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





NTP is located administratively at NIEHS. Rick Woychik, Ph.D., was appointed director of NIEHS and NTP in June 2020 after serving as acting director since October 2019. Brian Berridge, D.V.M., Ph.D., is NTP associate director and scientific director of the Division of NTP (DNTP) at NIEHS, which is a focal point for many NTP activities. NIEHS and NTP support best research practices and embrace developments in technology to discover how the environment affects people and to maintain leadership in the field of environmental health sciences.

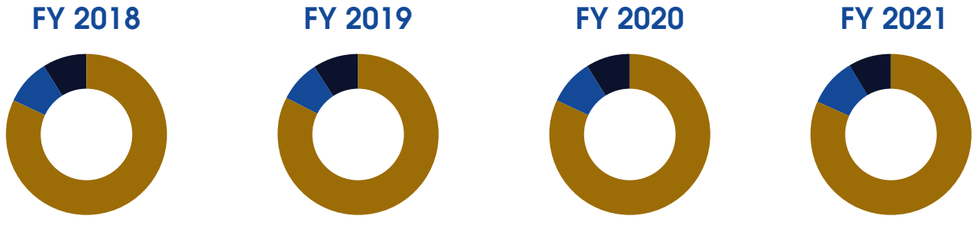
John Howard, M.D., is director of NIOSH. Staff from two NIOSH divisions participate in NTP activities. Elizabeth Whelan, Ph.D., chief of the Field Research Branch, and Cheryl Estill, Ph.D., Exposure Assessment Team Lead, manage NTP activities within the Division of Field Studies and Engineering. Donald Beezhold, Ph.D., oversees activities in the Health Effects Laboratory Division. 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 the center. As a center within FDA, NCTR also is under the leadership of FDA's chief scientist, Rear Admiral Denise Hinton, M.S. Scientists at NCTR provide innovative technologies, methods development, vital scientific training, and technical expertise that support the NTP mission, as well as the regulatory roles of FDA product centers. 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

Funding

NTP relies on voluntary allocations from the three core agencies —NIEHS, FDA, and NIOSH—to support its activities. These allocations are specified after annual appropriations have been determined. The total NTP budget for FY 2020 was $140 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 2020, NIEHS funded the contracts listed in the table. CDC and FDA may have additional contracts that support some of their voluntary NTP efforts.

Description	Contractor
Administrative and scientific staff	Kelly Government Services
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
Botanicals Safety Consortium	Department of the Interior/Federal Consulting Group/National Business Center
Collaborative work with Ramazzini Institute	Department of Energy with Oak Ridge Institute for Science and Education
Evaluation of alternative toxicological methods	Integrated Laboratory Systems
Evaluation of toxicity after 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
Pathology support	Experimental Pathology Laboratories Integrated Laboratory Systems PAI/Charles River Laboratories
Peer review of revised NTP monograph	National Academies of Sciences, Engineering, and Medicine
Production of B6C3F$_1$ mice	Taconic Biosciences
Project Portfolio Meeting Software (PPMPro)	Planview
Quality assessment support/audits and inspections	CSS-Dynamac Corporation
Scientific information management and literature-based evaluations for NTP	ICF International, Inc.
Statistical support	Social and Scientific Systems
Support for toxicological data	Vistronix
Tissue Chip Consortium (TCC) Membership	Texas A&M University
Toxicological and carcinogenic potential of chemicals	Battelle Memorial



3.0

Publishing, Collaboration, and Science Communications

Working together with the scientific community and reporting on ideas and findings is essential for protecting public health. Each year, the National Toxicology Program (NTP) publishes dozens of reports and hosts scientific panels, workshops, meetings, and trainings to advance the field.

3.1

Completed NTP Reports and Publications

NTP scientists published 260 journal articles and book chapters in FY 2020. Citations for these publications are provided in Appendix I. In addition, 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 for 2 years
- Developmental and reproductive toxicity reports, which document the potential of agents to affect test animal development and reproduction
- NTP toxicity reports, which document shorter-term studies, generally no longer than 13 weeks
- Immunotoxicity reports, which evaluate the potential of environmental and occupational substances to damage the immune system
- NTP research reports, which provide the results of research studies, rapid communications, and literature surveys that do not fall under the scope of the other report series
- NTP monographs, which assess the evidence that exposure to a substance causes adverse health effects or presents the state of the science
- Report on Carcinogens monographs, which are prepared for candidate substances selected for review and consist of cancer evaluations and substance profiles

All published NTP reports are peer reviewed by experts who are screened for conflicts of interest before their service. In 2020, NTP completed two technical reports, four developmental and reproductive toxicity reports, four toxicity reports, one immunotoxicity report, three research reports, and one monograph, as listed below.

Report Series	Report Number	Substance or Topic Addressed	Published Date
Technical Reports	597	2-Hydroxy-4-methoxybenzophenone	May 2020
	598	Perfluorooctanoic Acid	May 2020
Developmental and Reproductive Toxicity Reports	01	Tris(chloropropyl) Phosphate	June 2020
	02	4-Methylcyclohexanemethanol	June 2020
	03	Vinpocetine	June 2020
	04	Dimethylaminoethanol Bitartrate	June 2020
Toxicity Reports	87	Fullerene C60 (1 µm and 50 nm)	July 2020
	91	Abrasive Blasting Agents	July 2020
	94	1020 Long Multiwalled Carbon Nanotubes	November 2019
	99	Gum Guggul Extract Formulation	June 2020
Immunotoxicity Report	01	4-Methylcyclohexanemethanol	July 2020
Research Reports	15	Scoping Review of Potential Human Health Effects Associated with Exposures to Neonicotinoid Pesticides	September 2020
	16	Scoping Review of Paraquat Dichloride Exposure and Parkinson's Disease	September 2020
	17	Scoping Review of Prenatal Exposure to Progestogens and Adverse Health Outcomes	September 2020
Monograph	07	Traffic-related Air Pollution and Hypertensive Disorders of Pregnancy	December 2019



HIGHLIGHT

Botanical Dietary Supplements

Botanical dietary supplements, sometimes called herbals or herbal dietary supplements, are products made from plants, plant parts, or plant extracts that people take for a range of enhancement purposes.





Approximately

18%

of U.S. adults use natural products, including dietary supplements. U.S. consumers spent $8.8 billion on herbal dietary supplements in 2018.[1]

[1] Smith T, Gillespie M, Eckl V, Knepper J, Morton-Reynolds C. 2019. Herbal Supplement Sales in US Increase by 9.4% in 2018. HerbalGram. 2019;123:62-73.

Why Does NTP Study Dietary Supplements?

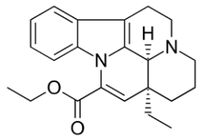
Not all products from plant sources are safe. The safety of a botanical depends on many factors, such as its chemical structure, how it works in the body, how it is prepared, and the dose taken. These supplements may contain active ingredients with strong biological effects that could interact with over-the-counter and prescription drugs.

The Food and Drug Administration (FDA) regulates dietary supplements under a different set of regulations than those covering conventional foods and drug products. Under the law, manufacturers and distributors of dietary supplements and ingredients are responsible for evaluating the safety of their products and accurately labeling them before making them available to the public. FDA is responsible for acting against adulterated or misbranded products only after they reach the market.

NTP tests the safety of supplements and their ingredients in response to requests from the public and other federal agencies. The studies help identify any harm from short- and long-term exposure to these substances.

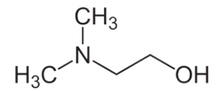
Public concern about botanical dietary supplements includes questions about labeling, product quality, health benefits, and risk of harmful effects. NTP studies offer scientific data that can inform decisions by health care providers, government regulators, and consumers.

FY 2020 Reports on Dietary Supplements



Vinpocetine

Vinpocetine is a synthetic derivative of the vinca alkaloid vincamine, which is extracted from either the seeds or the leaves of specific plants. The substance is marketed for uses such as improvement of brain function and weight loss. In June 2020, NTP published a report on its study that found harmful reproductive effects in animals exposed to vinpocetine. Because of these findings, FDA advised companies marketing vinpocetine products to include a warning on the label for women of childbearing age to avoid vinpocetine because it may cause miscarriage or harm fetal development.



Dimethylaminoethanol bitartrate

Dietary supplements containing dimethylaminoethanol bitartrate are marketed to improve memory and general cognitive function. Human exposure may also occur through occupational and industrial routes such as spray painting and lacquering. In June 2020, NTP published a report on its prenatal development study in rats, which demonstrated only marginal or discordant developmental effects that may or may not be related to the compound.

3.2

Advisory Groups

NTP strives to advance toxicological research and promote the development and use of innovative approaches and, as such, relies on advice from external federally chartered advisory groups to help guide its science.

The Board of Scientific Counselors (BSC) provides advice on the merits of NTP's intramural and collaborative programs. At its meeting on February 21, 2020, the BSC heard how microphysiological systems, such as organs-on-a-chip or 3D organ constructs, offer an opportunity to get closer to the complexity of in vivo systems to potentially reinvent how toxicology is done. The BSC was briefed on recent developments in research on human exposure to nanoplastics and microplastics, including activities by national and international regulatory agencies to address this problem. They learned about a recently completed systematic review of the published literature that confirmed an association of traffic-related air pollution to hypertensive disorders of pregnancy and about NTP's portfolio of studies on PFAS. Materials from the meeting are available on the Past BSC Meetings page on the NTP website.

The Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) advises the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), and the NIEHS and NTP director about statutorily mandated duties of ICCVAM and NICEATM activities related to promoting 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. The opening session of SACATM's annual meeting on September 2–3, 2020, focused on the past, present, and future of ICCVAM on its 20th anniversary. Other sessions summarized international partner activities and focused on nonanimal-derived antibodies, curation and characterization of data, and computational resources. SACATM members praised advances in use of computational methods to reduce animal use for chemical safety testing. They suggested resources and approaches that could be developed to further advance these tools and identified testing areas where they could be applied. Information about the meeting is available on the Past SACATM Meetings page on the NTP website.

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 use in making credible decisions about human health hazards, setting research, and testing priorities, and in evaluating test methods for toxicity screening.

NTP technical reports provide the results of long-term NTP toxicology and carcinogenicity studies that usually involve exposing laboratory animals (rats and mice) to a substance for a period of two years. For each report, the panel is charged with reviewing and assessing the methods, results, and conclusions about the toxicologic potential, including carcinogenic activity, of selected substances.

The draft technical reports listed below underwent peer review in FY 2020. Additional information about past reports' peer-review meetings is available on the NTP Technical Reports web page.

Report	Report Number	Uses of Study Substance	Peer Review Information
Peer Review of Draft NTP Technical Report on the Toxicology and Carcinogenesis Studies of 2-Hydroxy-4-methoxybenzophenone	TR-597	NTP assessed adverse effects in rodents of the UV filter often added to sunscreens.	Actions Peer-Review Report
Peer Review of Draft NTP Technical Report on the Toxicology and Carcinogenesis Studies of Perfluorooctanoic Acid	TR-598	NTP assessed the contribution of combined gestational and lactational exposure to the chronic toxicity and carcinogenicity of perfluorooctanoic acid (PFOA).	Actions Peer-Review Report

3.4

Workshops and Meetings

NICEATM Meetings and Workshops

Symposium Webinar: Use of the Kinetically Derived Maximum Dose in Toxicity Testing

The kinetically derived maximum dose (KMD) refers to the dose at which a departure from dose proportionality or linear pharmacokinetics is observed. Toxicity findings at doses above a KMD may not be relevant to human health risks when potential exposure concentrations are orders of magnitude lower.

In contrast to its routine use in pharmaceutical development, consideration of the KMD in the design or interpretation of animal toxicity studies for environmental chemicals is relatively rare. Interest is growing in using the KMD to interpret animal dose-response data or to set top dose in chronic toxicity studies of these chemicals, but many technical and scientific issues hinder its proper use. This symposium highlighted these commonly raised issues and provided the background information needed to develop more consistent, transparent approaches to support broader KMD application in risk assessment.

The symposium, organized by NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), the U.S. Environmental Protection Agency (EPA) Office of Pesticide Programs, and the Health and Environmental Sciences Institute, was presented virtually on September 30, 2020, and attended by more than 400 viewers. Presentations in this symposium reviewed the application of KMD in toxicity testing. They summarized commonly raised technical and scientific issues related to the use of KMD as an approach to select doses in toxicology testing studies or to interpret dose-response study results.

Workshop: Mind the Gaps: Prioritizing Activities to Meet Regulatory Needs for Acute Systemic Lethality

Acute systemic toxicity tests are the most commonly performed type of safety test worldwide. Participants in this October 30–31, 2019, workshop considered approaches available to assess acute lethality associated with chemicals and chemical mixtures, with the purpose of designing comprehensive strategies to predict toxicity while avoiding animal tests. The workshop was organized by NICEATM and the Physicians Committee for Responsible Medicine.

The specific areas discussed included:

- Defining how to calculate the LD50 (median lethal dose) of a chemical mixture/formulated product
- Identifying gaps for which model (or assay) development or optimization is needed
- Understanding regulatory needs for model (or assay) outputs
- Pinpointing the types of mechanistic information that would be useful
- Establishing the feasibility of using artificial intelligence in model development

Group discussions focused on issues specific to particular classes of chemicals, mechanisms of acute toxicity, prioritization of activities, and recommending next steps. Follow-up actions included an analysis of variability in the in vivo test to establish confidence in toxicity predictions, a comprehensive assessment of an additivity equation for predicting mixtures toxicity, and exploration of the addition of biological and mechanistic information to complement in silico predictions.

Antibody Webinar Series

Affinity reagents such as antibodies are used in a range of research, diagnostic, and regulatory applications. Because traditional practices for producing such reagents require immunization of laboratory animals, even when they are applied to nonanimal test methods, their use is inconsistent with the spirit of replacing, reducing, or refining animal use. Use of animal-based affinity reagents also introduces variability to the methods that use them, which contributes to increased interest in promoting the acceptance and use of nonanimal-derived antibodies.

NICEATM, the People for the Ethical Treatment of Animals International Science Consortium (PETA-ISC), and the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) co-organized a series of free webinars in which experts addressed the application and benefits of recombinant antibodies.

- In the first webinar, presented July 23, 2020, researchers described the role of nonanimal-derived antibodies in development of therapeutics for COVID-19 and other diseases.
- In the second webinar, presented September 16, 2020, João Barroso of EURL ECVAM shared a summary of the findings from a 2018 EURL ECVAM Scientific Advisory Committee meeting that reviewed the validity and benefits of using animal-free technology to produce affinity reagents and the subsequent EURL ECVAM recommendation.

The webinar series will continue into FY 2021. Information about upcoming webinars and recordings of past webinars are available on the PETA-ISC website.

ICCVAM Meetings

NICEATM supported 10 teleconferences held by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) in FY 2020. NICEATM also provided support for three ICCVAM-sponsored public meetings.

The sixth annual ICCVAM Communities of Practice webinar was held on January 21, 2020. The webinar reviewed the usefulness and limitations of nonanimal-derived affinity reagents and their potential to replace animal-derived reagents. Alejandra Solache, Ph.D., Abcam, presented an overview of production, use, and characteristics of monoclonal and polyclonal antibodies. Rebecca Clewell, Ph.D., 21st Century Tox Consulting, summarized a review by a working group of the Scientific Advisory Committee of the European Union Reference Laboratory for Alternatives to Animal Testing on the scientific validity and benefits of animal-free technologies to produce affinity reagents. Presentations from the webinar are available on the NTP website.

ICCVAM held its seventh annual public forum on May 21, 2020. Usually an in-person event, the 2020 public forum was presented as a webinar that was attended by more than 400 viewers. Representatives of ICCVAM member agencies described work to advance new approaches to safety testing of chemicals and medical products and to reduce the amount of testing required. Commenters at the forum commended the agencies' progress and noted the importance of federal agencies taking leadership roles in reducing animal testing. In addition to government agencies, viewers represented pharmaceutical and chemical companies, animal welfare organizations, academic researchers, and test method developers. Presentations from the public forum are available on the NTP website.



3.5

Collaboration and Scientific Leadership

NIEHS supports NTP collaborations with other federal agencies through interagency agreements. FY 2020 NTP activities made under these agreements are outlined here.

FDA/NCTR

Under an interagency agreement, NIEHS provided financial resources to the FDA to conduct toxicology studies on issues of mutual interest to NIEHS and FDA at the National Center for Toxicological Research (NCTR). 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. This research supports an increased understanding of the pharmacokinetics, mode-of-action, and dose-response relationships of the substances and refinements to risk assessment models. This research is included among the 60 peer-reviewed journal articles published in FY 2020 by NCTR scientists in support of NTP (included in Appendix I). Further information about NTP research at NCTR in FY 2020 can be found in Section 5.2.

CDC/NIOSH

NIEHS provides support to the National Institute for Occupational Safety and Health (NIOSH) of the Centers for Disease Control and Prevention (CDC) through multiple interagency agreements. In FY 2020, research under these agreements evaluated occupational exposure to graphene and other two-dimensional nanomaterials, molds and mycotoxins, and per- and polyfluoroalkyl substances (PFAS). This research is included among the 76 peer-reviewed journal articles published in FY 2020 by NIOSH scientists in support of NTP (see Appendix I). More information about NTP research at NIOSH in FY 2020 can be found in Section 5.3.

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 PreClinical Innovation (DPI) to evaluate high-throughput and high-content screening assays in support of Toxicology in the 21st Century (Tox21). Tox21 is a collaboration among federal agencies to characterize the toxicity of chemicals by using cells and isolated molecular targets instead of laboratory animals. This interagency agreement between NIEHS 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 is working with the 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 the effectiveness of NTP's work with stakeholders, to whom the work is disseminated. ORNL is also developing tools to apply natural language processing and machine learning methods to determine specific data elements in the full text of scientific publications.

EPA/NCCT

Under this interagency agreement, the EPA 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 2020 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 and the EPA 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.

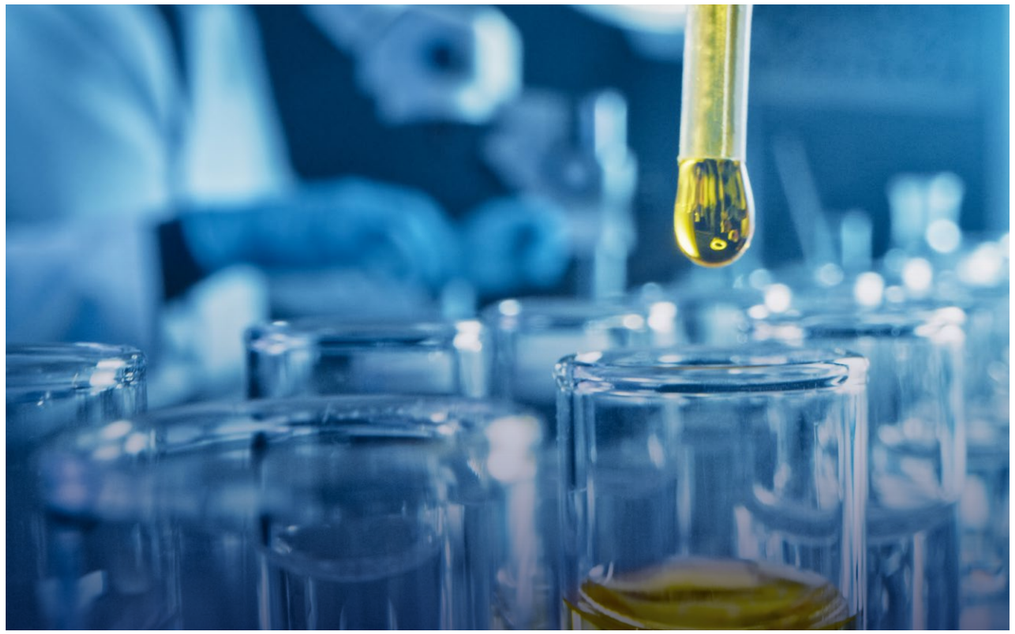
NIST

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

3.6

Training Opportunities

The Division of NTP (DNTP) at NIEHS offers a limited number of postdoctoral training fellowships to prepare trainees for careers in pharmaceutical and chemical industries, regulatory agencies, and academia. In FY 2020, NIEHS/DNTP staff mentored 20 postdoctoral fellows at NIEHS in seven focal areas. Full details on opportunities, benefits, and the application process is on the NIEHS training website.



4.0

Science, Innovation, and Methods Development

The National Toxicology Program (NTP) develops and tests innovative methodologies that advance toxicological science while also decreasing reliance on animal studies. By providing leadership in the state of the science, NTP and its partners are addressing today's public health challenges and preparing to meet the emerging challenges of tomorrow.

4.1

Development of New Approach Methodologies

NTP is a leader in the development of new approach methodologies (NAMs) that use human-based in vitro systems and computational approaches to assess harmful effects of substances without using animals.



Stem cells in developmental toxicology and the developmental basis of adult disease

Erik Tokar

NIEHS/DNTP scientists are investigating the role of environmental chemical exposures in birth defects. A rapid and accurate screening system for predicting developmental toxicants to humans in the environment is needed to test chemicals that have had little to no safety testing. Embryoid bodies are free-floating 3D spheres that spontaneously differentiate into three germ layers that recapitulate many early embryogenic processes. A transcriptomic-based, high-throughput platform using human embryonic stem cells/embryoid bodies is being developed to screen for environmental teratogens and developmental toxicants to gain insights into the chemical effects on pluripotency and early linage commitment.

Time optimizing in vitro human liver model for high-throughput screening

Katelyn Lavrich, Stephen Ferguson

Hepatocyte spheroids composed of 1,000 HepaRG human liver cells can efficiently metabolize chemicals and show liver functions better than regular, flat cell cultures. Ongoing work seeks to optimize the maturation time of spheroids for their "adult" hepatocyte functionality over time to optimally assess chemical toxicity screening, determine changes in media environment and composition, and estimate the reversibility of chemical changes. Time and concentration dependence of reference chemicals for toxicity will establish the relationship of molecular changes to pathological cellular toxicity.

NICEATM Activities

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 that supports activities of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and NIEHS/DNTP projects, especially those related to Toxicology in the 21st Century (Tox21).

Integrated Chemical Environment

Successful computational toxicology projects depend on freely available, high-quality data formatted for use in computational workflows. The NICEATM 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.

Improvements in FY 2020 to the ICE in vivo extrapolation (IVIVE) tool included

- Ability for users to upload their own data for IVIVE analyses.
- A new physiologically based toxicokinetic model.
- Selection of assays and filtering of results based on mode of action or toxicity endpoint.
- Improved output graphics.

Other updates to ICE in FY 2020 included

- Principal component analysis plots that provide additional visualization options in the Chemical Characterization tool.
- The ability to send search query results to the Environmental Protection Agency (EPA) CompTox Chemicals Dashboard.
- User interface improvements and additional metadata provided in results downloads.
- Organizing curated high-throughput screening assays based on mechanistic targets and modes of action and mapping these assays to controlled terms from the National Cancer Institute's Metathesaurus.
- New data sets and chemical lists, including chemical lists relevant to carcinogenesis.

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 used models developed for the project that met qualitative and quantitative review criteria to generate consensus predictions for the acute oral toxicity endpoints of interest. It combined these models using a weight-of-evidence 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), described in more detail below under "Computational Toxicology." The consensus predictions generated for the project are also available in ICE. Partnerships with federal agencies to apply CATMoS were described in a presentation at the 2020 meeting of the Scientific Advisory Committee on Alternative Toxicological Methods.

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 conducted a retrospective analysis of triple pack reports and compared in vivo with in vitro results. Results suggest that using either human in vitro data alone or human in vitro in combination with rat in vitro data would provide an estimate of dermal absorption that is either close to or more conservative than an estimate derived from triple pack data.

Additivity approaches to predicting toxicity of formulations

Using data obtained from EPA, NICEATM evaluated the use of the Globally Harmonized System of Classification and Labeling of Chemicals additivity equation to predict acute toxicity of chemical mixtures. NICEATM compared toxicity predictions generated by the additivity equation for antimicrobial cleaning products and agrochemical formulations with experimental measurements from in vivo data. The analysis suggested that toxicity of these mixtures was not well represented by the sum of their ingredients' toxicity, either because interactions between components increase the toxicity of the mixtures or because the additivity calculation does not adequately account for variability of the animal studies.

Acute fish toxicity

To assess hazards to wild fish species, EPA currently requires testing of each of three different fish types: warm water, cold water, and marine/estuarine. NICEATM and the EPA Office of Pollution Prevention and Toxics evaluated acute fish toxicity data from pesticide safety data submitted to the EPA office to determine whether fewer species could be used for this testing. The analysis revealed that cold water species were most often the most sensitive species, and thus it may be possible to run these tests in only one species. This would substantially reduce the number of fish needed to meet this testing requirement while still meeting risk protection goals.

Variability analysis of in vivo acute toxicity data

NICEATM assessed the reproducibility of the rat acute oral lethality test using a comprehensively curated data set of more than 2,000 chemicals. It conducted quantitative and categorical analyses and established a global confidence interval to characterize the inherent variability of the test method. These analyses revealed that independent studies can yield LD50 values that are orders of magnitude apart, which can have significant implications from a regulatory perspective due to the resulting impact on hazard classification and labeling. NICEATM did not find any evaluated parameter to correlate with variability, suggesting that the observed variability may be inherent to the animal test. The resulting publicly available data set can be used for modeling, and the calculated confidence interval can be applied when assessing performance of new approach methodologies (NAMs).

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 quantitative structure-activity relationship (QSAR) models, to screen drugs and chemicals more efficiently for their potential to be toxic to the heart or circulatory system. The initiative is supported jointly by NIEHS/DNTP, NICEATM, the Food and Drug Administration (FDA) Center for Drug Evaluation and Research, and the Health and Environmental Sciences Institutes. 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 determine human-relevant data. It is also 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 2020 identified molecular and cellular events contributing to cardiovascular failure modes, which 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), now maintained by NICEATM. 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.

In FY 2020, OPERA added a JAVA library to enable live predictions on tools such as ICE and the EPA Chemicals Dashboard.

SEAZIT

The small size and rapid development of zebrafish make it a useful vertebrate model for assessing 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 NIEHS/DNTP and NICEATM scientists.

NICEATM is coordinating the establishment of ontologies (standardized nomenclature systems) for zebrafish screening. Three laboratories tested the same 90-chemical set using similar study designs and morphology assessments. Zebrafish images were posted online, and researchers were asked to evaluate the phenotypes using in-lab terminology. Results were collected and compiled, and terms were mapped to the Zebrafish Phenotype Ontology. A second online evaluation was completed in FY 2020 and employed controlled vocabulary.

The interlaboratory study conceived during the initial information gathering phase of SEAZIT began in 2019. The study is designed to determine the effect of chorion removal and exposure media renewal on study outcomes. Participating laboratories are conducting dose range-finding experiments.

Automating identification of developmental toxicity reference data

To establish confidence in alternative approaches, it is critical to determine and extract information from the full text of scientific publications when developing reference databases. However, manually extracting protocol details such as species, route of administration, and dosing regimen is labor-intensive and can introduce errors. NICEATM is collaborating with FDA and the Department of Energy's Oak Ridge National Laboratory to apply natural language processing and machine learning methods to determine specific data elements in the full text of scientific publications. NICEATM is applying an approach trained on a curated set of data from the rodent uterotrophic database (Kleinstreuer et al. 2016) to developmental toxicity studies from the published literature.

Extraction and annotation of legacy developmental toxicity study data

To support the evaluation of nonanimal approaches for developmental toxicity assessment, NICEATM scientists extracted information from approximately 250 NTP legacy prenatal developmental toxicity animal studies and a subset of about 50 studies submitted to the European Chemicals Agency that were deemed high quality by NIEHS/DNTP subject matter experts. Study details extracted included species, strain, administration route, dosing duration, and treatment related effects. Efforts are underway to standardize the effects extractions by applying controlled vocabularies and ontologies to facilitate computational analyses and integration with other structured databases such as EPA's ToxRefDB.



HIGHLIGHT

Skin Sensitization

Before marketing, manufacturers test pesticides, cosmetics, household cleaners, and other chemical products to identify substances that can cause allergic reactions, known as skin sensitizers.

Regulatory agencies use skin sensitization test data to assess risks from exposure to chemicals and to develop product hazard labeling to protect consumers and workers. While these toxicology tests are often performed on animals, scientists are increasingly using biochemical or cell-based (in vitro) systems to determine skin-sensitizing substances.

Under U.S. laws and policies, scientists must consider alternative methods before using animals for toxicology research and testing. In some parts of the world, including Europe, testing cosmetic products and their ingredients on animals is banned. These efforts have led to increased development of in vitro tests for skin sensitization.

The ICCVAM established a Skin Sensitization Workgroup to help develop and validate nonanimal methodologies for skin sensitization tests. The NICEATM conducts many of these research activities, including the following work during FY 2020.

Nonanimal Risk Assessment for Isothiazolinones

In FY 2020, the NIEHS/DNTP and NICEATM responded to an EPA request for input on the use of an in vitro and artificial neural network-based defined approach to evaluate skin sensitization risks for products containing isothiazolinones, which are used in plastics, household cleaners, and laundry detergents. NIEHS/DNTP and NICEATM conducted studies, and the ICCVAM Skin Sensitization Workgroup reviewed the reports before delivering them to EPA to develop risk assessments. It was the first use of this type of in vitro data and defined approaches to support a regulatory risk assessment.

Expanding Approaches to Identify Skin Sensitizers

In FY 2020, NIEHS/DNTP tested more than 200 chemicals nominated by ICCVAM agencies, including pesticides, industrial chemicals, and others, using three in vitro methods: the KeratinoSens method, the direct peptide reactivity assay, and the human cell line activation test. NICEATM coordinated the testing, which began in 2017 and was completed in early FY 2020. The study data will allow NICEATM and ICCVAM to evaluate whether it's appropriate to use these three in vitro methods for various regulatory applications.

Human Reference Data for Skin Sensitization

At the 2020 annual meeting of the American Society for Cellular and Computational Toxicology, presenters described work by NICEATM, the German Federal Institute for Risk Assessment, and other collaborators to evaluate nonanimal approaches for skin sensitization assessment. The group had collected data for more than 2,300 human predictive patch tests from more than 1,700 publications. Human predictive patch test data that are based on single doses make determining thresholds uncertain and these data difficult to use as reference data. To overcome this challenge and help resolve ambiguity in results, classification criteria were extended using a decision tree to a reference list of 200 substances to support the evaluation of defined approaches for skin sensitization. This database will be publicly available to help develop additional methods and models.

Pred-Skin Web Portal for Predicting Human Skin Sensitizers

In FY 2020, NICEATM collaborated with scientists at the University of North Carolina at Chapel Hill and the Universidade Federal de Goiás to update a web tool implemented in Pred-Skin version 3.0, an application based on externally predictive QSAR models of skin sensitization (Borba et al. 2020). The updates integrate multiple QSAR models developed with in vitro, animal in vivo, and human ex vivo data into a consensus naïve Bayes model that predicts human effects (Braga et al. 2017).

Endocrine Disruption

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 stand-alone OPERA application. A paper describing the CoMPARA project was published in FY 2020.

Eye Irritation

Human-relevant approaches to assess eye corrosion/irritation potential of agrochemical formulations

In vitro and ex vivo methods are at least as reflective of human biology and more reproducible than the currently used in vivo rabbit eye test. Accordingly, NICEATM, in conjunction with scientists from EPA, the European Union Reference Laboratory for Alternatives to Animal Testing, the Institute for In Vitro Sciences, and People for the Ethical Treatment of Animals International Science Consortium (PETA-ISC) drafted a review article to detail these perspectives and make suggestions for optimizing the most promising methods to distinguish the complete spectrum of eye irritation effects. This review also considers including other information, such as physicochemical properties, consistent with the Organisation for Economic Co-operation and Development (OECD) Guidance Document 220 on an integrated approach to testing and assessing eye irritation.

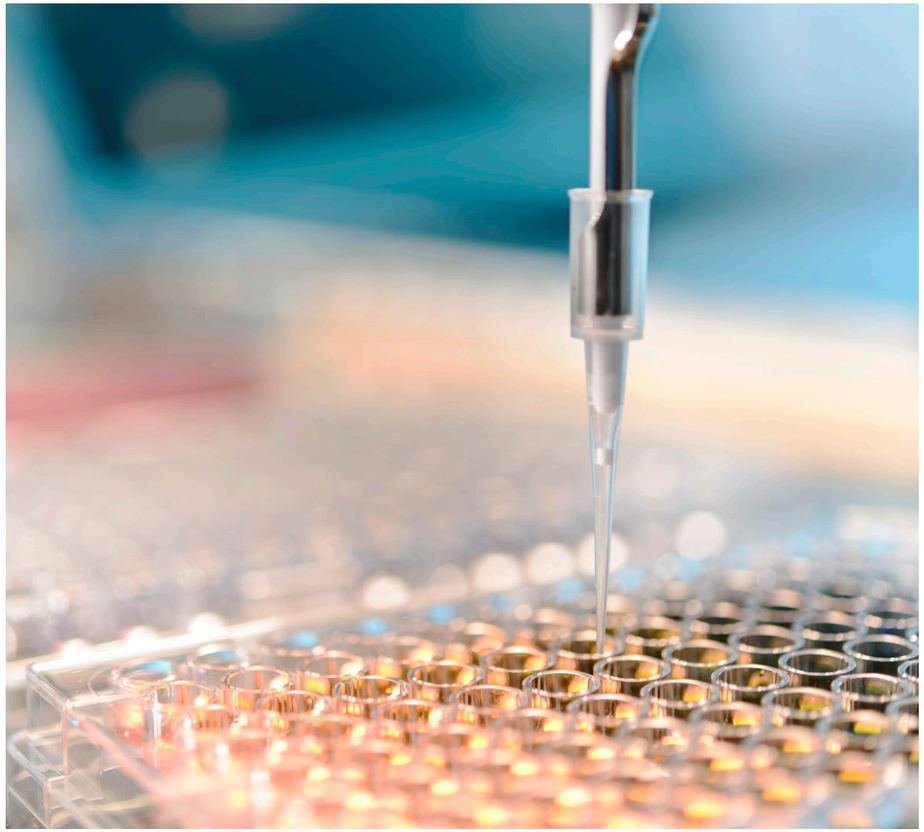
Statistical models for eye irritant classification

NICEATM developed statistical models to classify chemicals as eye irritants/corrosives or nonirritants/corrosives using an in silico approach. This approach differentiated chemicals falling into EPA hazard categories I and II (require eye protection when handling) from chemicals falling into hazard categories III and IV (do not require eye protection).

Skin Irritation

Variability analysis of in vivo skin irritation data

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 confounding factor for such comparisons. To better characterize the reproducibility of the in vivo assay, NICEATM compiled and curated a data set of more than 3,000 in vivo assay test records to assess variability in results from chemicals tested multiple times. The results show that, regardless of the classification system used, the extremes of the classification scales (corrosive or nonirritant) are the most reproducible, whereas substances classified as mild or moderate irritants are more likely to be classified in a different category when tested again. These analyses help provide much needed context not only to assess the reference test method but also to aid in setting expectations for performance of new approach methodologies.



4.2

Tox21

Tox21 is a unique federal collaboration among NIEHS/NTP, the National Center for Advancing Translational Sciences, EPA, and FDA. It develops 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 human health effects. Bioinformatics and computational toxicology support for Tox21 is provided by Tox21 members.





Test Method	Details
Using an in vitro pipeline to assess population toxicodynamic variability for chemicals suspected to cause developmental neurotoxicity	▪ Developed a computational and cellular testing framework for assessing genetic susceptibility to chemical agents suspected of causing developmental neurotoxicity ▪ Used approximately 200 diversity outbred mouse neural progenitor cell lines exposed to a chemical test battery ▪ Assessed cellular effect potency using high-content imaging techniques
Evaluating expansion of pathway coverage by Tox21 qHTS assays for better prediction of adverse effects from exposures	▪ Improved the prediction of adverse effects from exposures to drugs and chemicals ▪ Used additional assays to probe toxicologically important targets and pathways not captured in current Tox21 testing
Retrofitting existing Tox21 high-throughput screening assays with metabolic capability	▪ Refined existing methods to: • Imbue Tox21 assays with metabolic capability • 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 • Identify the particular cytochromes P450 responsible for observable shifts in bioactivity
Selecting cell lines for Tox21 screening	▪ Developed a data-driven approach to choose cell lines to maximize biological diversity ▪ Picked a diverse set of cell types based on publicly available gene expression data and baseline gene expression profiling

4.3

Validation and Adoption of NAMs

ICCVAM coordinated recent projects that provide U.S. federal agencies and stakeholders with new nonanimal approaches to safety and risk assessment of chemicals and medical products.

In vitro testing of agrochemical formulations

NICEATM, PETA-ISC, 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 in 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. Analysis of the data from these phases will determine the next steps of the study.

Optimization and validation of an in vitro botulinum neurotoxin assay

Tests to detect and measure botulinum neurotoxins are required by multiple federal agencies for a variety of purposes, such as measuring toxins in drug formulations or detecting toxins in possibly contaminated food or wildlife. Currently, the standard test for these endpoints is a mouse lethality assay that may use a large number of animals. NICEATM supported the optimization and validation of enzyme-linked immunosorbent assay that replace animal-based methods for diagnosing suspected avian botulism samples. Methods were developed for determining the presence or absence of botulinum neurotoxin serotypes C, D, and E in field-collected samples from a wide range of bird species. These methods may be used in the future to support testing requirements for the U.S. Geological Survey's National Wildlife Health Center.

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. For more information, see ICCVAM test method submissions.

Test Method Evaluations

Electrophilic allergen screening assay

ICCVAM Recommendations/Agency Status: This test method, nominated by the National Institute for Occupational Safety and Health, is an in chemico assay intended to identify skin sensitizers.

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.

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 accessibility. Planned testing of 20 chemicals using the 96-well assay has been delayed due to lab closures during the COVID-19 pandemic but is expected to be completed in FY 2021.

OptiSafe

ICCVAM Recommendations/Agency Status: 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. The study was completed in FY 2018, and a report on the study was published in FY 2020 (Choksi et al. 2020).

The study demonstrated that the OptiSafe test method is useful for identifying nonsurfactant substances not requiring classification for ocular irritancy and thus can reduce the use of animals for this type of testing.

EpiAirway™

ICCVAM Recommendations/Agency Status: 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 toxicity of inhaled chemicals. Testing of reference chemicals to determine the usefulness and limitations of EpiAirway for this purpose has been completed. Several ICCVAM agency representatives are members of the cooperative agreement steering committee.

ICCVAM International Validation Activities

NICEATM and ICCVAM participate in international test method validation activities through 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.

In FY 2020, ICCVAM members and NICEATM staff supported the OECD Test Guidelines Programme during FY 2020 by:

- Serving on an expert group developing a guideline for defined approaches for skin sensitization, with respect to hazard identification or potency subcategorization;
- Serving on expert groups reviewing various guidelines related to skin sensitization and skin and eye irritation;
- 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; and
- Contributing to a retrospective review of available information to support a draft OECD test guideline for a reconstructed human epidermis model for phototoxicity testing.

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 2019, representatives of NICEATM and ICCVAM attended a workshop to celebrate ICATM's 10th anniversary. Workshop participants discussed the future of alternatives in the different jurisdictions represented by ICATM partners. Representatives from a range of European Union regulatory agencies also attended the workshop and shared their perspectives on the role of alternatives in future activities. At the subsequent coordination meeting, attendees discussed ways to move forward with new validation approaches. Specifically, they discussed the need to move away from validating protocols and toward setting performance standards.

ICATM partners are also collaborating with NICEATM and the Physicians Committee for Responsible Medicine on a paper summarizing the acute systemic toxicity testing needs of regulatory authorities in the countries and regions represented by ICATM.

In FY 2020, ICCVAM and NICEATM scientists participated in two validation studies coordinated by the Japanese Center for the Validation of Alternative Methods:

- ICCVAM agency scientists served on the peer-review panel for a study evaluating the use of the LbL-3D Skin SIT in classifying skin irritants. The study will help ensure that the LbL-3D Skin SIT meets performance standards described in OECD Guidance Document 220 for reconstructed human epidermis test methods.
- A NICEATM scientist served on the management team for a validation study for the multi-immunotox screening assay, which identifies immunotoxic chemicals by assaying the activity of T cells and dendritic cells.

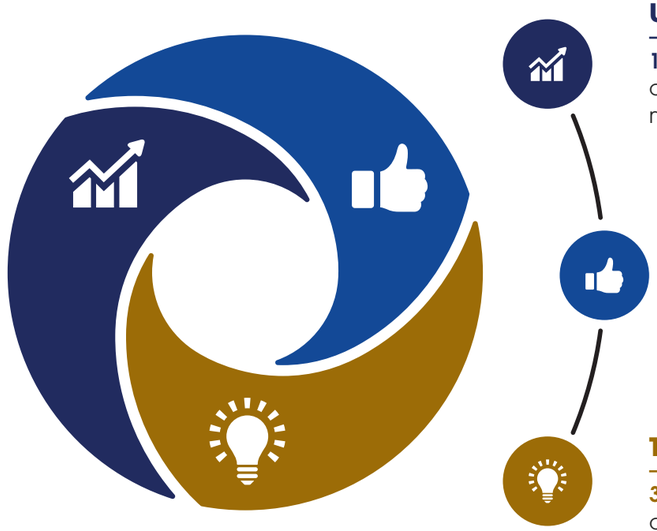
In addition, a NICEATM scientist served on the peer-review panel for a validation study coordinated by the Korean Center for the Validation of Alternative Methods. The study is evaluating the use of KeraSkin™ SIT, an in-vitro skin irritation test method, to ensure that it meets performance standards described in OECD Guidance Document 220.



Progress toward Strategic Roadmap Goals

The Interagency Coordinating Committee on the Validation of Alternative Methods (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 for enabling technologies and promoting 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 to address three strategic goals:



UTILIZATION

1. Connect users with developers of new approach methodologies.

CONFIDENCE

2. Foster the use of efficient, flexible, and robust practices to establish confidence in new methods.

TECHNOLOGY

3. Encourage the adoption and use of new methods and approaches by federal agencies and regulated industries.

The NTP website describes activities underway to address roadmap goals in the following areas:

- Acute systemic toxicity
- Skin and eye irritation
- Skin sensitization

Measuring Progress in Adopting Alternatives and Reducing Animal Use

In response to a request by Congress, the Government Accountability Office (GAO) investigated how federal agencies ensure that alternative methods are being considered and used. In its report, the GAO confirmed that federal agencies actively promote the use of alternative methods; however, the GAO also found that federal agencies have not routinely developed or reported metrics that demonstrate how their efforts to encourage the use of alternative methods affect animal use. The report recommended that ICCVAM establish a workgroup to develop metrics that member agencies could use to help reduce, refine, or replace animal use in testing. Furthermore, the report recommended that such metrics be incorporated into ICCVAM biennial progress reports.

In response, ICCVAM established its Metrics Workgroup in FY 2020. The workgroup includes members from nine ICCVAM agencies and receives support from NICEATM. The workgroup is currently developing recommendations on how member agencies can determine, compile, and report metrics that track progress in reducing animal use.



5.0 Testing and Assessment

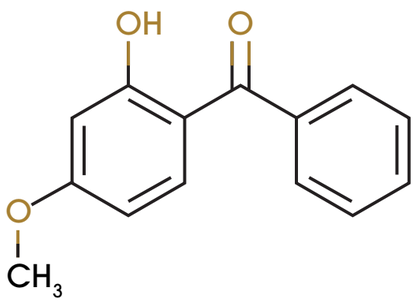
The National Toxicology Program (NTP) evaluates substances and circumstances for a variety of cancer and noncancer health-related effects using well-established rodent models and study protocols as well as new, nonanimal methods. In addition, NTP evaluates scientific literature to identify conditions that could pose a hazard to human health.



HIGHLIGHT

Sunscreen/Ultraviolet Filters

Millions of people use sunscreen lotions, creams, and sprays on their skin to prevent sunburn and resulting skin damage. Ingredients in these products, known as ultraviolet (UV) filters, are added to sunscreens to absorb or block UV radiation from the sun. UV filters have been found in urine, indicating they make their way into the body.



Chemical structure of 2H4MBP

NTP Studies

NTP is assessing the safety of UV filters utilizing a variety of model systems. In FY 2020, NTP reported findings from its long-term studies on 2-hydroxy-4-methoxybenzophenone (2H4MBP)—one of the most common UV filters—to test the toxicity and carcinogenicity of chronic exposure to this chemical in rats and mice. Also included were studies that characterized endocrine activity. NTP concluded that 2H4MBP had no carcinogenic activity in mice and the findings in rats were equivocal, or uncertain, as to whether they were due to the 2H4MBP. NTP could not conclude if 2H4MBP alters endocrine activity.

What's next?

NTP is assessing and documenting the reproductive and developmental toxicity data on ethylhexylmetoxycinnimate, another UV filter. It is also working on a report summarizing cell-based and short-term animal studies conducted on additional UV filters: avobenzone, ensulizole, homosalate, padimate-o, octylmethoxycinnamate, octylsalate, and octocrylene.



5.1

NTP at NIEHS/DNTP

Scientists with the Division of NTP (DNTP) at the National Institute of Environmental Health Sciences (NIEHS) use a variety of approaches to better understand how factors in our environment may impact our health.

5.1.1 Chemical Screening

NIEHS/DNTP scientists apply tools of modern toxicology to screen individual chemicals or chemical classes and evaluate their effects on human health. Results from these approaches can be used to prioritize testing of hazardous chemicals or documented in published literature to help make public health decisions. See NIEHS/DNTP Investigative Projects in Appendix III for a complete list of chemical screening project summaries in FY 2020.



Assessing cardiovascular liabilities associated with HIV therapeutics

Janine Santos

Combination HIV therapeutics are important in HIV treatment and prophylaxis. The health effects of these therapeutics on early development are being investigated as are longer-term effects that extend into adulthood. Cardiovascular outcomes and molecular changes are being studied in the offspring of pregnant rodents exposed to combination HIV therapeutics. Appropriate in vitro cardiovascular assays will be developed from this work for use in rapid screening of these and other chemicals of NTP interest.

In vitro toxicity screening of aqueous film-forming foams

Stephen Ferguson

In vitro screening efforts are being conducted to evaluate qualified aqueous film-forming foam products and comparator per- and polyfluoroalkyl substances (PFAS) constituents to reference chemicals for binning and estimations of liver injury potential through benchmark concentration modeling.

Study of PFOA and GenX exposures on developmental and reproductive effects in mice

Suzanne Fenton

Perfluorooctanoic acid (PFOA) is a PFAS associated with adverse pregnancy outcomes in mice and humans. The potential for developmental and reproductive effects of two widely detected PFAS contaminants in the environment—GenX (ammonium perfluoro-2-proposypropionate) and PFOS (perfluorooctanesulfonate)—are being studied in pregnant mice and their offspring.

Toxicogenomic Studies

Toxicogenomics involves the use of advanced genomic technologies to solve problems in toxicology and to gain insights into the effects of chemical exposures on human health. Over the last two decades, toxicogenomics has grown from microarrays or "gene chips" to now include next-generation sequencing and metabolomic and proteomic analyses to determine adverse chemical effects and better understand an organism's response to environmental toxicants.

Measuring the entire genetic information of a cell or organism is daunting, but advances in technology have allowed production of genetic information to increase in speed and breadth at a rapid pace. NTP uses in vitro models with animal and human cells to rapidly screen environmental chemicals for toxicity and to better understand the mechanism of how chemical toxicity occurs. Animal models are used when needed to relate chemical exposure to toxicity and then extrapolate these findings to chemical exposure conditions that the public might face in the environment or workplace.

NTP continues to conduct chemical exposure experiments that affect differential genetic expression, animal and tissue variability, methods for tissue sampling, and standardized conditions for conducting toxicogenomic studies under high-quality laboratory conditions. Planned or ongoing NTP toxicogenomic studies from FY 2020 are listed below.

In vitro screen for developmental neurotoxicants

Alison Harrill

Chemicals that produce developmental neurotoxicity within early life exposure pose a challenge for in vitro test batteries that aim to predict human health effects and adult disease. One approach to address this issue is to use a genetically diverse mouse model called the DO diversity outbred, which simulates the complex genetics of people. Neural progenitor cells, as early nerve cells, were isolated from DO mice for screening with six known developmental neurotoxicants to find benchmark concentrations at which molecular transcript changes occur relative to toxicity observed using cell-based changes. The program hopes to find genes that are activated prior to traditional cell-based measures of toxicity and thereby help identify the underlying mechanisms of neurotoxicants and more effectively screen for developmental toxicity.

Tracking genetic changes in liver by hepatocarcinogens

Julie Foley, B. Alex Merrick

Aflatoxin B1 is a widespread liver cancer-causing chemical (hepatocarcinogen) that is a byproduct of molds that occur on grains, corn, peanuts, and many other foods consumed by humans and animals. After absorption, the liver can metabolize aflatoxin into DNA-damaging forms, causing mutations that, over time, can lead to liver cancer. Rats fed an experimental diet containing aflatoxin developed liver cancer. DNA sequenced by a specialized method called Exome-Seq was used to target the most critical regions of DNA to find mutations that might lead to cancer.

Extrapolation in vitro high-throughput screening data to animal toxicity

William Gwinn, Nancy Urbano

The parallelogram approach to toxicity assessment involves extrapolation of in vitro data to an in vivo model that accounts for interspecies variation and exposure levels between animal models and humans. Studies are underway to evaluate high-throughput transcriptomic in vitro data from human and animal liver spheroid cell models to establish in vitro dose-response relationships to in vivo rat toxicity data and use the ICE database in in vivo extrapolation for estimation of human chemical exposure and potential toxicity.

5.1.2. Chemical Testing

The NTP testing program evaluates substances for 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. See Appendix II for a complete list of testing and toxicology studies completed or in progress in FY 2020. Testing and toxicology studies with results published in FY 2020 are included in Appendix I.

5.1.3 Noncancer Research

NIEHS/DNTP scientists conduct literature-based evaluations using systematic review methods to assess the evidence that environmental substances may be associated with adverse, noncancer health effects. NTP provides opinions on whether these substances might be of concern because of what is known about current human exposure levels.

In FY 2020, three scoping reviews were published (see Appendix I) that determine the extent and limitations of the evidence base on challenging scientific topics to inform decisions on future research or analyses. These reviews apply transparent systematic review methods and present interactive evidence maps that advance scientific knowledge about the extent of the literature and enable researchers to explore the relevant health effects data that are identified. See Appendix III for a complete list of noncancer literature analysis projects in FY 2020.

Highlights

Exploring informatics and semi-automation in analysis workflow

Vickie Walker, Andrew Rooney

NIEHS/DNTP is implementing novel informatics methods to the literature-evaluation process by developing, refining, or adapting machine-learning and other informatics approaches to its project workflow and visualizations. Current efforts are focused on the labor-intensive literature screening steps (i.e., identifying relevant studies among tens of thousands of relevant publications) and data extraction, including capturing key study details, such as the exposure or chemical, experimental animal species, and health outcomes studied. An innovative semi-automated method for data extraction is under development and testing.

Personal care products: An example of evaluations of broad classes of exposures

Kyla Taylor, Kembra Howdeshell

The use of personal care products (e.g., soaps, lotions, cosmetics, and fragrances) is an important close-contact exposure to environmental chemicals, especially for susceptible life stages, including pregnant women and children. Two scoping reviews are underway to determine and categorize the evidence of associations between the wide range of chemicals found in personal care products or patterns of personal care product use and key reproductive outcomes. These reviews will serve as a foundational step in informing more in-depth analysis, such as selecting a chemical or subset of chemicals with more evidence or more widespread exposure, as well as determining areas of uncertainty that could be targets for further research.

Scoping reviews to support evidence-based research and analysis decisions on disease-focused environmental health questions

Brandy Beverly, Vickie Walker

As part of NIEHS/DNTP's approach to developing fit-for-purpose literature-based evaluations, broad-based scoping reviews are being conducted that examine potential environmental factors on human diseases (e.g., hypertensive disorders of pregnancy and cardiovascular disease). The goals of these evaluations are to identify published literature on environmental exposures associated with cardiovascular toxicity and cardiovascular effects and to develop an interactive evidence map that is a foundation for evidence-based decisions and informs future projects and researchers within and outside NIEHS/DNTP.

5.1.4 Cancer Health Effects Research

Identifying cancer hazards: Report on Carcinogens

The Report on Carcinogens (RoC) is a congressionally mandated, science-based, public health report that provides a cumulative list of known or reasonably anticipated human carcinogens. As the identification of carcinogens is a key step in cancer prevention, publication of the report is important to improve public health. Substances are listed in the report after a multi-step process using established listing criteria, with several opportunities for scientific and public input.

NTP has completed the reviews of eight substances for potential listing in the 15th RoC, and the report is in the fourth step of the RoC process to seek the Department of Health and Human Services Secretary's approval of new listings and release the report.

Evaluating cancer hazards: Scoping, evidence mapping, literature-based reviews, and studies

NTP conducts an extensive assessment of publicly available, relevant cancer studies to determine whether a substance should be recommended for listing in the RoC. The assessment is captured in an RoC monograph. NTP may also conduct cancer hazard evaluations that are independent of the RoC. These literature-based assessments use systematic review methods that integrate the relevant evidence across many different types of studies to reach conclusions about whether a substance is a cancer hazard.

NIEHS/DNTP has completed scoping and problem formulation activities and is conducting hazard assessments for several substances. See Appendix III for a complete listing of cancer literature analysis projects in FY 2020.

Highlights

Evaluating evidence for the carcinogenicity of polycyclic aromatic hydrocarbons and other types of polycyclic aromatic compounds

Polycyclic aromatic compounds (PACs) are a structurally diverse group of ubiquitous contaminants that exist in complex and dynamic mixtures and are often formed as a result of incomplete combustion or organic matter. Polycyclic aromatic hydrocarbons (PAHs) are PACs with carbon- and hydrogen-only chemicals with at least two aromatic rings in the fused part. Nitro-PAHs are PACs with at least one or more nitro-functional groups attached to a PAH. People are exposed to mixtures of PACs from multiple sources, including through certain occupations and from foods, tobacco smoke, and contaminated air.

NIEHS/DNTP is conducting research on PAHs and PACs, including an epidemiological study, evidence mapping and scoping activities, and literature-based cancer hazard evaluations. Some projects are evaluating PAHs mixtures (e.g., the epidemiological study and woodsmoke evidence mapping and literature-based cancer hazard evaluations), whereas others are evaluating classes, subclasses, or individual PACs (e.g., PAH and nitro-PAH literature-based cancer hazard evaluations). These projects complement other cross-DNTP projects on PAHs and PACs.

Urinary PAH metabolites and mortality in the United States: A prospective analysis

Suril Mehta

PAHs are associated with chronic disease in epidemiologic studies, although the contribution of PAH exposure on fatal outcomes in the United States is largely unknown. NIEHS/DNTP and other collaborators conducted a prospective epidemiological analysis of urinary hydroxylated PAH metabolites (OH-PAHs) with all-cause, cancer-specific, and cardiovascular-specific deaths in a representative sample (i.e., National Health and Nutrition Examination Survey 2001–2014) of the U.S. population. Mortality was ascertained using the National Death Index, followed up to 2015. Metabolites of commonly detected PAHs (pyrene, phenanthrene, fluorene, naphthalene) were evaluated as aggregate exposure and complex mixtures. NIEHS/DNTP presented the study's findings at the 32nd Annual Conference of the International Society of Environmental Epidemiologists.

Evidence mapping and cancer hazard evaluation of woodsmoke

Ruth Lunn

More than 2 million U.S. households use wood as a primary heat fuel, and there is an emerging concern about exposure to woodsmoke from increasing numbers of wildfires. Because it is a natural substance, which is often perceived as safe, there may be a disconnect between the public's perception and that of the scientific community on the health effects of woodsmoke.

To address and translate these concerns, NIEHS/DNTP is developing an evidence map and conducting a cancer hazard evaluation of woodsmoke and will communicate its findings about hazard concerns.

NIEHS/DNTP scientists completed the literature scoping and have largely completed a detailed evidence map of human cancer and mechanistic studies. (The protocol and cancer hazard evaluations are under development).

PAHs

Amy Wang

Among hundreds of PAHs, only 16 are commonly monitored for exposure assessment and 13 are listed in the RoC. NIEHS/DNTP has identified more than 250 animal studies on at least 35 PAHs. However, only a small number of these have an adequate database of studies for carcinogen classification (e.g., multiple species or tumor sites). An expanded database of PAHs of varying toxicities would provide an opportunity for increased use of structure- and bioactivity-based read across for toxicity and carcinogenicity prediction.

To determine a more comprehensive list of carcinogenic PAHs, NIEHS/DNTP is conducting a cancer hazard evaluation that includes assessment of cancer studies in animal and human studies and mechanistic data and is using computational models to predict carcinogenicity of PAHs (individual, class, subclass). NIEHS/DNTP completed a scoping report of the human and animal studies and presented the results at the 59th Annual Society of Toxicology Meeting in 2020. It also completed a state of the science review of the human epidemiological studies of PAHs and breast cancer and presented the results at the 32nd Annual Conference of the International Society of Environmental Epidemiologists. The protocol, cancer hazard assessment, and computational models are under development.

Nitro-PAHs

Gloria Jahnke

Nitro-polycyclic aromatic hydrocarbons (NPAHs) pose public health problems due to widespread exposure. Information on specific or classes of NPAHs is important for monitoring ways to decrease exposure and prevent health effects. Five NPAHs are listed in the RoC; however, new cancer data related to other NPAHs are now available. To identify cancer hazards for approximately 100 NPAHs, NIEHS/DNTP is conducting an evaluation that includes an assessment of animal cancer data and uses physio-chemical properties and mechanistic data to potentially group NPAH as a class or subclass(es).

NIEHS/DNTP has completed the scoping and study quality assessment of the animal cancer studies; the protocol and cancer hazard assessment are under development.



5.2

NTP at FDA/NCTR

The National Center for Toxicological Research (NCTR) plays a critical role in the missions of the Food and Drug Administration (FDA) and the Department of Health and Human Services to promote and protect public health. NCTR collaborates with researchers from elsewhere in FDA, other government agencies, academia, and industry to provide innovative technology, methods development, vital scientific training, technical expertise, and data generation for FDA decision-making. The unique scientific expertise and infrastructure of NCTR is critical in supporting FDA product centers and their regulatory roles. In 1979, the FDA Commissioner designated NCTR as FDA's principal partner to NTP, enabling a science-based interface between FDA's regulatory mission and NTP's research mission. Appendix III provides brief summaries of NTP projects completed or underway at FDA/NCTR in FY 2020.



Highlights

Research	Objectives
Somatic oncomutations as biomarkers for translating preclinical safety data to human cancer risk *Barbara Parsons*	▪ Develop a method for error-corrected sequencing of amplicons enriched in somatic hotspot cancer-driver mutations ▪ Establish how the data on cancer-driver mutation levels from humans, rats, and mice can be used to derive a metric of clonal expansion, predict future cancer risk, and extrapolate from sub-chronic, repeat-dose rodent studies to human cancer risk
Investigation of opioid-induced neural tube defects in a mouse model *Amy Inselman*	▪ Investigate the effects of morphine and methadone exposure on neural tube development when administered to mice during early pregnancy ▪ Focus on maternal toxicity, specifically hypoxia as a contributing factor to neural tube defect development ▪ Assess incidence rate of neural tube defects, fetal and maternal drug levels, maternal clinical blood gas parameters, and measurement of uterine artery blood flow
Enhance prediction of potential endocrine activity of chemicals by integrating multiple endpoints data *Huixiao Hong*	▪ Develop models integrating diverse endocrine-related endpoints for chemicals, including: • Binding activity of estrogen receptors alpha and beta • Androgen receptor • Rat alpha-fetoprotein • Human sex hormone binding globulin • Agonistic and antagonistic activity of estrogen receptor alpha
Assessing the developmental neurotoxicity of cannabidiol exposure in Sprague Dawley rats *Tim Flanigan*	▪ Quantitatively assess the neurotoxicity associated with developmental exposure to cannabidiol in rats ▪ Perform behavioral assessments, including measures of complex cognition, neuropathological and neurochemical endpoints, among many others
Evaluation of an in vitro testis organ system as an alternative model for male reproductive toxicology *Noriko Nakamura*	▪ Develop an in vitro model to determine the effects of drugs and chemicals on spermatogenesis ▪ Investigate whether the in vitro testes culture system can produce sperm and is responsive to known male reproductive toxicants, as assessed by steroid levels, immunohistochemistry, and metabolomic markers of injury

5.3

NTP at NIOSH

Occupationally Relevant Exposure Research: Overview of the NTP Program

Approximately 84,000 chemical compounds are found in the workplace, and thousands of new chemicals are introduced each year. Research at the National Institute for Occupational Safety and Health (NIOSH) is primarily driven by the National Occupational Research Agenda. Priorities for this research agenda are influenced by

- The number of workers at risk for a particular injury or illness,
- The seriousness of a hazard or problem, and
- The chance that new data or approaches can make a difference.

Through a set of exposure assessment studies, NIOSH characterizes inhalation and dermal exposures to high-priority chemicals at workplaces in the United States and determines the approximate number of workers exposed to these chemicals. Appendix III provides brief summaries of NTP projects completed or underway at CDC/NIOSH in FY 2020.

Immunotoxicology Research to Study Fungal Exposures

Inhalation exposure and associated health effects of aerosols, particularly those derived from biological sources such as fungi, continues to cause concern across the United States. This exposure is of particular concern in occupational environments (e.g., agriculture, forestry, service, manufacturing) where exposure can be significantly elevated and can lead to respiratory health effects, including allergic rhinitis, asthma, and hypersensitivity pneumonitis. The National Academy of Sciences (NAS) Committee on Damp Indoor Spaces and Health and the World Health Organization (WHO) have published consensus reports that identified associations between exposure to damp indoor environments, microbial growth, and adverse respiratory health effects and have called for additional research studies.

Because of the public health burden associated with fungal exposures, NIEHS and the NIOSH Health Hazard Evaluation Program frequently receive requests for information on the subject. Despite progress made in understanding health impacts, several knowledge gaps remain, including:

- The assemblage of fungi that contribute to personal exposure and the mechanisms by which fungi modulate adverse health effects, such as respiratory morbidity
- The effect of repeated fungal exposure on neurological and cardiac systemic effects

In 2019, an interagency agreement between NIEHS and NIOSH supported toxicological evaluations of potential health effects in humans exposed to quantifiable concentrations of fungal bioaerosols and other occupationally relevant aerosols encountered in the built environment or workplace. The agreement focused on two areas of study:

- The development of improved methodologies to detect microbial sources and potential biomarkers of personal and occupational exposure
- Pulmonary immunology and fungal toxicology studies nominated to the NTP in 2004

Projects directly address the knowledge gaps highlighted in the NAS and WHO reports. They also support the development of innovative exposure assessment methodologies to improve species-specific fungal hazard identification and better characterize the health effects associated with sub-chronic fungal exposures. The knowledge gained from these projects will assist in developing improved testing methods for the quantification of environmental fungi, as well as generate data that will strengthen the scientific base for fungal risk assessment.

Recent projects include the following:

- An Acoustical Generator System developed by NIOSH for these studies more accurately models the natural exposure a worker would encounter in a fungal-contaminated occupational environment.
- Studies to examine toxicological responses in rodents to repeated Aspergillus fumigatus exposure and repeated Stachybotrys chartarum exposure have been completed and data analysis is underway toward development of final reports for publication.



HIGHLIGHT

Flame Retardants

Flame retardants are chemicals that are applied to materials to prevent the start or slow the growth of fire. These chemicals have been used in many consumer and industrial products since the 1970s to decrease the ability of materials to ignite.

Flame retardants are often added or applied to furnishings, electronics, building and construction materials, and transportation products.

Many flame retardants have been removed from the market or are no longer produced. The most common type, polybrominated diphenyl ethers (PBDEs), were phased out in or before 2013 due to evidence of liver and thyroid effects, developmental changes, and neurotoxicity in humans. However, because they do not break down easily, these chemicals can remain in the environment for years. They can also build up in people and animals over time, a process known as bioaccumulation.

NTP Study on Occupational Exposure

In FY 2020, NIOSH conducted a comprehensive assessment that measured workers' exposures to PBDEs, organophosphate, and non-PBDE brominated flame retardants in the following industries and settings:

- Carpet installation
- Chemical manufacturing
- Electronic scrap
- Foam manufacturing
- Gymnastics studios
- Nail salons
- Rigid polyiso board installation
- Roofing
- Spray polyurethane foam installation

The exposure assessment included workers' personal breathing zone, hand-wipe sampling, and urine and serum collection.

Findings

Using results from the study, peer-reviewed journal articles were published on (1) polyurethane spray foam worker exposures to tris(1-chloro-2-propyl) phosphate (TCPP), (2) nail technicians' exposures to triphenyl phosphate (TPhP), and (3) all-industries, air- and hand-wipe sampling results and comparisons.

The study's results showed high TCPP exposure in spray polyurethane foam installation, foam manufacturing, chemical manufacturing, roofing, and rigid board installation relative to exposure in other industries. TPhP air concentrations were very high for chemical manufacturing workers compared with other industries. PBDE exposures were present among carpet installers and electronic scrap workers and in gymnastics studios, but they were lower than in previous studies as a result of the phaseout of PBDEs.





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