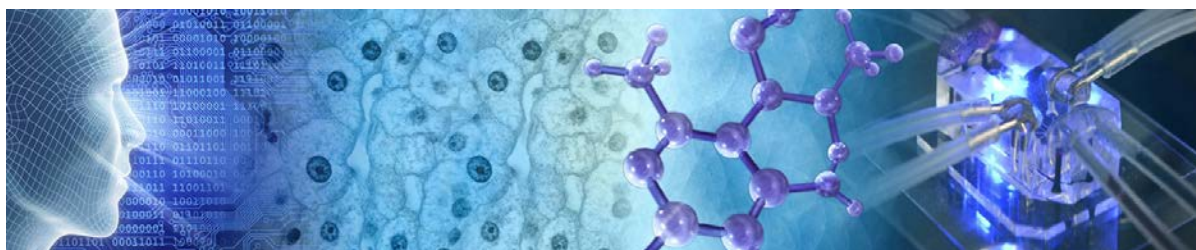# Biennial Progress Report 2022-2023

Interagency Coordinating Committee on the

Validation of Alternative Methods

September 2024

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About this Report

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) facilitates the development, validation, and regulatory acceptance of test methods that replace, reduce, or refine the use of animals in testing.

The ICCVAM Authorization Act of 2000 directed ICCVAM to prepare a progress report on its first anniversary and biennially thereafter. This 2022-2023 biennial report summarizes progress by ICCVAM agencies toward encouraging the development, utilization, and building of confidence in new methods as set forth in the 2018 ICCVAM report, A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States. The text is not copyrighted and can be reprinted without permission. If you use any part of the Biennial Report in a publication, please provide NICEATM a copy for our records.

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Send comments or suggestions to NICEATM

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Message from NIEHS and NTP

We are pleased to share with you the many accomplishments of the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), and the 17 ICCVAM agencies in the 2022-2023 Biennial Report. These accomplishments address needs and priorities identified in the "Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States."

One of the three goals stated in the Strategic Roadmap is to "foster the use of efficient, flexible, and robust practices to establish confidence in new methods." The ICCVAM Validation Workgroup, with participation from 10 of the 17 ICCVAM member agencies, provided an important resource supporting that goal with publication of its report, "Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies." In addition, publications coauthored by members of the workgroup informed the criteria and processes described in that report, including approaches for quality management (Petersen et al. 2023) and establishing confidence (van der Zalm et al. 2022) in new approach methodologies (NAMs). With these publications, ICCVAM assumed a central role in establishing criteria for the appropriate evaluation of NAMs.

To address specific testing needs in 2022-2023, NICEATM and ICCVAM continued to engage in collaborative efforts to replace, reduce, and refine animal use for acute toxicity testing. This report describes progress in replacement of animal use for skin sensitization testing, with information about the largest existing database of human reference data for skin sensitization and about broadening applicability of defined approaches for skin sensitization. Collaborations among ICCVAM agencies and their stakeholders produced computational models that can predict a variety of toxicities and demonstrated how these models can be useful in a regulatory context. An important example is the application of the Collaborative Acute Toxicity Modeling Suite to predict toxicity of pesticide active ingredients. Member agencies also conducted retrospective data analyses to identify opportunities to reduce the number of species needed for regulatory testing, which can have immediate impact in reducing animal use while maintaining public health protection.

A key area of the Strategic Roadmap is communication. During 2022-2023, NICEATM and ICCVAM continued to effectively connect with stakeholders, as demonstrated by the high attendance and strong participation at ICCVAM-sponsored virtual public events including the Communities of Practice webinars and the Public Forums. Increased interest in engaging with ICCVAM prompted the need for a forum specifically targeted to method developers, which will launch in 2024 and operationalize key concepts from the ICCVAM Validation Workgroup report. In addition, NICEATM and ICCVAM agencies such as the U.S. Environmental Protection Agency and the U.S. Food and Drug Administration sponsored virtual events to educate and engage stakeholders on progress toward the validation, acceptance, and use of alternatives to animal testing. Details and outcomes of many of these events are described herein.

The importance and relevance of ICCVAM's work is reflected in its continued growth as an interagency committee. Established in 2000 with 15 member agencies, ICCVAM grew to 18 member agencies in 2024 with addition of the National Center for Advancing Translational Sciences (NCATS). NCATS plays a central role in Tox21 and other U.S. federal efforts to advance alternatives to animal testing and has made valuable contributions to ICCVAM workgroups. We welcome their broader participation in ICCVAM's activities as an ICCVAM member.

The activities in this 2022-2023 Biennial Report illustrate the outstanding contributions of ICCVAM to advance alternative methodologies and the 3Rs. We invite you to read this report to learn about all that ICCVAM has been accomplished in the past two years.

Richard Woychik, Ph.D.
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Message from NICEATM and ICCVAM

On behalf of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), we are pleased to present the 2022-2023 ICCVAM Biennial Progress Report.

The acceptance and application of new approach methodologies (NAMs) that can reduce and replace animal use while improving public health protection require multifaceted efforts in scientific innovation, development of new approaches to validation, and critical evaluation of current test methods. This report describes how ICCVAM agencies are actively engaged in all these activities. The ICCVAM Validation Workgroup recently published "[Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies](#)," which we hope will become a key resource for the evaluation of NAMs. Now that this document is available, ICCVAM members are supporting international application of these principles by [contributing to the update of relevant guidance documents issued by the Organisation for Economic Co-operation and Development](#). Evaluation of current in vivo test methods is critical to ensuring that appropriate benchmarks are available for the validation of NAMs. This report describes efforts by NICEATM and ICCVAM to [appropriately characterize the limitations of current test methods](#) and sources of variability in their data.

NAMs produce data that can sometimes be challenging to interpret in a regulatory context, or that may rely upon complex computational models to produce information to support regulatory decisions. Over the past two years, ICCVAM, NICEATM, and ICCVAM agencies have worked together and with stakeholders to help make data readily available, characterize data quality appropriately, and provide tools to enable stakeholders to better understand those data. NICEATM has made improvements to the [Open (Quantitative) Structure-activity/property Relationship App](#) (OPERA) and the [Integrated Chemical Environment](#) (ICE) in response to stakeholder needs, and developed tools such as [ChemMaps](#), [STopTox](#), and [DASS App](#) to help stakeholders to explore and apply these data. Similarly, ICCVAM member agencies such as the U.S. Environmental Protection Agency and the U.S. Department of the Interior have developed computational tools tailored to the needs of their stakeholders and offered training to encourage their use.

ICCVAM agencies recognize the importance of communicating information needs and testing requirements to stakeholders, and over the past two years they have used different approaches to facilitate that communication. Five regulatory ICCVAM member agencies made or are making improvements to their webpages that discuss their testing requirements and options for alternatives to animal use. ICCVAM members contributed to publications that describe information needs for ecotoxicity and nanomaterials testing, and the newly formed ICCVAM PFAS Workgroup is considering federal agencies' testing needs specific to per- and polyfluoroalkyl substances. The U.S. Food and Drug Administration (FDA) continued its webinar series that enables test method developers to interact with FDA centers to explore appropriate opportunities for application of new testing methods. They also offered programs for qualification of new development tools for drugs and medical devices. Finally, several ICCVAM agencies presented webinars or webinar series, either independently or in collaboration with other agencies or nongovernmental partners, to explore application of NAMs in a variety of regulatory and nonregulatory contexts.

We would like to acknowledge the contributions of the representatives and interagency workgroup members from the 17 ICCVAM member agencies, NICEATM and its contract staff at Inotiv, members of ICCVAM's advisory committee, and our many other stakeholders. In particular we would like to thank John Gordon, Ph.D., U.S. Consumer Product Safety Commission, and Anna Lowit, Ph.D., U.S. Environmental Protection Agency, for serving as co-chairs of ICCVAM during the 2022-2023 reporting period. We recognize and appreciate their leadership and expertise, which were key to ICCVAM's effectiveness. We thank them for their past contributions and look forward to their continued engagement with ICCVAM and service on ICCVAM workgroups.

The activities summarized in this biennial report exemplify ICCVAM's ongoing commitment to working with U.S. and international partners to advance the development and acceptance of new scientifically valid test methods that will reduce and eventually replace animal use. These engagements, along with those of our many other stakeholders, are vital to ICCVAM, and we look forward to continued progress and effective interactions in the coming years.

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Co-Chair, ICCVAM

Center for Food Safety and Applied Nutrition
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Key NICEATM and ICCVAM Accomplishments and Impact

Tags: EPA, ICCVAM, NIEHS, acute toxicity, cardiotoxicity, defined approaches/IATA, IVIVE, QSAR, skin sensitization

Key accomplishments of ICCVAM and ICCVAM member agencies in support of the ICCVAM mission during 2022 and 2023 include:

- **Activities to support definition of evolving validation and confidence frameworks.** NICEATM and ICCVAM agency scientists published articles describing approaches for quality management (Petersen et al. 2023) and establishing confidence (van der Zalm et al. 2022) in new approach methodologies (NAMs). This work and other ongoing activities informed an update of ICCVAM criteria and processes for validation, qualification, and regulatory acceptance of toxicological test methods.

- **Publication of articles describing agency information needs and testing requirements.** ICCVAM workgroups published journal articles describing U.S. federal agency information needs and testing requirements for nanomaterials and ecotoxicity. An ICCVAM workgroup published an award-winning article describing federal agency application of in vitro to in vivo extrapolation. NICEATM scientists and collaborators also prepared a review of acute systemic toxicity testing requirements for jurisdictions that participate in the International Cooperation on Alternative Test Methods (ICATM).

- **Curation and publication of human skin sensitization database.** ICCVAM agencies and NICEATM collaborated with the German Federal Institute of Risk Assessment to compile the largest existing database of human reference data for skin sensitization.

- **Broadening applicability of defined approaches for skin sensitization.** Following international acceptance of defined approaches to skin sensitization, ICCVAM agency scientists evaluated use of defined approaches to characterizing sensitization potential of agrochemical formulations and isothiazolinones, a type of preservative used in consumer products.

- **Development of approaches to predict and characterize cardiotoxic potential.**
  National Institute of Environmental Health Sciences (NIEHS) scientists developed
  [assays](#) and [computational models](#) to identify chemicals with the potential to affect the
  cardiovascular system, as well as approaches to [predict the exposure levels](#) that might
  be of concern for this endpoint.

- **Expansion of the Integrated Chemical Environment.** NICEATM created the
  [Integrated Chemical Environment](#) (ICE) to provide curated data and tools for safety
  assessment of chemicals. [Updates to ICE tools](#) during 2022 and 2023 enabled
  searches using chemical names and a broader range of chemical identifiers, allowed
  users to upload their own data for modeling or visualization, expanded options for
  exploring chemical properties, and provided the option to model exposure to the
  developing fetus. In addition to updates to specific ICE datasets, [ICE data updates](#)
  facilitated user access to large data sets and added annotations to better characterize
  data quality and biological relevance.

- **Web tools for chemical exploration and toxicity prediction.** In addition to ICE,
  ICCVAM agencies released web tools to enable public access to leading-edge
  technology for chemical exploration and toxicity prediction. These include:

  - A broad range of tools provided by the U.S. Environmental Protection Agency
    for chemical exploration and toxicity prediction, along with a [training
    program](#) to support their use.

  - [STopTox web portal](#) for prediction of acute toxicity.

  - [DASS App](#) for application of defined approaches to predict skin sensitization
    potential.

  - Updates to the [ChemMaps](#) and [OrbiTox](#) tools enabling visual exploration of
    chemical properties.

About ICCVAM and NICEATM

The [ICCVAM Authorization Act of 2000](#) (42 U.S.C. 285l-3) established the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), which is supported by the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). The two groups work collaboratively to evaluate new and improved testing approaches applicable to the needs of U.S. federal agencies. The articles below provide background information on NICEATM and ICCVAM.

ICCVAM Establishment and Purpose
Tags: ICCVAM, NIEHS

U.S. regulatory agencies are charged to protect human and animal health and the environment. To do this, agencies must determine the hazards presented by substances such as pesticides, consumer products, and workplace chemicals. Testing these substances provides information about possible hazards and enables informed decisions about responsible use, storage, and disposal.

Many currently accepted test methods use laboratory animals. Alternative test methods are methods that replace animal use with nonanimal test systems or lower species, reduce the number of animals required for a specific test procedure, or refine animal use to enhance animal well-being and lessen or avoid pain and distress. Collectively, the principles of replacement, reduction, or refinement of animal use for scientific research or product safety testing are referred to as the 3Rs. More recently, the term "new approach methodologies (NAMs)" has been adopted as a broadly descriptive reference to any technology, methodology, approach, or combination of these that can be used to provide information on chemical hazard and risk assessment and that support the 3Rs.

The [ICCVAM Authorization Act of 2000](#) (42 U.S.C. 285*l*-3) established the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) to

> "Establish, wherever feasible, guidelines, recommendations, and regulations that promote the regulatory acceptance of new or revised scientifically valid safety testing methods that protect human and animal health and the

environment while reducing, refining, and replacing animal tests and ensuring human safety and product effectiveness."

The ICCVAM Authorization Act states that the purposes of ICCVAM are to:

- Increase the efficiency and effectiveness of federal agency test method review.

- Eliminate unnecessary duplicative efforts and share experiences between federal regulatory agencies.

- Optimize utilization of scientific expertise outside the federal government.

- Ensure that new and revised test methods are validated to meet the needs of federal agencies.

- Reduce, refine, and replace the use of animals in testing, where feasible.

ICCVAM is a permanent interagency committee of the National Institute of Environmental Health Sciences (NIEHS) under NICEATM.

ICCVAM Member Agencies
Tags: ICCVAM

- Agency for Toxic Substances and Disease Registry (ATSDR)
- National Cancer Institute (NCI)
- National Institute for Occupational Safety and Health (NIOSH)
- National Institute of Environmental Health Sciences (NIEHS)
- National Institute of Standards and Technology (NIST)
- National Institutes of Health (NIH)
- National Library of Medicine (NLM)
- Occupational Safety and Health Administration (OSHA)
- U.S. Consumer Product Safety Commission (CPSC)
- U.S. Department of Agriculture (USDA)
- U.S. Department of Defense (DoD)
- U.S. Department of Energy (DOE)
- U.S. Department of the Interior (DOI)

- U.S. Department of Transportation (DOT)
- U.S. Department of Veterans Affairs Office of Research and Development (VA ORD)
- U.S. Environmental Protection Agency (EPA)
- U.S. Food and Drug Administration (FDA)

ICCVAM Duties and Activities

Tags: ICCVAM

The ICCVAM Authorization Act directs ICCVAM to carry out the following duties:

- Coordinate the technical review and evaluation of new, revised, or alternative test methods.
- Foster interagency and international harmonization of test protocols that encourage replacing, reducing, and refining animal test methods.
- Assist with and provide guidance on validation criteria and processes.
- Promote the acceptance of scientifically valid test methods.
- Promote awareness of accepted test methods.
- Submit ICCVAM test method recommendations to appropriate U.S. federal agencies.
- Consider requests from the public to review and evaluate new, revised, or alternative test methods that have evidence of scientific validity.
- Make ICCVAM's final test recommendations available to the public.
- Prepare reports on ICCVAM progress and accomplishments under the Act and make them available to the public.

ICCVAM Workgroups

Tags: ICCVAM, acute toxicity, ecotoxicity, IVIVE, PFAS, structure similarity

ICCVAM establishes ad hoc workgroups to perform specific tasks important for the development or validation of alternatives to animal testing. The workgroups are composed of representatives from agencies that use or require data from the topic of interest.

Representatives of partner organizations in the [International Cooperation on Alternative Test Methods](#) may also be invited to participate in a workgroup.

ICCVAM workgroups play a key role in carrying out ICCVAM activities, including implementing the goals of the [Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States](#). When a workgroup completes its charge tasks, it transitions to an expert group to facilitate continued communication and engagement in that area of interest.

During 2022-2023, ICCVAM had active workgroups focused on the following areas:

- Acute toxicity: co-chairs Donald Cronce, Ph.D. (DoD) and Grace Patlewicz, Ph.D. (EPA)

- Consideration of alternative methods: co-chairs Jessie Carder (USDA), Matthew Johnson, D.V.M., DACLAM (DoD; through March 2022), and Shannon Marko, D.V.M. (DoD)

- Ecotoxicology: co-chairs William Eckel, Ph.D. (EPA) and Natalia Garcia-Reyero Vinas, Ph.D. (DoD)

- In vitro to in vivo extrapolation: transitioned to expert group status in 2023; co-chairs Moiz Mumtaz, Ph.D. (ATSDR) and Cecilia Tan, Ph.D. (EPA)

- PFAS: co-chairs Natalia Garcia-Reyero Vinas, Ph.D. (DoD), Kelly Carstens, Ph.D. (EPA), and Shruit Kabadi, Ph.D. (FDA)

- Read across: transitioned to expert group status in 2022; chair Grace Patlewicz, Ph.D. (EPA)

- Validation: co-chairs Suzanne Fitzpatrick, Ph.D., D.A.B.T. (FDA), John Gordon, Ph.D. (CPSC), and Elijah Petersen, Ph.D. (NIST)

How NICEATM Supports ICCVAM

Tags: ICCVAM, NIEHS

NICEATM, an office within the NIEHS Division of Translational Toxicology (DTT), provides technical and scientific support for ICCVAM and ICCVAM workgroup activities, peer review panels, expert panels, workshops, and validation efforts.

In addition to providing support for ICCVAM, NICEATM:

- Supports activities of NIEHS DTT, especially those contributing to the U.S. government's interagency Tox21 initiative.

- Conducts analyses and evaluations and coordinates independent validation studies on novel and high-priority alternative testing approaches.

- Provides information, data, and tools to test method developers, regulators, and regulated industry through its website and workshops on topics of interest.

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ICCVAM Advisory Committee

Tags: ICCVAM

The ICCVAM Authorization Act established the [Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)](#). SACATM advises the Director of NIEHS, NICEATM, and ICCVAM about NICEATM and ICCVAM activities.

SACATM, which is directed by its charter to meet at least once each fiscal year, [met in September 2022 and September 2023](#).

Roster of SACATM Members 2022-2023

Name	Title	Company	Appointment End Year
Antonio Baines, Ph.D.	Associate Professor, Department of Biological and Biomedical Sciences	North Carolina Central University, Durham, NC	2025
Szczepan W. Baran, V.M.D., M.S.	Chief Scientific Officer	VeriSIM Life, San Francisco, CA	2024
Ellen Berg, Ph.D.	Principal	Alto Predict LLC, Palo Alto, CA	2025
Joseph L. Charest, Ph.D.	Director, Strategic Innovation	Biogen, Cambridge, MA	2022
Amy Clippinger, Ph.D.	Director	PETA Science Consortium International e.V., Washington, DC	2022
K. Nadira De Abrew, Ph.D. (Chair 2022)	Senior Scientist (Toxicologist)	The Procter & Gamble Company, Cincinnati, OH	2022
Denis Fourches, Ph.D.	Computational Chemist	Oerth Bio, Raleigh, NC	2023

Sean C. Gehen, Ph.D., D.A.B.T.	Human Safety Leader	Corteva Agriscience, Indianapolis, IN	2022
Sue Leary, M.S.	President	Alternatives Research and Development Foundation, Jenkintown, PA	2024
Adrian Nañez, Ph.D.	Senior Medical Science Liaison	Servier, Inc., San Antonio, TX	2025
Kathryn E. Page, Ph.D., D.A.B.T. (Chair 2023)	Product Safety Toxicologist (Senior Scientist), Global Stewardship	The Clorox Company, Pleasanton, CA	2024
Priyanka Sura, D.V.M., M.S., D.A.B.T.	Senior Occupational Toxicologist, Nonclinical Safety and Pathobiology	Gilead Sciences, Inc. Foster City, CA	2024
Tamara Tal, Ph.D.	Senior Scientist, Group Leader	Helmholtz Center for Environmental Research – UFZ, Leipzig, Germany	2023
Misti Ushio, Ph.D.	Managing Partner	Digitalis Ventures, New York, NY	2024

Technology

Many ICCVAM member agencies are developing new technologies and resources to replace the use of animals for chemical safety testing. These include new platforms such as microphysiological systems (MPS), data resources to support the development of predictive models and quantitative structure–activity relationships (QSARs), and web tools to facilitate data access and visualization.

Assay Development

Many ICCVAM member agencies are developing new in vitro technologies and resources to replace animal use for chemical safety testing. Many of these include technologies to address important endpoints such as carcinogenicity, ecotoxicity, and inhalation toxicity.

Design and application of an adept aerosol lung-on-chip and aerosol/vapor delivery system
Tags: DoD, inhalation toxicity, MPS

The U.S. Army Chemical Biological Center (CBC) has established cooperative research and development agreements (CRADAs) with the tissue chip manufacturers Emulate and TissUse to apply their products to investigation of toxicity of aerosols.

Aerosolization of chemical agents is a health concern that presents specific technical challenges in addressing. Generation of aerosols in specific environments raises the possibility that the agent could be deposited on surfaces and then be reaerosolized into the environment due to the elements, foot traffic, or passing vehicles. This is particularly likely with substances that are easily disseminated, persist in the environment, and are hydrophobic and thus remain in the surface soil environment. In a first-of-its-kind study, CBC scientists investigated the potential of reaerosolization of pharmaceutical-based agents (PBAs) in a soil with high sand content. Investigation into this concept required novel experimental system design and unique approaches for data collection. A vibration system with air inlet and outlet ports was developed to allow sampling of the reaerosolized soil-PBA, and work is ongoing to determine a worst-case scenario concentration. Once identified, the PBA concentration will be assessed using receptor-based in vitro screening tools. Toxicity of substances of concern will be assessed using an open-top TissUse organ-on-a-chip system that is amenable to exposures from an aerosolized agent. The system functions with an established Emulate

microfluidic delivery system and a novel aerosol chip exposure apparatus called the Independent Holistic Air-Liquid Exposure System (InHALES). This system accurately replicates the entire human respiratory tract in vitro. Experimental work with the Emulate and TissUse systems is planned for 2024.

eDNA surveillance to characterize biotic diversity
Tags: DOI, ecotoxicity

The U.S. Geological Survey (USGS) of the Department of the Interior (DOI) develops and tests environmental DNA (eDNA) tools for the detection of both invasive and native species in the nation's waterways. Using eDNA for surveying or monitoring aquatic species can reduce costs and be less intrusive than methods that require direct capture of an organism. Throughout 2022 and 2023, efforts focused on the development and implementation of eDNA methods for native freshwater mussel species, including those that are federally listed species. These activities are providing insight into best practices for eDNA survey design, including optimal sampling time for mussel eDNA and identification of factors affecting eDNA detection. USGS also assisted with a study of eDNA detection of the Missouri state-listed longnose darter that indicated possible expansion of the species' range of distribution.

Development of in vitro systems for testing of volatile chemicals
Tags: EPA, inhalation toxicity, IVIVE

Inhalation is the most relevant route of volatile organic chemical (VOC) exposure. However, due to unique challenges posed by properties of VOCs and their poor solubility in aqueous solutions, in vitro chemical safety testing is predominantly performed using direct application dosing or submerged exposures. To address the difficulties in screening toxic effects of VOCs, scientists in the U.S. Environmental Protection Agency (EPA) Office of Research and Development developed a cell culture system that permits cells to be exposed to multiple concentrations at air-liquid interface (ALI) in a 24-well format. A pilot study with eight volatile compounds was conducted to determine whether high-throughput transcriptomics (HTTr) using the TempO-Seq assay would predict toxicity at sub-cytotoxic exposure concentrations delivered to human airway cells at the ALI. The publication describing these results (Speen et al. 2022) was recognized as a "Top 10 Best Paper Demonstrating an Application of Risk Assessment" in March 2023 by the Society of Toxicology Risk

Assessment Specialty Section. To expand the applications of the ALI exposure system, computer-aided design and computational fluid dynamics modeling were conducted to optimize operational parameters. Results indicate that a redesign of the system would improve VOC delivery and expand its applications to include aerosols. Planned 2024 activities include the evaluation of a broad array of VOCs and nonvolatile compounds with a focus on direct quantification of deposition and cellular uptake to improve in vitro to in vivo extrapolation (IVIVE). Furthermore, comparisons between ALI and direct liquid application studies are underway to determine best practices for in vitro new approach methodologies (NAMs).

Development of a bioprinter-based method for adding metabolic competence to high-throughput screening assays

Tags: EPA, endocrine disruptors, metabolism, Tox21

Certain chemicals used in commercial products and present in the environment have the potential to interfere with biological systems. Identifying human health effects using in vitro NAMs is challenged by the absence of metabolism in most test systems, which may lead to the under- or overestimation of potential health hazards. To retrofit existing high-throughput assays with metabolic competence, EPA developed the alginate immobilization of metabolic enzymes platform (AIME; Deisenroth et al. 2020) and used it to screen the ToxCast library to evaluate chemicals' estrogenic potential (Hopperstad et al. 2022). The AIME platform improves upon many conventional high-throughput screening assays by incorporating encapsulated hepatic S9-alginate microspheres to allow for consideration of the effects of liver metabolism. The ToxCast library data demonstrated a range of metabolism-dependent effects across a diverse chemical library. These results support the utility of the AIME platform for identifying false positive and false negative target assay effects and reprioritizing hazards based on metabolism-dependent bioactivity. They also highlight the need to evaluate the role of intrinsic xenobiotic metabolism for endocrine and other toxicity-related health effects.

Throughput and accessibility of the lid-based AIME method were improved by incorporating automated bioprinting to deposit S9-encapsulated microspheres directly into standard microplates with requisite cofactors for phase I and II hepatic metabolism (Hopperstad and Deisenroth 2023). The AIME bioprinting metabolism method will be a useful tool for the

tiered-testing paradigm outlined in the CompTox Blueprint (Thomas et al. 2019), and is also more amenable to method transfer because it uses commercial hardware rather than custom proprietary lids. Planned activities for 2024 and beyond include an active cooperative research and development agreement with Proctor & Gamble to transfer the AIME method to a commercial contract research organization, application of the AIME method to additional endocrine assays, and application of the method to broad high-throughput phenotypic assays and transcriptomic profiling.

In vitro test battery for developmental neurotoxicity
Tags: EPA, developmental neurotoxicity

Developmental neurotoxicity (DNT) testing is traditionally done using animals, which is resource-intensive and fails to provide information on cellular processes affected by chemicals. EPA scientists have been evaluating use of NAMs to assess DNT potential (Carstens et al. 2022), focusing on the microelectrode array neuronal network formation assay and high-content imaging to evaluate proliferation, apoptosis, neurite outgrowth, and synapse formation. Data for 92 chemicals with a range of potential DNT activities screened in 57 assay endpoints were sourced from publicly available data. From these data, a proposed DNT NAM battery was constructed that provides a sensitive marker of DNT bioactivity, particularly for evaluating cytotoxicity and network connectivity. This multi-dimensional assay suite may also provide insight into specific functional processes affected by chemical exposure.

Screening-level information for developmental neurotoxicity using new approach methodologies
Tags: EPA, NIEHS, defined approaches/IATA, developmental neurotoxicity, small model organisms

In 2023, the Organisation for Economic Co-operation and Development (OECD) published the guidance document "Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In-Vitro Testing Battery" (OECD 2023). The DNT in vitro battery consists of multiple NAMs that evaluate key processes of neurodevelopment such as proliferation, migration, differentiation, neuronal network formation, and function and locomotor activity in zebrafish (*Danio rerio*) embryos.

To explore a broader chemical space and increase the applicability domain, there is a need to screen additional compounds. The [DNT Health Effects Innovation program](#) within the NIEHS Division of Translational Toxicology is generating screening-level information on chemicals using the DNT in vitro battery and behavioral assays in small model organisms. Currently about 220 chemicals have been selected and distributed for testing with half of them being finalized and analyzed using a unified data analysis pipeline to combine data from the individual assays. To further build confidence in using the DNT in vitro battery for regulatory applications, the screening-level information is being used to develop specific case studies of integrated approaches to testing and assessment (IATA).

Gene expression biomarker to predict estrogen receptor activity
Tags: EPA, NIEHS, endocrine disruptors

High-throughput transcriptomics (HTTr) has the potential to support efforts to reduce or replace some animal tests. EPA and NIEHS scientists developed a computational approach utilizing MCF-7 breast cancer cells and a biomarker assessing expression of 46 genes to predict estrogen receptor activity after chemical exposure ([Ryan et al. 2016](#)). To further explore the utility of this model, they investigated whether it could identify estrogen receptor activities of chemicals examined by Endocrine Disruptor Screening Program (EDSP) Tier 1 screening assays ([Corton et al. 2022](#)). For the 62 chemicals examined, the estrogen receptor biomarker model accuracy was 97% for in vitro reference chemicals and at least 76% for in vivo assays. These accuracies were similar or slightly better for the same chemicals than those of a previously described ToxCast estrogen receptor model based on 18 in vitro assays. These results indicate that the HTTr biomarker model can correctly identify active and inactive estrogen receptor reference chemicals, and is potentially useful to rapidly identify chemicals with potential estrogen receptor bioactivities for additional screening and testing.

Safety testing strategy for cell-based human cancer therapies
Tags: NCI, stem cells

Preclinical safety assessment for chimeric antigen receptor (CAR) T cell-based therapies is necessary because non-targeted binding can have serious adverse consequences in healthy tissues. Three potential scenarios are of concern:

- A cell that binds to its intended target on the tumor ("on-target/on-tumor") could potentially trigger cytokine release syndrome or tumor lysis syndrome.

- A cell that binds to its intended target off the tumor ("on-target/off-tumor") could destroy healthy cells that express the target of interest.

- A cell that binds to an unintended target off the tumor ("off-target/off-tumor") could damage healthy cells that do not express the target of interest.

For CAR T cell-based therapies, safety testing in animal models is generally limited or impossible; therefore, robust in vitro assays that address on- or off-target binding and subsequent cytotoxic consequences are necessary as part of the preclinical safety information for these therapies. To evaluate the safety of CAR T cell drug candidates, National Cancer Institute (NCI) scientists are performing co-culture experiments using human pluripotent stem cell-derived cell types as models of healthy human cells that broadly represent various cell types that could be targeted by CAR T cell therapy. Potential readouts from such co-culture experiments include cell imaging for morphologic signs of cell stress/cytotoxicity, cytotoxicity assays, cytokine release assays, and impedance monitoring. Special consideration is given to the setup and execution of each readout to ensure that each assay is adequately controlled. The orthogonal data generated by these co-culture experiments will collectively support a good weight-of-evidence approach for assessing on- and off-target binding and potential cytotoxicity of CAR T cells in healthy human tissues. A paper describing this project is in preparation for submission in 2024.

NIEHS activities to develop new approaches for identifying potential immunotoxicants
Tags: NIEHS, population variability

The NIEHS Division of Translational Toxicology (DTT) conducts testing and research to determine potential human health effects of chemicals, drugs, food additives, dietary supplements, or environmental agents. One DTT area of study is how environmental factors that alter immune responses may contribute to human disease. Changes in immune function can affect susceptibility to infectious disease or cancer, contribute to the development of respiratory or dermal allergic responses resulting from xenobiotic exposures, and induce or exacerbate autoimmune disease. The DTT immunotoxicity testing and research program investigates the ability of xenobiotics to alter the normal structure and/or function of the

immune system. Current efforts within the research program focus on using in vitro approaches to assessing potential immunotoxicity. A major effort during 2022 and 2023 used an in vitro human whole blood culture system to investigate how interindividual susceptibility factors and environmental risk factors impact the response to viral infection. Over 200 individual human samples have been screened, and data are being analyzed to examine how intrinsic factors such as age, gender, and ethnicity influence the response of peripheral blood leukocytes to influenza and SARS-CoV-2 antigens. Preliminary results suggest that males have a higher natural killer cell activity in peripheral blood than females, and data are being further analyzed to determine if this effect is due to males having higher abundance of natural killer cells or more active natural killer cells than females. A second phase of this study is investigating responses to influenza and SARS-CoV-2 antigens following in vitro exposure to known immunotoxicants and how exposure to these environmental agents may affect susceptibility to viral infection. As proof of concept, whole blood cultures were unstimulated or stimulated with anti-T cell receptor antibodies or viral peptide pools in the presence of dexamethasone, a known immunosuppressive drug. Dexamethasone treatment resulted in inhibition of natural killer activity, cytokine production, and T cell activation following stimulation with the positive control. This work demonstrated that the in vitro immunotoxicity platform could detect immune suppression and alterations in responses to SARS-CoV-2 peptides. A second proof-of-concept study to examine the effect of benzo(a)pyrene exposure in the presence of metabolizing enzymes resulted in potent suppression in immune endpoints. Work planned for 2024 will develop additional endpoints for this culture system to facilitate interrogation of antibody-mediated responses and cytotoxic T cell driven immunity. This in vitro toolbox will be critically important for providing direct human relevance of methods used to identify chemicals that have the potential to modulate immune function and reduce the use of animals in toxicology testing.

NIEHS activities to develop new approaches for identifying potential cardiotoxins
Tags: NIEHS, NIH, cardiotoxicity, exposure, IVIVE, PFAS

Cardiotoxicity, or toxicity to the heart or cardiovascular system, is a major cause of failure of new drugs in mid- to late-stage development. Chemicals found in these drugs or in the environment may also contribute more broadly to human cardiovascular disease. The NIEHS DTT conducts testing and research to determine potential human health effects of chemicals,

drugs, food additives, dietary supplements, or environmental agents. Activities during 2022 and 2023 in the DTT's Cardiovascular Health Effects Innovation Program focused on how environmental factors can affect human susceptibility to or development of cardiovascular disease.

- In vitro to in vivo extrapolation (IVIVE) was used to derive human-equivalent administered doses (EADs) from test chemical concentrations that induce effects in in vitro cardiotoxicity assays. These EADs were then compared with U.S. human exposure biomonitoring, prediction models, and data from geospatial mapping to prioritize chemicals for further study. Reports on this project (Krishna et al.) were presented at the 2023 annual meeting of the Society of Toxicology (SOT) and the 12th World Congress on Alternatives and Animal Use in the Life Sciences. A publication describing this work is in preparation for submission in 2024.

- In vitro assays representing a broad range of cardiovascular-relevant activities were used to characterize potential cardiotoxicity hazard of a group of chemicals including botanicals, flame retardants, insecticides, polycyclic aromatic hydrocarbons, quaternary ammonium salts, and per- and polyfluoroalkyl substances (PFAS). A report on this project (Ramaiahgari et al.) was presented at the SOT 2023 annual meeting.

- Program scientists developed an interactive systematic evidence map to integrate data from human, animal, and in vitro studies of effects of environmental exposures on cardiotoxicity to support prioritization of future cardiotoxicity studies. A publication describing this systematic evidence map is in preparation for submission in 2024. A similar map was constructed to integrate data from human and animal studies on hypertensive disorders of pregnancy.

- In collaboration with the National Center for Advancing Translational Sciences (NCATS), the program is testing chemicals with potential vascular toxicity in human umbilical vein endothelial cells and in co-culture and flow models.

Future activities include collaborative efforts with external cardiovascular researchers using various models such as tissue-engineered blood vessels and cardiomyocytes to evaluate potentially cardiotoxic chemical activity.

Profiling transcription factor transactivation with P450 metabolism integration

Tags: NIEHS, metabolism

Profiling chemical effects on transcription factor activity can help characterize the mechanisms by which chemicals may perturb biological systems. The Attagene cis-FACTORIAL™ assay uses a reporter system to detect transactivation of 46 transcription factors to provide a quantitative assessment of chemical effects. A new version of this assay, CYP-FACTORIAL, adds nine key cytochrome P450 (CYP450) enzymes to enable the evaluation of chemical effects on transcription factor activity with and without CYP-mediated Phase 1 metabolism. These two complementary assay formats support a better understanding of whether CYP-mediated oxidation results in an altered bioactivity profile between parent and metabolite compounds. To explore the application of this system to predicting toxicity, NIEHS and Attagene collaborators tested 24 chemicals of regulatory concern in both systems. Profiling across all 46 transcription factors produced toxicity signatures for chemicals, and applying a biological read-across approach identified chemicals with similar effects. Additionally, profiles for "toxic" vs. "nontoxic" chemicals yielded insight into the biological mechanisms underlying adversity. This study exemplifies how integrating metabolism into a multiplexed in vitro assay system can provide additional mechanistic insight needed to understand chemical-elicited bioactivity, thereby facilitating the development of human-relevant test systems. A poster describing this work (Karmaus et al.) was presented at the [12th World Congress on Alternatives and Animal Use in the Life Sciences](#), and a manuscript is under development.

Data Resources

As momentum grows toward adoption of alternatives to animal use for chemical safety testing, curated data are needed to support method validation and establish scientific confidence in new approaches. ICCVAM agencies address that need by compiling data and making them publicly available.

EPA Ecotoxicology Knowledgebase (ECOTOX)

Tags: EPA, metrics, PFAS

EPA's Ecotoxicology Knowledgebase ([ECOTOX](#)) provides public access to comprehensive information on adverse effects of single chemical stressors to ecologically relevant aquatic

and terrestrial species. ECOTOX includes curated data compiled from over 54,000 references from the scientific literature and over 1.1 million test records covering nearly 14,000 aquatic and terrestrial species and 13,000 chemicals.

There were eight updates to the ECOTOX during 2022-2023. [Recent additions](#) include information on 6-PPD quinone, cyanotoxins, and PFAS. In 2023, the ECOTOX Knowledgebase had over 16,000 average monthly users and over 2,000 new users. An [ECOTOX training session](#) presented in February 2023 attracted over 500 participants. Additional training resources for ECOTOX are available on the [EPA NAMs Training webpage](#).

Updates to CompTox Chemicals Dashboard data
Tags: EPA, exposure, metrics

The [CompTox Chemicals Dashboard](#) is the primary web-based application that provides access to data and algorithms from EPA Center for Computational Toxicology and Exposure (CCTE). The Dashboard is a widely used resource for chemistry, toxicity, and exposure information for over a million chemicals. There were seven updates to the Dashboard released during 2022 and 2023. Data updates included in these releases added data on 300,000 new chemicals and enabled users to explore exposure predictions, production volumes, and analytical quality control data for PFAS. The next Dashboard update is planned for April 2024. A [October 2022 virtual training](#) on the Dashboard gave an overview of Dashboard functions and highlighted new features from the 2022 updates. Additional training resources for the Dashboard are available on the [EPA NAMs Training webpage](#).

Updates to computational toxicology and exposure data APIs
Tags: EPA, exposure

Application programming interfaces (APIs) allow programmatic access to EPA's computational toxicology and exposure data resources. APIs provided by EPA enable users to extract specific data from various databases and integrate them into their applications. APIs can effectively automate the process of accessing and downloading the data that populates the CompTox Chemicals Dashboard. As part of EPA's commitment to provide "open data," all Computational Toxicology and Exposure APIs and computational toxicology data resources are publicly available for anyone to access and use. These APIs are hosted on

cloud.gov, a secure cloud environment managed by the General Services Administration specifically for U.S. federal government applications. Data are free of all copyright restrictions and are fully and freely available for both non-commercial and commercial use. These APIs are documented on the CCTE API webpage and all data are also available for download on EPA's Downloadable Computational Toxicology Data webpage.

Development of ChemExpo Knowledgebase
Tags: EPA, exposure

Evaluation of chemical risk requires understanding of both the hazard presented by a chemical, via toxicity, irritation, or other harmful effects, and how much or how often the organism of interest is exposed to the chemical. ChemExpo is a publicly accessible data search and visualization tool for exploring chemical data relevant to exposure assessment that have been curated from public documents. It provides data collected by EPA about how chemicals are used in commerce and how they occur in consumer and industrial products. The ChemExpo team actively works to curate these data to harmonize consumer and occupational product categories, chemical functional categories, and exposure-relevant keywords, as well as to substance identifiers (DTXSIDs) used by EPA and the CompTox Chemicals Dashboard. These curated data are collectively known as EPA's Chemicals and Products Database (CPDat).

The beta version of ChemExpo was released in September of 2023. The beta release allows users to explore, search, and download CPDat data, aligning with bulk releases to enhance accessibility and integration with the CompTox Chemicals Dashboard.

Release of accessible bioactivity data in ToxCast's InvitroDB
Tags: EPA, developmental toxicity, Tox21

The EPA Toxicity Forecasting (ToxCast) program makes in vitro medium- and high-throughput screening (HTS) assay data publicly available for prioritization and hazard characterization of thousands of chemicals. The assays employ a variety of technologies to evaluate the effects of chemical exposure on diverse biological targets, from distinct proteins to more complex cellular processes like mitochondrial toxicity, nuclear receptor signaling, immune responses, and developmental toxicity. The ToxCast data pipeline (tcpl) is an open-

source R package that stores, manages, curve-fits, and visualizes ToxCast data and populates the linked MySQL database, [invitroDB](#).

In 2022-2023, major updates were made to tcpl and invitroDB to accommodate a new curve-fitting approach ([Feshuk et al. 2023](#)). The original tcpl curve-fitting models (constant, Hill, and gain-loss models) have been expanded to include Polynomial 1 (linear), Polynomial 2 (quadratic), Power, Exponential 2, Exponential 3, Exponential 4, and Exponential 5, which are based on BMDExpress and encoded by the R package dependency, tcplfit2. Inclusion of these models impacted invitroDB (beta version v4.0) and tcpl v3 in several ways: (1) long-format storage of generic modeling parameters to permit additional curve-fitting models; (2) updated logic for winning model selection; (3) continuous hit calling logic; and (4) removal of redundant endpoints as a result of bidirectional fitting. The tcpl and invitroDB resources provide a standard for consistent and reproducible curve-fitting and data management for diverse, targeted in vitro assay data with readily available documentation, thus enabling sharing and use of these data in myriad toxicology applications.

The [CompTox Chemicals Dashboard](#) v2.3.0 (release planned for 2024) will display data from invitroDB version 4.1, which was released in September 2023. In 2024, EPA will continue iteratively improving the tcpl software, releasing new data in invitroDB, and providing additional ways to access ToxCast data.

Semi-automated extraction of literature data using machine-learning methods
Tags: DOE, ICCVAM, NIEHS, developmental toxicity, machine learning

NICEATM, other scientists within NIEHS DTT, and the ICCVAM Developmental and Reproductive Toxicity Expert Group collaborated with the Oak Ridge National Laboratory (U.S. Department of Energy [DOE]) to automate the process of identifying high-quality developmental toxicity studies in the published scientific literature. The approach applied natural language processing and machine-learning methods to identify specific data elements in the full text of scientific publications using both unsupervised and supervised approaches. This work is being extended to investigate application of large language models to further refine the approaches to extract study protocol information. A publication describing this work is being drafted for submission in 2024.

Projects to extract and annotate legacy developmental toxicity study data
Tags: NIEHS, developmental toxicity

To support the evaluation of nonanimal approaches for developmental toxicity assessment, NICEATM scientists extracted information from more than 100 legacy National Toxicology Program (NTP) prenatal developmental toxicity animal studies and a subset of about 50 studies submitted to the European Chemicals Agency that were deemed high-quality by NTP subject matter experts ([Foster et al. 2024](#)). Study details extracted included species, strain, administration route, dosing duration, and treatment-related effects. The extracted data were standardized by applying controlled vocabularies and ontologies to facilitate computational analyses and integration with other structured databases such as EPA's Toxicity Reference Database (ToxRefDB). Elements of three controlled vocabularies (the Unified Medical Language coding system, the German Institute for Risk Assessment DevToxDB ontology, and the OECD Harmonised Template 74 terminologies) were combined with automation code to programmatically standardize primary source language of extracted developmental toxicology endpoints. Of all the standardized extracted end points, about half required manual review for potential extraneous matches or inaccuracies. Extracted end points that were not mapped to standardized terms tended to be too general or required human logic to find a good match. It was estimated that this augmented intelligence approach saved over 350 hours of manual effort and yielded valuable resources including a controlled vocabulary crosswalk, organized related terms lists, code for implementing an automated mapping workflow, and a computationally accessible dataset. Application of such approaches can reduce manual labor, facilitate further analyses (e.g., systematic review, model-building, NAM validation), and uphold findability, accessibility, interoperability, and reusability (FAIR) principles.

DNT-DIVER: integration and visualization of DNT assay data
Tags: NIEHS, developmental neurotoxicity

NIEHS DTT launched the [Developmental NeuroToxicity Data Integration and Visualization Enabling Resource](#) (DNT-DIVER) in 2018. DNT-DIVER allows users to analyze, compare, and visualize multiple DNT assays in an interactive web application. Initially, this resource provided data from cell-based assays and alternative animal models generated using a

targeted set of 91 compounds provided by DTT. In 2019, DTT updated DNT-DIVER to allow it to be a permanent web-based application to allow public access and visualization of all data from compounds screened by the DTT's Developmental Neurotoxicity Health Effects Innovation Program. The updated version includes different tabs including experimental design summary, quality control, chemical-specific concentration–response curves, ranking of chemical toxicity per lab/assay, and comparison of results across assays. It will also accept data from novel screening assays in as they become available and accepted by the international DNT community. A testing version of DNT-DIVER was published internally in October 2023, and the resource is being modified to address team member feedback. The updated version will be launched at the SOT 2024 annual meeting.

Integrated Chemical Environment data updates
Tags: NIEHS, acute toxicity, endocrine disruptors, exposure, QSAR, skin sensitization, Tox21

NICEATM's [Integrated Chemical Environment](#) (ICE) provides data and tools to help develop, assess, and interpret chemical safety tests. The March 2022 ICE 3.6 update added quality control annotations to curated high-throughput screening (cHTS) data and provided flat files for easy download of entire data sets via the ICE webpages. Implementation of application programming interfaces supporting the representational state transfer architectural style (REST APIs) in the July 2022 ICE 3.7 update enabled users to access ICE data more easily outside of the ICE environment. Other updates and improvements to ICE data sets during 2022 and 2023 include:

- References for acute oral toxicity data.

- Endpoints for skin sensitization data.

- Addition and harmonization of endocrine data.

- Harmonization of structure and data fields in dermal irritation/corrosion data.

- Updates of OPERA predictions from [OPERA version 2.8](#).

- Addition of exposure prediction data from EPA's Systematic Empirical Evaluation of Models (SEEM3).

NICEATM is developing an annotation scheme for HTS assays that will provide biological context to the cHTS data and enable toxicological interpretation. The scheme incorporates annotations from the [Open Biological and Biomedical Ontology Foundry](#), a harmonized and interoperable database consisting of multiple knowledge areas to encompass a broader range of biologic and toxicologic processes. An abstract describing the annotation scheme (Hill et al.) has been accepted for a poster presentation at the [SOT 2024 annual meeting](#). NICEATM is also aligning the ICE cHTS annotations to the [OECD's Harmonized Template 201](#).

Formatting ToxCast and ICE cHTS data into OECD reporting templates
Tags: EPA, NIEHS, Tox21

OECD has developed internationally agreed-upon formats for reporting of intermediate effect and mechanistic information from NAM studies. OECD Guidance Document 211 serves as a standard for comprehensive assay documentation describing non-guideline in vitro test methods and their interpretation. OECD Harmonized Template 201 (OHT201) is a harmonized template for reporting chemical test result summaries for intermediate effects.

[ToxCast assay description documentation](#) aligns with Guidance Document 211 standards to describe experimental systems, protocols, performance metrics, and assay quality statistics. Major software and database enhancements to tcpl and invitroDB warranted a complete overhaul to existing assay description documentation. ToxCast annotations are being leveraged to populate stipulated fields for automated report generation and direct additional curation efforts. A compiled report is expected to be released in fall 2024 and accompany ToxCast's invitroDB v4.2 release.

It is envisioned that widespread use of OHT201 will harmonize data at an international level, facilitate international adoption of standardized NAMs data and make data more accessible. However, cHTS data available in [NICEATM's ICE](#) do not conform to the OHT201 format. To address this, NICEATM began working with EPA and European Commission Joint Research Centre (JRC) collaborators in October 2023 to apply the OHT201 formatting to ICE cHTS data. Collaborators are identifying fields and data points that need to be populated in OHT201 form using annotations from ICE, EPA annotations retrieved from the CompTox Chemicals Dashboard, and OECD's "Guidance Document for Describing Non-Guideline In Vitro Test Methods." The group is also developing a formatting automation pipeline to apply

a KNIME workflow to the European Chemicals Agency's International Uniform Chemical Information Database software to map these annotations to the OHT 201 standardized template. Completed OHT201 forms for active chemical-assay pairs within the ICE cHTS dataset are anticipated to be available at the end of 2024.

Compilation and curation of an acute inhalation toxicity data set
Tags: ICCVAM, NIEHS, inhalation toxicity

Chemical safety evaluation has traditionally relied on animal models to identify potential acute inhalation toxicants and define safety standards that protect human health. NAMs that include in vitro and computational approaches have been proposed as complementary resources that can be integrated to identify and/or mechanistically evaluate such toxicants and also yield human-relevant insight into inhalation toxicity. Developing and evaluating such approaches requires robust, well-curated, and chemically diverse reference data. NICEATM has curated a database with in vivo rat acute inhalation data for approximately 1,200 unique substances. Data were compiled from six open-access sources: the National Institute for Occupational Safety and Health Pocket Guide; European Chemicals Agency Registration, Evaluation, Authorisation and Restriction of Chemicals Database; EPA Acute Exposure Guideline Levels; U.S. Department of Defense; and PubChem/ChemIDPlus. In addition to LC50 values (exposure concentration of a toxic substance estimated to be lethal to half of the test animals), metadata collected for each entry included exposure type, exposure route, species, sex, and number of animals tested when available. The diversity of chemical space represented in the database was characterized using predicted chemical properties and functional use categories obtained from the EPA Chemical and Products Database (CPDat). hazard categories (e.g., nontoxic, toxic, highly toxic) were assigned based on LC50 values and exposure phase data following various agency-specific classification schemes. To evaluate categorical variability, conditional probabilities were calculated, representing the probability that a chemical would be assigned a specific hazard category given that it was previously assigned the same or another category. The final curated database contains 2,565 entries for 1,209 unique substances. Of these, 1,020 unique chemicals (2,076 entries) have a QSAR-ready structure. These chemicals showed robust coverage across physicochemical properties and functional use categories. Preliminary variability analysis of the data showed lognormally distributed LC50 point data with broad coverage across multiple chemical

descriptors. Ongoing characterization of the database includes variability analysis of phase-dependent hazard classifications. This characterization will be used to contextualize potential modeling endpoints. The database can be downloaded from NICEATM's [Integrated Chemical Environment](#).

SEAZIT ontologies, database, and data analysis pipeline
Tags: NIEHS, developmental toxicity, small model organisms

The zebrafish embryo is a useful alternative research model for assessing the effects of substances on growth and development. However, cross-laboratory developmental toxicity outcomes can vary due to lack of standardization both in laboratory procedures and terminology used to describe outcomes. Thus, reported developmental defects in zebrafish may not be directly comparable between laboratories. To enable broader adoption of zebrafish for toxicological screening, NIEHS established the [Systematic Evaluation of the Application of Zebrafish in Toxicology](#) (SEAZIT) program.

Discussions among scientists participating in SEAZIT considered how variability in results could be addressed by implementing standardized nomenclature systems known as ontologies. A collaborative exercise was conducted to evaluate how the application of ontologies improved data consistency ([Thessen et al. 2022](#)). Analysis of the results suggested that the use of ontology terms increased consistency and decreased ambiguity, and that utilizing a common data standard should reduce the heterogeneity of reported terms and potentially increase agreement and repeatability between different laboratories.

A key element of SEAZIT is an interlaboratory study to investigate how experimental protocol differences can influence chemical-mediated effects on developmental toxicity. Three laboratories were provided a common and blinded set of 42 substances to evaluate chemical effects on developmental toxicity in the embryonic zebrafish model. Laboratory work was completed in 2022, and a paper has been published ([Hsieh et al. 2023)](#) describing the relational database developed to store the data, which features harmonization of the above-described ontologies for altered phenotype endpoints, and the data analysis pipeline. Data are available in the NIEHS Chemical Effects in Biological Systems (CEBS) data resource, and a [web application](#) is being developed to allow users to interactively explore the data. A second paper describing the study design is being prepared for publication in 2024.

Human data set for skin sensitization methods evaluation
Tags: CPSC, FDA, NIEHS, skin sensitization

Appropriate evaluation of NAMs requires reference data for assessing the method's ability to predict an outcome of interest. Human data provide the most relevant basis for such comparisons, but they are rarely available due to obvious ethical issues associated with toxicology testing in humans. One exception is data from skin sensitization tests, which are routinely conducted using a wide range of materials. For this project, U.S. Consumer Product Safety Commission (CPSC), FDA, and NICEATM scientists and collaborators in the German Federal Institute of Risk Assessment collected data from human predictive patch tests conducted under two protocols: the human repeat insult patch test and the human maximization test. Data were collected from more than 1,500 publications. The data collection process also captured protocol elements and positive or negative outcomes, calculated traditional and non-traditional dose metrics, and developed a scoring system to evaluate each test for reliability. The resulting database ([Strickland et al. 2023](#)), which represents the largest set of human data ever assembled for the purpose of evaluating nonanimal approaches for chemical safety testing, was characterized for physicochemical properties, chemical structure categories, and protein binding mechanisms. The data are publicly available via [ICE](#) to serve as a resource for the development and evaluation of NAMs for skin sensitization testing.

Mapping of Tox21/ToxCast assays onto characteristics of cancer
Tags: EPA, NIEHS, carcinogenicity, Tox21

Carcinogenesis is a multistep process in which healthy cells acquire properties that allow them to form tumors or malignant cancers. The concept of key characteristics of carcinogens has been developed to describe 10 properties that are shared by viruses and chemicals that induce human cancers, properties that can encompass various mechanistic endpoints. Mapping these characteristics onto assays used in the Tox21/ToxCast program could be instrumental in developing NAMs for carcinogenicity, defining associated mechanisms, and identifying data gaps in carcinogenicity. To develop a consensus mapping of key characteristics of carcinogens onto Tox21/ToxCast assays, NIEHS organized a working group including scientists from EPA, NIEHS, and collaborating organizations. The working

group started meeting in September 2023 and is currently engaged in annotating and reviewing assay annotations, which will consider data available in a new release of InvitroDB. The final mapping is anticipated to be completed in 2024 and will be available in an upcoming release of the Integrated Chemical Environment.

Development of the bioinformatics repository BioBricks
Tags: NIEHS, structure similarity

BioBricks (Insilica) is an application that makes large bioinformatics databases programmatically accessible. Because of the potential usefulness of this tool for toxicological data acquisition and analysis, NICEATM began working in 2022 with Insilica to provide support and testing of the BioBricks platform. A specific goal was to develop a BioBrick to query the Protein Data Bank, a U.S. government resource that represents the largest database of 3D protein structures. The Protein Data Bank includes binding affinity data for many protein-ligand complexes, which is an important resource to anticipate chemical actions. These binding affinity data are a key resource for a subsequent NICEATM activity, development of a biological similarity metric wherein chemicals that share a large number of biological effects will be considered similar. This metric may be useful for evaluating chemical hazard molecular mechanisms. NICEATM is also supporting testing and expanding the BioBricks interface through activities such as development of a Python version. All databases integrated into BioBricks are fully and freely available; the code is open-source and available via a GitHub repository.

Perspectives on variability and reproducibility of in vivo toxicology studies
Tags: EPA, NIEHS, acute toxicity

Understanding the variability and reproducibility of reference animal data and how it may affect the NAM evaluation process is of utmost importance to the development, integration, and implementation of NAMs into regulatory decision-making. To better understand these factors, NICEATM and EPA have conducted multiple retrospective evaluations that have shown substantial variability for several standardized in vivo toxicology test methods, including both single (e.g., Karmaus et al. 2022) and repeat-dose (e.g., Pham et al. 2020) study designs.

NICEATM has undertaken a broader assessment of these evaluations to provide a more realistic context to existing data streams and to help set appropriate expectations for the overall performance of NAMs in the context of existing in vivo reference data. Additional assessments of the validation status of multiple in vivo guideline studies have also been undertaken. A lack of validation can impact the robustness and reproducibility of a method, thus impacting the variability within the method. This work was presented in a poster (Oyetade et al.) at the [12th World Congress on Alternatives and Animal Use in the Life Sciences](#) in 2023 and a paper will be submitted for publication in 2024.

An EPA study estimated benchmarks for NAM performance in predicting organ-level effects in repeat-dose studies of adult animals based on variability in replicate animal studies ([Paul Friedman et al. 2023](#)). Treatment-related effect values from the Toxicity Reference database (v2.1) for weight, gross, or histopathological changes in the adrenal gland, liver, kidney, spleen, stomach, and thyroid were used. In brief, findings suggest the following:

- Variance explained by study metadata was similar for organ and study findings.

- Organ effects were unlikely in a chronic study if no organ findings were observed in a subchronic study.

- Mean differences in lowest-effect level by exposure duration were similar in size to replicate study variance.

- For most chemicals, administered equivalent doses derived from in vitro methods were within an order of magnitude of organ lowest-effect levels observed in in vivo studies with respect to liver and kidney effects, with larger differences (up to three orders of magnitude) for a smaller number of chemicals.

Computational Tools Development

Computationally generated predictions of toxicity endpoints can inform decisions about testing priorities and sometimes eliminate the need for laboratory testing. ICCVAM agencies are developing tools to predict toxicity endpoints such as cardiotoxicity, carcinogenicity, skin sensitization, and genotoxicity, as well as tools for cross-species extrapolation and application of defined approaches (DAs).

QSAR machine-learning models for NRF2 activation and PPARg inhibition to support prediction of lung toxicity

Tags: DoD, inhalation toxicity, machine learning, QSAR

Identifying and applying mitigation strategies for oxidative stress can reduce adverse health effects from operational stressors and improve U.S. Air Force (USAF) aircrew and guardian readiness. Nuclear factor erythroid 2–related factor 2 (NRF2) is a key element of the cellular antioxidant defense system, because it regulates transcription of antioxidant proteins and detoxifying enzymes. Scientists in the USAF Force Health Protection program's Predictive Risk Team (PRT) developed a computational approach to predict potential activators of NRF2 using structural alerts and machine-learning QSAR modeling ([Chushak and Clewell 2024](#)). Once developed, the approach was used to screen a list of approved drugs collected in DrugBank to identify potential novel NRF2 activators. Currently, PRT scientists are working on development of QSAR models to identify inhibitors of peroxisome proliferator-activated receptor gamma (PPARg). Chemicals that inhibit PPARg increase the risk of long-term lung damage from inhaled toxins. The developed models will be used together with in vitro data and other mechanistic models to predict whether exposure to chemicals in the operational environment may cause acute or chronic lung injury.

Development of a multitask machine-learning model for diverse toxicity data

Tags: DoD, machine learning, Tox21

Machine-learning methods enable data-driven development of predictive models for health-effects screening of novel chemicals. Single-task machine-learning models train on one endpoint and lack transferability to similar endpoints. These models also require large, homogeneous data sets. Health-effects screening needs machine-learning tools that can handle multiple small, noisy data sets. USAF PRT is collaborating with a team from Johns Hopkins University Applied Physics Laboratory to investigate a novel machine-learning pipeline for molecular representation learning based on a multitask machine-learning paradigm. ToxCast assays with the same target were consolidated to minimize missing entries, and a machine-learning model was trained simultaneously on multiple tasks from moderate-sized data sets. To predict novel tasks from small data sets, the pretrained multitask model was combined with a dimension-reducing map and a task-specific predictor. The multitask model performed better than single-task models on six of 11 training tasks and six

of seven distinct non-training tasks. This novel machine-learning pipeline generates molecular representations, leverages reduced dimensionality for greater efficiency, and combines information on multiple effects, including highly specific ligand binding and nonspecific systemic responses, to provide a more generalizable chemical risk model. The pipeline is being completed and prepared for publication in 2024.

Citizen science project for biosurveillance of blotchy bass syndrome
Tags: DOI, ecotoxicity, metrics

The public is often aware of and interested in fish and wildlife diseases, particularly those that are highly visible, change the appearance of animals, and affect species of high recreational or commercial value like black bass (*Micropterus* spp.). "Blotchy bass syndrome" is a term used to describe external hyperpigmentation (melanosis) in black basses. This condition has received increased attention from anglers and resource managers in recent years and is a popular topic of discussion and reporting on angling websites and blogging platforms. Crowdsourced data collection can be used to increase community engagement and buy-in, as well as expand geographical and temporal sampling beyond those provided by state agencies. Recognizing a need to understand the geographical extent, seasonality, and biological threat of blotchy bass syndrome, USGS established crowdsourcing efforts to monitor this condition. Approaches used included traditional solicitation, smartphone applications, and virtual fishing events. Multiple discrete but overlapping efforts were undertaken starting in 2021. In March 2022, state agency partners solicited reports of blotchy bass syndrome via social media, requesting that anglers submit photos and information on catch location. Between June and November 2022, a virtual BioBlitz was conducted using the Angler's Atlas MyCatch smartphone application, incentivized with prizes. A "Blotchy Bass Bonanza" participatory science effort was launched in July 2022 and encompassed all freshwater waterbodies within the United States and Canada. The Bonanza was reinitiated in March 2023 and will continue through February 2024. Overall, efforts yielded data from 31 states, six Canadian provinces, and some submissions from Mexico, Spain, and South Africa. Anglers submitted a total of 1,077 digital photographs of individual fish with presumptive blotchy bass syndrome for scientific review. USGS valued the 52,399 donated personnel hours by participating anglers at $1,153,000.

ECOSAR: computational tool to predict aquatic toxicity

Tags: EPA, acute toxicity, ecotoxicity, QSAR

[Ecological Structure Activity Relationships](#) (ECOSAR) is a computerized predictive system that estimates aquatic toxicity. The program estimates a chemical's acute and chronic toxicity to aquatic organisms, such as fish, aquatic invertebrates, and aquatic plants, by using computerized structure–activity relationships (SARs). ECOSAR software is available for free without licensing requirements. Key characteristics of the program include:

- Grouping of structurally similar organic chemicals with available experimental effect levels that are correlated with physicochemical properties to predict toxicity of new or untested industrial chemicals.

- Programming of a classification scheme to identify the most representative class for new or untested chemicals.

- Continuous update of aquatic QSARs based on collected or submitted experimental studies from both public and confidential sources.

ECOSAR version 2.2 was released in March 2022. Updates included two new chemical classes, a module to predict toxicity of cationic polymers, user interface updates, and user input of melting point, octanol–water partition coefficient, and water solubility.

Updates to CompTox Chemicals Dashboard tools

Tags: EPA, metrics

The [CompTox Chemicals Dashboard](#) is the primary web-based application that provides access to data and algorithms from the EPA CCTE. It is a widely used resource for chemistry, toxicity, and exposure information for over a million chemicals. There were seven updates to the Dashboard released during 2022 and 2023. The next Dashboard update is planned for April 2024. In 2023, the Dashboard had over 21,000 average monthly users and over 11,000 new users. An [October 2022 virtual training](#) on the Dashboard gave an overview of Dashboard functions and highlighted new features from the 2022 update. Additional training resources for the Dashboard are available on the [EPA NAMs Training webpage](#).

Continued development of httk

Tags: EPA, IVIVE, metabolism

To fully characterize the potential human health risk of a substance, data are often needed on that substance's toxicokinetics: how a substance is absorbed, distributed, metabolized, and eliminated in the body. Traditional approaches for obtaining toxicokinetics data use animals, but alternative approaches are being developed to computationally estimate relevant parameters. EPA researchers have developed toxicokinetic models within an R software package called high-throughput toxicokinetics (httk) to estimate chemical concentrations in humans and support IVIVE. The package currently uses human in vitro data to make predictions about the fate of chemicals in humans, rats, mice, dogs, and rabbits. The latest version of httk, v2.3.0, is now available. This version incorporates new in vitro measures of gut absorption for over 400 chemicals. There is also new in vitro clearance and binding data, new quantitative structure–property relationship (QSPR) predictions including gut absorption, and many other new features. New models are being developed to describe absorption through the skin, exposure to aerosols (clouds of droplets), partial oral absorption, and human pregnancy. More information is available on the EPA httk website.

Generalized Read-Across (GenRA) application
Tags: EPA, structure similarity

Read-across is a computational technique that uses toxicity data from one or more known (source) chemicals to predict toxicity for another (target) chemical, usually but not always on the basis of structural similarity. EPA's Generalized Read-Across (GenRA) application is a standalone tool linked to the CompTox Chemicals Dashboard that performs read-across algorithmically, helping researchers to make informed decisions about chemicals with little toxicity data available.

GenRA had two major updates during 2022-2023.

- In September 2022, a new update allowed users to evaluate the relevance of similar substances using predicted physical property information. It provided predictions of toxicity and bioactivity that users could download for further analysis.

- Version 3.2 was released in March 2023. This update included data updates and over 30 minor improvements and bug fixes. Improved indexing in this release enabled increased processing speed.

In 2023, EPA presented [virtual training on GenRA.](#) Approximately 725 people attended the main session and 215 attended the breakouts, representing a record for attendance at an EPA NAMs training session. Additional training resources for GenRA are available on the [EPA NAMs Training webpage](#).

High-throughput transcriptomics and high-throughput phenotypic profiling
Tags: EPA, IVIVE

Researchers at EPA are using two high-throughput profiling assays, high-throughput transcriptomics (HTTr) with the human whole transcriptome TempO-Seq assay and high-throughput phenotypic profiling (HTPP) with the Cell Painting assay to characterize the biological activity of chemicals across a variety of human-derived cell types. Computational workflows have been established for determining biological pathway-altering concentrations and phenotype-altering concentrations from these two assay types ([Harrill et al. 2021](#); [Nyffeler et al. 2023](#)). The high-dimensionality data have been organized for display and distribution to the public through the [CompTox Chemicals Dashboard](#), under the "Bioactivity, HTTr: Summary" and "Bioactivity, HTPP:Summary" views. HTTr and HTPP results for hundreds of chemicals in a variety of cell types (MCF-7 breast adenocarcinoma, U-2 OS osteosarcoma and the 2-D differentiated HepaRG liver cell model) became available on the Dashboard in 2022 and will be updated in future releases.

Continued development of SeqAPASS
Tags: EPA, ecotoxicity, structure similarity

EPA's [Sequence Alignment to Predict Across Species Susceptibility](#) (SeqAPASS) is a fast online screening tool that allows researchers to extrapolate toxicity information across species. SeqAPASS version 7.0, released in September 2023, allows users to incorporate protein structural evaluations of conservation in the SeqAPASS analysis. Using the integrated Iterative Threading ASSEmbly Refinement tool, users can generate protein structures. They can then use those structures or incorporate additional structures from tools like the Research Collaboratory for Structural Bioinformatics Protein Data Bank and AlphaFold to align them to their chosen species, typically a known sensitive species. From there, users can add evidence based on structural similarity to their sequence similarity data in their chemical susceptibility predictions. This work has been leveraged to support agency

decision-making relative to pollinators, the Endangered Species Act, and EDSP. SeqAPASS version 7.1, which will incorporate data and tool updates, is planned for release in early 2024.

Geospatial modeling approaches to link in vitro data with geographic exposure
Tags: EPA, NIEHS, exposure, mixtures toxicity

Traditional risk assessments based on in vivo animal studies typically use a chemical-by-chemical approach and apical disease endpoints. However, in the real world, individuals are exposed to chemicals from sources that vary over space and time. EPA and NIEHS scientists collaborated to develop a workflow (Eccles et al. 2022) to integrate human exposure data for 41 chemicals in the EPA National Air Toxics Assessment with cHTS assays to identify counties where exposure to the local chemical mixture may perturb a common biological target. The workflow used the estimated blood plasma concentration and the concentration–response curve from the cHTS data to determine the chemical-specific effects of the mixture components. Three mixture modeling methods were used to estimate the joint effect from exposure to the chemical mixture on the activity levels, which were geospatially mapped. This workflow demonstrates how NAMs can be used to predict early-stage biological perturbations that can lead to adverse health outcomes that result from exposure to chemical mixtures. As a result, this work will advance mixture risk assessment and other early events in the effects of chemicals.

Computational models for hazard identification of flavor compounds in tobacco products
Tags: FDA, inhalation toxicity, structure similarity

Flavor chemicals contribute to the appeal and toxicity of tobacco products, and the assortment of flavor chemicals available for use in tobacco products is extensive. However, potential harms from inhaling these substances and their byproducts have not been extensively studied. To help address this data gap, U.S. Food and Drug Administration (FDA) scientists (Goel et al. 2022) used a chemistry-driven computational approach to evaluate flavor chemicals based on intrinsic hazardous structures and reactivity of chemicals. A library of 3,012 unique flavor chemicals was compiled from publicly available information, and a structure-based analysis was done to characterize their (1) physicochemical properties, (2) United Nations Globally Harmonized System of

Classification and Labelling of Chemicals (GHS) health hazard classification, (3) structural alerts linked to the chemical's reactivity, instability, or toxicity, and (4) substructures shared with chemicals characterized by FDA as respiratory toxicants. Computational analysis of the constructed flavor library flagged 638 chemicals with GHS classified respiratory health hazards, 1,079 chemicals with at least one structural alert, and 2297 chemicals with substructural similarity to chemicals on the respiratory toxicant list. A subsequent analysis was performed on a subset of 173 chemicals in the flavor library, from which four general structures with an increased potential for respiratory toxicity were identified. This study indicated that computational methods are efficient tools for hazard identification and understanding structure-toxicity relationship. With appropriate context of use and interpretation, in silico methods may provide scientific evidence to support toxicological evaluations of chemicals in or emitted from tobacco products.

Integrated Chemical Environment tools updates
Tags: NIEHS, exposure, IVIVE, structure similarity, Tox21

The [Integrated Chemical Environment](#) (ICE), developed by the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), provides data and tools to help develop, assess, and interpret chemical safety tests. Improvements made in the March 2023 version 4.0 update allowed users to query ICE using chemical names and their synonyms, as well as with existing chemical identifier options. Other updates to ICE during 2022 and 2023 added help videos, new Chemical Quick Lists, and REST APIs. The updates also increased the utility and versatility of the resource's tools.

- An updated Results view for the **Search** tool improved data navigation and provided query summary visualizations.

- The March 2023 update of the **IVIVE** tool incorporated models from EPA's httk version 2.2.2, including a new gestational model allowing modeling of maternal and fetal chemical distribution. Users can now upload their own in vitro data for modeling, as well as their own in vivo data for comparison to predictions based on in vitro data. Users can compare predictions to population level exposure predictions from EPA's Systematic Empirical Evaluation of Models (SEEM3). The tool's

inhalation model has been updated to allow the input of chemical concentration in parts per million per unit volume.

- A rebranded "Curated Product Use Explorer" in the **Chemical Characterization** tool expands and improves upon the former "Consumer Use Explorer." A new Functional Use Explorer allows the graphical distribution of chemical lists across functional use categories.

- The **Curve Surfer** tool, which allows users to view and interact with concentration–response curves from cHTS data, has new options to view and filter results.

- Updates to the **Physiologically Based Pharmacokinetics (PBPK)** tool incorporated models from the EPA's httk version 2.2.2, including the new gestational model. The inhalation model has been updated to allow the input of chemical concentration in parts per million per unit volume. The tool output download files were also updated to include predicted half-life and area-under-curve values.

- The **Chemical Quest** tool has been fully implemented and updated to allow users to identify chemicals in the ICE database having similar structures to a query chemical, as well as new options to filter results. Users can now perform similarity searches based on user-defined chemical lists.

Open (Quantitative) Structure–activity/property Relationship App (OPERA) updates
Tags: EPA, NIEHS, IVIVE, PFAS, QSAR

The [Open (Quantitative) Structure–activity/property Relationship App](#) (OPERA) is a free and open-source/open-data suite of QSAR models developed to support a range of research and regulatory purposes. In addition to physicochemical and environmental fate properties, OPERA offers a number of models predicting absorption, distribution, metabolism, and excretion (ADME) endpoints that are important to PBPK modeling and IVIVE studies. All OPERA models were built using curated data sets split into training and test sets and molecular descriptors calculated based on standardized QSAR-ready chemical structures. Modeling adhered to the five principles for QSAR model development adopted by OECD. These principles support development of scientifically valid, high-accuracy models with minimal complexity that support mechanistic interpretation, when possible. For consistency

and transparency, OPERA provides a tool for standardizing chemical structures, an estimate of prediction accuracy, an assessment of applicability domain, and incorporation of experimental values when available. Technical and performance details are described in OECD-compliant QSAR Model Reporting Format reports.

Existing OPERA models are updated regularly when new experimental data are available. Version 2.9, released in September 2022, updated a number of physicochemical properties and ADME parameters covering different classes of chemicals including PFAS. OPERA predictions are available through the EPA [CompTox Chemicals Dashboard](#) and NICEATM's [Integrated Chemical Environment](#). The OPERA application can also be downloaded from the [NIEHS GitHub repository](#) as a command-line or graphical user interface for Windows and Linux operating systems. To enable broader access, in September 2022 OPERA became [available as an extension](#) to [OECD's QSAR Toolbox](#), a resource provided by OECD and the European Chemicals Agency to support animal-free chemical hazard assessment.

QSAR models of ocular toxicity
Tags: NIEHS, eye irritation, mixtures toxicity, QSAR

NIEHS scientists developed of a set of computational models to predict eye irritation and corrosion ([Sedykh et al. 2022](#)). The models were developed using a curated database of in vivo eye irritation studies from the scientific literature and stakeholder-provided data. The database contained over 500 unique substances, including many mixtures, tested at different concentrations. Substances were categorized according to GHS and EPA hazard classifications. Two modeling approaches were used to predict classification of mixtures. A conventional approach generated predictions based on the chemical structure of the most prominent component of the mixture. A mixture-based approach generated predictions by using weighted feature averaging to consider all known components in the mixture. Results suggest that these models are useful for screening compounds for eye irritation potential. Future efforts to increase the models' utility will focus on expanding their applicability domains and using them in conjunction with other input variables (e.g., in vitro data) to establish DAs for eye irritation testing.

STopTox: computational tool to predict acute toxicity
Tags: NIEHS, acute toxicity, eye irritation, inhalation toxicity, QSAR, skin sensitization

The "six-pack" battery of tests uses animals for acute toxicity assessment of chemicals used as pesticides, pharmaceuticals, or in cosmetic products. Endpoints include skin sensitization, skin irritation and corrosion, eye irritation and corrosion, and acute oral toxicity, acute inhalation toxicity, and acute dermal toxicity. To provide an option for replacing or reducing animal use for these endpoints, NICEATM scientists and collaborators created a publicly accessible Systemic and Topical chemical Toxicity (STopTox) web portal, a comprehensive collection of computational models that can predict the toxicity hazard of small organic molecules (Borba et al. 2022). Publicly available data were compiled, curated, and integrated, then used to develop an ensemble of QSAR models for all six endpoints. In addition to high internal accuracy assessed by cross-validation, all models demonstrated an external correct classification rate ranging from 70% to 77%. Scientists and regulators can use the STopTox portal to identify putative toxicants or nontoxicants in chemical libraries of interest.

DASS App for skin sensitization prediction using defined approaches
Tags: NIEHS, defined approaches/IATA, skin sensitization

In June 2021, OECD issued Guideline 497, Defined Approaches on Skin Sensitisation, the first internationally harmonized guideline to describe a nonanimal approach to predict skin sensitization potential. In March 2023, NICEATM launched the DASS App, which computationally applies the defined approaches outlined in Guideline 497 through a user-friendly interface (To et al. 2024). The user uploads data from the test methods used in the defined approach to the web application, which then generates skin sensitization predictions for chemicals of interest. The user selects the analysis variables, and the application dynamically provides feedback about the user's data set to identify problematic data values. This open-access web-based implementation of internationally harmonized regulatory guidelines for an important public health endpoint is designed to support broad user uptake and consistent, reproducible application.

SARA-ICE model for identification of skin sensitizers
Tags: NIEHS, defined approaches/IATA, skin sensitization

NICEATM is collaborating with consumer products company Unilever to test and further develop their Skin Allergy Risk Assessment predictive model (Reynolds et al. 2019) using

data from NICEATM's [ICE](#) resource (SARA-ICE). SARA-ICE is a computational model that uses a variety of input data to estimate a probability that a chemical will cause an allergic skin reaction in humans. It improves upon other similar models by providing a point-of-departure for quantitative risk assessment applications. The model uses publicly available data on 443 chemicals from the ICE database and Unilever SARA and Cosmetics Europe databases and has been applied in several case studies focused on different chemical classes. The SARA-ICE model is under evaluation for inclusion in OECD Test Guideline 497 as a defined approach for derivation of points-of-departure.

One project undertaken during 2022 and 2023 explored the application of SARA-ICE to a diverse set of chemicals nominated by multiple U.S. federal agencies for testing in in vitro skin sensitization assays. The study showed that for this challenging chemical set the SARA-ICE model performs as well as or better than other skin sensitization DAs that are already accepted for regulatory use, and has the advantage of providing a point-of-departure for quantitative risk assessment applications. In a second project, the SARA-ICE model was then applied to provide point-of-departure estimates and hazard classifications for six isothiazolinones, a group of broad-spectrum preservatives. This case study demonstrated that the SARA-ICE model can accurately categorize skin sensitization hazard and potency using in vitro and in vivo data inputs and provide quantitative estimates of human potency that include uncertainty. This work is described in a publication being prepared for submission in 2024. This model is an important tool to assess the probability that exposure to a chemical of interest is "low risk" and to support diverse regulatory decision frameworks.

Toxicokinetics tools to connect metabolism and variability
Tags: NIEHS, metabolism, population variability

Chemicals that enter the body are metabolized via several pathways. Rates of metabolism can vary across human populations due to genetic variability of metabolic enzymes, such that some populations are more sensitive to effects of parent chemicals or metabolites. Risk assessors apply physiologically based kinetic (PBK) models to predict the dynamics of tissue concentrations for parent chemicals and their metabolites, but it is difficult to use these models to characterize the effects of enzymatic pathway-related variability. NIEHS scientists developed a generalized workflow to incorporate pathway-related variability for specific

metabolic enzymes across human populations into PBK models. The workflow includes metabolite structures generated using SimulationsPlus ADMET Predictor®, PBK models from EPA's httk package, estimates of interindividual enzyme variability from European Food Safety Authority reports, and parameter predictions from OPERA (v2.8). Parent chemical dynamics were simulated following initial exposure, and the amount of parent metabolized was scaled by percent yield to provide an intravenous time series for metabolite models. Ranges of parent and metabolite concentrations were estimated by Monte Carlo sampling of enzymatic variability in intrinsic clearance. A case study to demonstrate the utility of the workflow used 10 parent chemicals and their metabolites, and efforts are ongoing to incorporate additional chemicals. In quantifying the range of tissue concentrations resulting from metabolic pathway variability, this work facilitates a more health-protective risk assessment for susceptible population groups. The case study was described in an oral presentation (Hull et al.) at the [12th World Congress on Alternatives and Animal Use in the Life Sciences](#).

Updates to ChemMaps

Tags: NIEHS, Tox21

Access to visualization tools to navigate chemical space has become more important due to the increasing size and diversity of publicly accessible compendiums of HTS and other descriptor and effects data. Construction of such tools relies on complex projection techniques using molecular descriptors. However, application of these techniques requires advanced programming skills that are beyond the capabilities of many stakeholders. Inspired by the Google Maps application, NICEATM developed the [ChemMaps.com webserver](#) to easily navigate chemical space. The first version of ChemMaps.com was limited to exploration of drugs and drug candidates. ChemMaps.com v2.0, released in 2022, added data on approximately one million environmental chemicals from the EPA Distributed Structure-Searchable Toxicity (DSSTox) inventory ([Borrel et al. 2023](#)). ChemMaps.com v2.0 incorporates mapping to HTS assay data from the U.S. federal Tox21 research collaboration, which includes results from approximately 2,000 assays tested on up to 10,000 chemicals. Users can visualize chemical activity both by assay and target directly on the map and compare chemical spaces occupied by active and inactive chemicals. ChemMaps.com v2.0

also has new navigation options, including an on-the-fly distance measurement between two chemicals selected on the 3D map and a map screenshot button.

Computational models for cardiotoxicity via hERG inhibition

Tags: NIEHS, NIH, cardiotoxicity, QSAR, Tox21

Cardiovascular disease is the leading cause of death for people of most ethnicities in the United States. The human ether-a-go-go-related gene (hERG) potassium channel plays a pivotal role in cardiac rhythm regulation, and drug molecules and environmental chemicals can potentially induce cardiotoxicity via hERG inhibition. An evaluation of the effect of environmental chemicals on hERG channel function can help inform the potential public health risks of these compounds. NICEATM and NCATS scientists employed several machine-learning approaches to develop QSAR prediction models for the assessment of hERG inhibition for drug-like and environmental chemicals screened in the Tox21 federal research program ([Krishna et al. 2022](#)). The data and scripts used to generate the hERG prediction models are provided in an open-access format as key in vitro and in silico tools that can be applied in a translational toxicology pipeline for drug development and environmental chemical screening.

Novel artificial intelligence models to predict carcinogenicity

Tags: NIEHS, carcinogenicity, machine learning, QSAR, Tox21

Carcinogenesis is a multistep process in which healthy cells acquire properties that allow them to form tumors or malignant cancers. The concept of key characteristics of carcinogens has been developed to describe 10 properties that are shared by viruses and chemicals that induce human cancers. QSAR models that rely on structural or physicochemical properties to predict carcinogenesis potential endpoints usually perform poorly, likely because they lack sufficient information on the complex mechanisms involved in carcinogenicity. NICEATM scientists and collaborators combined a novel imputation profile QSAR modeling approach with modern machine learning to analyze data on 10,000 Tox21/ToxCast chemicals and 2,000 in vitro assay endpoints associated with key characteristics of carcinogens. Because limited experimental data were available, data gaps were filled by imputing assay results for the Tox21/ToxCast inventory using structural and physicochemical properties and novel artificial intelligence modeling. Various machine-learning approaches including a multitask

deep learning model were applied to predict each chemical's likelihood of inducing cancer based on the imputed in vitro data. Results included output metrics on the quality of imputation, defined by grouping of assays, and performance computed per chemical. Work is ongoing to validate the prediction model results against literature data, develop confidence scores for the imputation modeling, and map assay data to the key characteristics of carcinogens.

PBPK modeling to predict chemical distribution in brain and adipose tissues
Tags: EPA, NIEHS, IVIVE, neurotoxicity

PBPK modeling is used to facilitate decision-making in drug discovery and risk assessment. PBPK models are based on various assumptions and simplifications to make them computationally tractable. Most existing high-throughput, open-source PBPK models predict chemical concentrations in major body compartments such as the liver, kidney, and gut. However, estimates for additional organs require specialized models. As an example, for neurotoxicity evaluations, chemical concentrations in the brain depend upon the activity of the blood-brain barrier. Incorporating the blood-brain barrier in a PBPK model and evaluating whether a chemical can cross this barrier is an important step in assessing the potential neurotoxicity of the chemical. Another limitation of existing open-source PBPK models is that they often do not include an explicit adipose tissue compartment. Adipose tissue plays a critical role in toxicokinetics by acting as a storage compartment for lipophilic chemicals and a source of continuous internal exposure as the chemical is released.

To better estimate chemical concentrations in these two toxicologically relevant compartments, NIEHS and EPA scientists and collaborators added brain and adipose tissue compartments to the existing generic PBPK model from EPA's httk R package (v2.2.2). Concentration–time profiles generated by the model for both hydrophilic and lipophilic chemicals were compared with in vivo data and also with predictions from commercial models. The alignment between the model's predictions against predictions from both commercial models and experimental data indicated that the PBPK model is robust and may be applicable to various aspects of drug development. The project is described in an abstract (Unnikrishnan et al.) accepted for a poster presentation at the [2024 annual meeting of the Society of Toxicology.](#)

Interpretable chemical grouping using an automated KNIME workflow
Tags: NIEHS, structure similarity

With the increased availability of chemical data in public databases, innovative techniques and algorithms have emerged for the analysis, exploration, visualization, and extraction of information from these data. One such technique is chemical grouping, where chemicals with common characteristics are categorized into distinct groups based on physicochemical properties, use, biological activity, or a combination. However, existing tools for chemical grouping often require specialized programming skills or the use of commercial software packages. To address these challenges, NIEHS scientists developed a user-friendly chemical grouping workflow implemented in KNIME, a free open-source low/no-code and data analytics platform. The workflow serves as an all-encompassing tool, expertly incorporating a range of processes such as molecular descriptor calculation, feature selection, dimensionality reduction, hyperparameter search, and incorporates supervised and unsupervised machine-learning methods, enabling effective chemical grouping and visualization of results. The workflow also has tools for interpretation, identifying key molecular descriptors for the chemical groups, and using natural language summaries to clarify the rationale behind these groupings. The workflow was designed to run seamlessly in both the KNIME local desktop version and KNIME Server WebPortal as a web application. It incorporates interactive interfaces and guides to assist users in a step-by-step manner. The workflow is being implemented as part of the Modeling and Visualization (MoVIZ) Pipeline, which is described in an abstract (Moreira-Filho et al.) accepted for a poster presentation at the 2024 Society of Toxicology meeting.

Derivation of an adverse outcome pathway linking VEGF and cardiotoxicity
Tags: NIEHS, AOP, cardiotoxicity, endocrine disruptors, structure similarity, Tox21

Dysregulation of vascular endothelial growth factor (VEGF) and its receptor (VEGFR) contributes to the development of atherosclerosis and cardiovascular disease. This makes the VEGF pathway a potential target for cardiovascular risk assessment of pharmaceuticals and environmental chemicals. Adverse outcome pathways (AOPs) represent a logical sequence of biological responses that contribute to toxicity phenomena and are useful in informing chemical risk assessments. The advent of HTS has made available large-scale in vitro

bioassay data that provides mechanistic information that can help assess chemical toxicity and identify AOP molecular initiating events. This in turn can enable the development of human-relevant NAMs for assessing toxicity without the need for extensive animal experimentation. NIEHS scientists applied AOP frameworks to gain a better understanding of the relationship between VEGFR signaling and the development of atherosclerosis. A data-driven approach was developed to find environmental chemicals linked to the bioactivity of the VEGF signaling pathway, and to investigate their links to other regulatory proteins like estrogen receptor alpha and endpoints like atherosclerosis. ToxCast, Tox21, and PubChem data were evaluated to obtain bioprofiles of 4,165 compounds with bioactivity in assays targeting different VEGFR. An AOP hypothesis was developed by coupling the mechanistic relationships highlighted by HTS data with literature review findings. These linked estrogen, serotonin, and vasopressin receptor targets with VEGFR activity mediated by several endocrine-disrupting chemicals, such as bisphenols, triclosan, dichlorodiphenyltrichloroethane, and polychlorinated biphenyls. Structure-based clustering was performed on relevant bioactive chemicals to evaluate potential molecular initiating events and analyze associations with use-case classes. Computational toxicology profiling of in vitro HTS bioassay facilitates the development of mechanism-driven AOPs and associated chemical perturbants to better understand the link between environmental chemical exposures and potential adverse cardiovascular outcomes. A paper describing this project is in preparation for submission in 2024.

OrbiTox: a computational translational discovery platform for data mining and read-across

Tags: NIEHS, carcinogenicity, cardiotoxicity, genotoxicity, QSAR, structure similarity, Tox21

Visualization tools to navigate chemical space have become more important due to the increasing size and diversity of publicly accessible compendiums of HTS and other effects data. OrbiTox uniquely addresses this need by offering an interactive and immersive 3D environment for visualization of millions of chemicals and their known or predicted activity against gene targets along with available animal study data. By organizing activity in "data domains" as concentric circles, the tool facilitates translational discovery by inferring knowledge from connections across multiple data domains. OrbiTox has a rich, user-friendly

interface, offering almost instantly refreshing visualizations along with extensive gap-filling capabilities. The repository contains 37 QSAR models for Tox21 assays at 10 uM and 100 uM thresholds, 13 Ames mutagenicity models (including ten bacterial strain-specific assays), and five cardiotoxicity assays. These models use Saagar molecular fingerprints ([Sedykh et al. 2021](#)), which provide chemistry-backed reasoning for each prediction. Based on the structural features and motifs responsible for a prediction, the user can hypothesize mechanistic steps for a given chemical or identify favorable or unfavorable chemotypes for a desired property profile and/or prioritize experimental testing. The January 2024 release will include major architecture and feature updates, with QSAR reports generated as PDFs, an additional 29 Saagar features, and the addition of QSAR models for Ames mutagenicity and cardiotoxicity. The June 2024 release will add 304 ToxCast assays, six cardiotoxicity models, carcinogenicity models, and metabolic similarity evaluations for read-across.

Tox21 Cross-partner Projects

[Tox21](#) is a collaboration among groups within four U.S. federal organizations aimed at developing more efficient approaches to predict how chemicals may affect human health. Tox21 studies use assays that are run at higher throughput than traditional tests. Test approaches developed and data collected via this initiative may enable agencies to reduce reliance on animal data for assessing chemical safety.

The four groups participating in the Tox21 collaboration are ICCVAM members:

- U.S. Environmental Protection Agency
- U.S. Food and Drug Administration
- NIEHS Division of Translational Toxicology (DTT)
- National Center for Advancing Translational Sciences (NCATS; within the National Institutes of Health)

Tox21 projects and projects using Tox21 data are described below and [throughout this report](#).

High-throughput transcriptomic analysis of Tox21 chemicals to further develop toxicity pathways

Tags: EPA, NIEHS, AOP, Tox21

Gene expression profiling with alternative toxicological cell systems is a valuable tool to explore mechanisms of chemical interactions in biological systems. However, interpretation

of these data for decision-making is challenging. EPA and NIEHS scientists are addressing this gap by building interpretable bridges between environmental chemicals and specific molecular targets of biological response such as nuclear receptors, enzymes, and transporters. A set of over 300 reference chemicals has been identified and procured to build robust transcriptomic data sets in various cell systems, including the U-2-OS human osteosarcoma cell line and differentiated cultures of HepaRG and 3D microtissues of primary hepatocytes, using the TempO-Seq™ technology for next-generation sequencing of cell lysates. Experiments survey a broad range of exposures to capture the initial stages of biological response to environmental chemicals that translate transcriptomic landmarks into reference chemical similarity relationships for elucidating mechanisms with data-poor chemicals. This efficient strategy holds the potential to greatly increase mechanistic understanding of adaptive responses to environmental chemicals at the gene expression level and inform next steps in toxicology research investigations.

Incorporating genetic susceptibility into developmental neurotoxicity screening via population diversity

Tags: EPA, FDA, developmental neurotoxicity, neurotoxicity, population variability, Tox21

The potential for neurotoxicity in children following exposure to environmental chemicals is of concern due to recent increases in the prevalence of neurological disorders such as attention deficit hyperactivity disorder and autism. Neurotoxicity risk for an individual can be influenced both by genetic background and by exposures to neurotoxic chemicals in the environment. To investigate the role of genetic diversity in susceptibility to neurotoxicity, scientists at EPA and FDA are using a genetically diverse set of cells to evaluate a curated set of chemicals with neurotoxic potential. Neural progenitor cells were derived from a set of mice bred to maximize genetic diversity, and a set of 135 cell lines was established, considered to be the minimum number of cell lines needed to quantitatively assess diversity in population responses. The panel of cell lines was exposed to eight concentrations of a 12-chemical test set. The intracellular structure of each treated cell was visualized using a high-content imaging assay called Cell Painting, which uses six fluorescent dyes to quantitatively describe cell features such as cell membranes, mitochondria, DNA and RNA, cytoskeleton, and Golgi bodies (Bray et al. 2016). Data analysis has enabled derivation of points-of-departure for chemical-induced intracellular morphometric endpoints and characterization of

the variability in these points-of-departure across cell lines for each chemical. Analysis is ongoing to utilize a probabilistic framework to inform data-driven uncertainty factors that describe interindividual variability, allowing for adequate protection of genetically sensitive subpopulations. A paper describing the project is planned for submission in early 2025.

Performance-based validation of Tox21 assays

Tags: EPA, NIEHS, NIH, developmental toxicity, endocrine disruptors, Tox21

To use data generated by HTS initiatives such as Tox21 and ToxCast in regulatory applications, the assays and models built from the assays must be validated based on their performance against the biological targets they query. This requires developing sets of reference chemicals that consistently yield reproducible results when assayed for these biological targets. Furthermore, the development of reference chemical sets needs to be streamlined and rapid enough to manage the tens to hundreds of assays that can help inform regulatory toxicity endpoints. To address this need, scientists at EPA and NIEHS developed a process to identify reference chemicals that consistently produce positive or negative results when tested in defined assays ([Judson et al. 2019](#)). Current work under this project in partnership with NCATS seeks to automate the generation of reference chemical lists without the need for new experimental tests or upfront literature review. The targets being examined are androgen receptor, estrogen receptor, glucocorticoid receptor, peroxisome proliferator-activated receptor gamma, progesterone receptor, retinoic acid receptors, thyroid hormone receptor, tumor protein P53, mitochondria toxicity, and cell stress pathways. The list of assays and reference chemicals generated through this process will be used to validate new assays or AOPs, which will improve chemical screening abilities when predicting toxicity. The project will produce a reference chemical selection workflow and an associated paper to be submitted for publication in 2024.

Retrofitting existing Tox21 HTS assays with metabolic capability

Tags: EPA, NIEHS, NIH, endocrine disruptors, metabolism, Tox21

HTS assays that have been run in the Tox21 testing program to date generally lack the metabolic activity found in living systems, which can potentially increase or decrease the toxicity of chemicals. As a result, HTS results may not accurately reflect in vivo activity. Scientists at EPA, NCATS, and NIEHS are using several approaches to address this problem:

adding human or rat liver microsomes into the existing assays ([Ooka et al. 2022](#)), transfecting cells with mRNAs encoding human metabolic enzymes, or using metabolically capable human HepaRG cells. The retrofitted assays are then used to screen the Tox21 10K chemical library to identify chemicals that are either bioactivated or detoxified by metabolic activity. Chemicals that exhibit metabolism-mediated shifts in bioactivity are further screened to identify the responsible metabolic enzymes. This screening data can be used to build predictive models for identifying the CYP inhibition. A paper describing the results of the screening is planned for publication in 2024. Retrofitted assays are also being used to explore how metabolism might affect interaction of chemicals with the androgen receptor pathway; screening of candidate chemicals will be ongoing in 2024.

Expansion of pathway coverage by Tox21 HTS assays for better prediction of adverse drug effects

Tags: FDA, NIEHS, NIH, AOP, cardiotoxicity, hepatotoxicity, Tox21

To date, Tox21 HTS assays have focused primarily on selected nuclear receptor and stress response pathways. This relatively limited focus suggests that activity in other toxicity pathways has not been adequately assessed; it is likely that some unexplored pathways relate to unanticipated adverse drug effects. Therefore, expanding the coverage of biological responses by adding assays that probe under-represented pathways in the current Tox21 assay portfolio may improve the predictivity of Tox21 data. Scientists at FDA, NCATS, and NIEHS are systematically identifying these under-represented pathways in a data-driven approach and nominating assays for development and Tox21 chemical screening. The data generated will be used to build models for human toxicity prediction, focusing on common adverse drug effects such as drug-induced liver injury and cardiotoxicity. The initial survey identified targets and pathways of interest, including cytochrome P450 metabolic enzymes and G protein-coupled receptors. In 2022 and 2023, HTS assays for the proposed new targets and pathways were screened against the Tox21 10K chemical library. Predictive models for hepatotoxicity and cardiotoxicity were built using chemical structure and Tox21 in vitro assay data ([Ye et al. 2022](#)). Predictions from the models are currently being validated in relevant assays. A second manuscript evaluating the impact of the newly generated assay data on improving model performance is under preparation and planned to be submitted in 2024.

Machine-learning models to predict nonspecific thiol reactivity in high-throughput screening assays

Tags: EPA, NIH, machine learning, structure similarity, Tox21

HTS programs such as Tox21 gather data on chemical bioactivity in a variety of targets and activities to prioritize assays for further testing or better understand mechanisms of toxicity. However, interpreting such data is complicated by nonspecific chemical reactivity. To help address this problem, HTS assays have been developed to measure autofluorescence and data from these assays used to train machine-learning models to predict reactivity. EPA and NCATS scientists used a fluorescence-based HTS assay that identifies thiol-reactive compounds to screen 7,872 unique chemicals in the Tox21 10K chemical library (Patlewicz et al. 2023). Active chemicals were compared with profiling outcomes using structural alerts encoding electrophilic information. Random Forest classification models based on chemical fingerprints were then developed to predict assay outcomes. The model developed shows promise as a tool to screen untested chemicals for their potential electrophilic reactivity based solely on chemical structure.

Predictive toxicology of the retinoid signaling pathway

Tags: EPA, FDA, NIEHS, NIH, AOP, developmental toxicity, IVIVE, metabolism, Tox21

The developing child is vulnerable to genetic, pharmacological, or chemical disruption of the retinoid biochemical pathway, especially during early growth and differentiation of embryonic tissues. Susceptibility of this pathway to chemical disruption is an important regulatory concern for developmental and reproductive hazard identification. In this study, which involves all four Tox21 participating offices, Tox21 data are being mined and modeled to identify potential retinoid pathway disruptors. Results from a dozen Tox21 molecular targets mapping to retinoid pathway targets identified over 100 structurally diverse chemicals with relevant bioactivity. Computational tools and approaches are now being built to integrate these data with embryological knowledge and construct data-driven models for developmental hazard prediction. Recent publications from the project described:

- Identification of candidate reference chemicals for use in in vitro assays relevant to the pathway (Baker et al. 2022).

- A computational model for fetal skeletal defects potentially linked to disruption of retinoic acid signaling ([Pierro et al. 2022](#)).

- Application of IVIVE to characterize potency of chemicals interacting with the pathway ([Chang et al. 2022a](#)).

- Development and validation of a high-throughput assay measuring activity of the metabolic assay CYP26A1 ([Sakamuru et al. 2024](#)), and screening of a library of pharmacologically active chemicals with the assay.

Planned activities in 2024 include analysis of the CYP26A1 assay data, development and validation of assays measuring activity of additional CYP enzymes, further application of quantitative IVIVE for potency assessment, and evaluation of a cytokine array for profiling microglia activation.

Investigation of environmental determinants of pubertal timing in girls
Tags: NIH, NIEHS, developmental toxicity, endocrine disruptors, Tox21

Over the past decade, there has been a worldwide trend toward earlier breast development in girls. The rapid pace of this trend suggests the involvement of environmental factors. Scientists at NIEHS and NCATS are examining the potential effects of endocrine-disrupting chemicals on important components of the biochemical pathway responsible for pubertal timing. In these studies, 58 chemicals in the Tox21 10K collection were tested in human cell-based assays that measure activation or inhibition of the gonadotropin-releasing hormone receptor and kisspeptin receptor. These receptors are expressed in the hypothalamus and are important players in the control of pubertal timing. Chemicals that exhibited activity included chemicals known to be active in these pathways and chemicals for which this activity was novel; these were characterized further in other cell-based assays and molecular docking studies. Further studies are necessary to investigate whether exposure to these chemicals might play a role in the increasing prevalence of early puberty in girls.

Confidence

Establishing confidence in new testing methods requires engagement by both federal agencies and stakeholders. To support this goal, ICCVAM agencies evaluate the suitability of new methods for specific purposes and facilitate communication and education about new methods.

Assay Application

ICCVAM and its member agencies conduct, support, and evaluate studies investigating the suitability of new laboratory methods for specific purposes. Methods evaluated during 2022 and 2023 leveraged complex in vitro approaches such as MPS, as well as DAs and applied integrated approaches to testing and assessment (IATAs) to areas such as topical toxicity, ecotoxicity, inhalation toxicity, and identifying endocrine-active substances.

Validation of 96-well EASA assay to detect potential skin sensitizers
Tags: CPSC, DoD, FDA, NIEHS, NIOSH, NIST, AOP, skin sensitization

The electrophilic allergen screening assay (EASA) is a promising in chemico method to identify substances that covalently bind to skin proteins, the first key event in the AOP for skin sensitization. This assay assesses the depletion of either of two probe molecules in the presence of a test compound. The initial version of the EASA, developed by the National Institute for Occupational Safety and Health (NIOSH), used a cuvette format, which presented multiple measurement challenges such as low throughput and the inability to include adequate control measurements. Scientists with the National Institute of Standards and Technology (NIST), CPSC, and NIEHS redesigned the EASA into a 96-well plate format that incorporates in-process control measurements to quantify key sources of variability each time the assay is run ([Petersen et al. 2022](#)). The paper also describes a measurement science approach that provides steps that can be taken during assay development to increase confidence of in chemico assays and other new approach methodologies (NAMs) by characterizing sources of variability and potential biases and incorporating in-process control measurements.

A subsequent validation study assessed intra- and interlaboratory of the 96-well EASA assay. In addition to the CPSC/NIST lead lab, laboratories sponsored by FDA, Department of

Defense (DoD), and NIEHS participated in the validation study. Each participating laboratory tested 10 positive and negative control substances and 20 reference chemicals. Within- and between-laboratory reproducibility met performance criteria established by the validation management team. A report of the validation study has been prepared and a peer review of the study will be conducted in 2024.

Use of EpiDerm skin models for risk assessment of textile-integrated liquid metal electrodes

Tags: DoD

Next-generation textile-based wearable sensing systems will require flexibility and strength to maintain capabilities over a wide range of deformations. However, materials currently used for textile-based skin contacting electrodes lack these key properties, hindering applications such as electrophysiological sensing. U.S. Air Force (USAF) scientists and collaborators developed a facile spray coating approach to integrate liquid metal nanoparticle systems into textile form factors for conformal, flexible, and robust electrodes ([Li et al. 2022](#)). The system employs functionalized liquid metal nanoparticles that provide a simple "peel-off to activate" means of imparting conductivity. The spray coating approach combined with the functionalized liquid metal system creates long-term reusable textile-integrated liquid metal electrodes. To perform risk assessment on these materials, USAF investigators utilized an in vitro reconstructed tissue model (EpiDerm FT). They demonstrated biocompatibility of textile-integrated liquid metal electrodes in an in vivo skin environment and improved sensing performance compared to previously reported textile-based dry electrodes. The "spray on dry—behave like wet" characteristics of the textile-integrated liquid metal electrodes opens opportunities for textile-based wearable health monitoring and augmented/virtual reality applications that require the use of flexible and conformable dry electrodes.

Use of gut-on-a-chip models to screen engineered synbiotics

Tags: DoD, MPS

Synbiotics are a new class of live therapeutics employing engineered genetic circuits. The rapid adoption of genetic editing tools has catalyzed the expansion of possible synbiotics,

exceeding traditional testing paradigms in terms of both throughput and model complexity. USAF conducted two projects using gut-on-a-chip models to evaluate candidate synbiotics.

- SYN is a cortisol sensing tryptamine-producing synbiotic for cognitive performance sustainment. It can sense cortisol at physiological concentrations and activates a genetic circuit that produces tryptophan decarboxylase and converts bioavailable tryptophan to tryptamine. The gut-chip provided a stable environment to characterize the sensitivity of the cortisol sensor and dynamic range by altering cortisol and tryptophan dosimetry. Collectively, the human gut-chip provided human-relevant apparent permeability to assess tryptophan and tryptamine metabolism, production, and transport, and enabled host analyses of cellular viability and proinflammatory cytokine secretion, providing a successful efficacy test of a novel synbiotic ([Nelson et al. 2023](#)).

- The ECN synbiotic is a medical countermeasure taken to prevent traveler's diarrhea, an illness caused by *Shigella flexneri* infection that affects nearly 80% of all deployed warfighters, impacting readiness and performance. In an ongoing study, USAF used a gut-on-a-chip to model traveler's diarrhea infection. Host assessments of barrier integrity, cellular viability, cytokine production, and transcriptomic responses were conducted, showing that *S. flexneri* challenges reduced barrier integrity by 2-fold, and increased host-cell cytotoxicity by 1.5-fold. In addition, high-resolution confocal microscopy of host tissue morphology and phenotypic alterations in tight-junctions and mucin production were evaluated in response to *S. flexneri* challenge, showing that the pathogen was able to effectively invade and replicate within the intestine-chip. When the ECN synbiotic was administered as a prophylactic, *S. flexneri* was unable to infect, and was undetectable in samples after 4 hours.

Impact of dynamic oxygen conditions on a human neurovascular unit-on-a-chip
Tags: DoD, MPS, inhalation toxicity, neurotoxicity

Aircrew piloting high-performance aircraft face environmental stressors during missions that could reduce efficiency and compromise success. One of those stressors is hypoxia. The military has addressed the issue of hypoxia by supplying high levels of oxygen to the pilots. Cognitive symptoms have been observed that may be related to pilot oxygenation, leading to

concern over negative impacts directly from hyperoxia or from hypoxia that may be caused by a mismatch between the pilots and the equipment. To study the mechanisms that may be affected, USAF scientists used a neurovascular-unit-on-a-chip model built in Emulate microfluidic chips and exposed it to either hypoxia or hyperoxia. Oscillatory hypoxia and hyperoxia exposures were included to address the potential mismatch between pilot breathing and oxygen supply from the equipment. Both hypoxic and hyperoxic conditions were found to disrupt the blood-brain barrier, although viability was not affected very much by hypoxia or hyperoxia in the vascular compartment. In the brain compartment, both hypoxia and hyperoxia appeared to improve viability of the cells.

Stimulation of hiPSC neuron co-cultures using a 3D printed platform
Tags: DoD, MPS, neurotoxicity, stem cells

It is not unusual for Air Force pilots to experience cognitive fatigue, which has the potential to compromise mission success. USAF scientists are developing transcranial alternating current or direct current therapies that use electrical stimulation to promote neural plasticity and mitigate fatigue. A 3D-printed platform using human induced pluripotent stem cell-derived neurons and astrocytes is being used to investigate the impact of applying direct and alternating currents on stress levels and gene expression. Transcriptional alterations, viability, and morphological changes were assessed to determine the impact of low current stimulation, emulating transcranial therapies. Extrapolation of this technique to include high-density microelectrode arrays will enable an understanding of how low current stimulation impacts neuronal firing, bursting, networking, and synaptic plasticity. Taken together, this in vitro platform allows for both functional and molecular analyses of electrical stimuli representing transcranial therapies with the goal of optimizing future therapies to combat military cognitive fatigue.

Alveolus-on-a-chip reveals distinctive soot-based toxicity biomarkers in extracellular vesicles
Tags: DoD, inhalation toxicity, mixtures toxicity, MPS

Rapid advancements in engineering have driven the need to understand human exposure to ultrafine particles and volatiles. To this end, USAF scientists developed an in vitro inhalation model to quickly assess toxicity. This model has been utilized to identify unique biomarkers

of soot-based exposures, an emerging respiratory global concern to human health and performance. An alveolus-on-a-chip platform was developed using primary alveolar epithelial and microvascular endothelial cells cultivated at an ALI. The platform incorporates cyclical stretch and breathing mechanics to better emulate human physiology in a laboratory model. A primary goal of this effort is to establish and optimize extracellular vesicle isolation, enumeration, and molecular cargo analysis workflows using a relatively low number of cells and conditioned medium. Extracellular vesicles carry cellular material such as microRNAs to facilitate cell-to-cell communications in response to a variety of stress conditions. These are found in nearly every biological fluid, providing a rich opportunity for investigating tissue specific biosignatures of exposure. Preliminary results treating the alveolus-on-a-chip platform with diesel particulate matter resulted in increased cytotoxicity and a statistically significant decrease in lung-blood barrier integrity. These preliminary findings drove the development of a "mini-jet engine" soot generator, an in vitro aerosol deposition platform, and the integration of the alveolus-on-a-chip to form a platform termed AERO-TOX (Aerosol Exposure of Respiratory Organ-on-a-Chip for Toxicology). The soot generator has been optimized to produce soot of relevant size distribution and mixtures with common combustion reaction gases such as nitric dioxide and carbon monoxide, and has been statistically shown to emulate F-22 jet exhaust soot profiles. Investigators now aim to utilize the AERO-TOX platform to discover novel extracellular vesicle-derived biomarkers of exposure to aid in next-generation diagnostic and countermeasure development.

Incorporation of organ-on-a-chip technology to streamline drug candidate characterization

Tags: DoD, inhalation toxicity, hepatotoxicity, MPS

U.S. Army Chemical Biological Center (CBC) scientists are validating a highly complex and human-relevant multiorgan chip in its ability to serve as a model system for antimicrobial drug efficacy and safety and to recapitulate physiologically based pharmacokinetic parameters.

While MPS technologies are gaining traction as a viable alternative model for toxicity studies, further characterization is necessary to explore their full translational potential, as well as the utility of these platforms to serve as a diagnostic tool. For human-relevant omics data to be truly reliable, samples must be collected and analyzed from patients or individuals

infected by or exposed to the drugs or agents in question. CBC scientists investigated how well bioinformatic data collected from organ chips matches the bioinformatic data collected from human samples, with the hope that organ chips will provide an alternative approach for improved biomarker discovery for toxicity assessment and exposure identification. TissUse Chip3 multiorgan chips seeded with kidney organoids, liver organoids, and full thickness lung tissue were exposed to acetaminophen and analyzed to characterize effects on protein content and metabolism. Data from the organ chips largely matched what was found in the published literature, including the identification of several known acetaminophen metabolites and biotransformation products. These data suggest that organ chips may be a suitable surrogate for human biomarker identification and drug exposure diagnosis.

CBC is also incorporating immune components into commercially available chip systems. The pulmonary system is an ideal exposure route for chemical and biological agents making mast cells a prime choice to assimilate into a lung-on-a-chip. Mast cells rapidly release a variety of mediators such as histamine, heparin, proinflammatory cytokines, and chemokines in response to foreign substances or endogenous damage associated molecular patterns. These factors facilitate immune cell recruitment and play a key role in pulmonary toxicity due to mast cells location within airways and mucosal surfaces of the lungs. CBC is collaborating with the University of Colorado Anschutz Medical Campus to integrate a mast cell immune component into the Emulate system. This work will allow substantial future work to incorporate other immune cells into the system to build toward a complete model of immune system infection prevention and pathogen defense.

Validation of human reconstructed lung tissue in vitro models for acute lung injury from gas exposures
Tags: DoD, AOP, inhalation toxicity, IVIVE

A comparison of in vitro results to in vivo data is necessary to establish suitability of the in vitro models as a replacement for in vivo acute lung toxicity for volatile chemical hazards. USAF Predictive Risk Team (PRT) is evaluating the mechanistic predictivity of respiratory in vitro models by comparing the in vitro dose-response curves for key events in acute respiratory to in vivo rodent and human data following exposure to prototype respiratory toxicants. Key events in the acute lung injury AOP are being measured in a monoculture cell model (A549) and human reconstructed tissue models (EpiAirway, EpiAlveolar). IVIVE will

be performed using in silico models describing gas disposition in the lung. Assays and measured endpoints that are determined to be most predictive of in vivo response will be incorporated into a rapid chemical testing protocol. These NAMs are expected to facilitate scientifically sound chemical risk assessments on a timescale that would realistically support operational and acquisitions decisions. The project was selected for funding by the Military Operational Medical Research Program in 2023, with experimental work planned to begin in 2024.

Case study for NAM-based rapid risk assessment of acute toxicity using a tiered-testing strategy

Tags: DoD, acute toxicity, IVIVE, MPS

USAF PRT is developing a NAM-based rapid approach to better support risk assessment of operational exposure to novel and poorly characterized chemicals. This is a tiered process where lower tiers use in silico and high-throughput in vitro models to identify hazards and prioritize testing, and higher tiers use organotypic in vitro models to perform quantitative risk assessments. PRT is also building models and validating assays to support this approach, focusing initially on acute toxicity. To test the utility of this NAM-based approach and developed models, the PRT is performing case studies with several occupationally relevant chemicals. These chemicals are being tested with available in silico models (Tier 0) to evaluate mode-of-action and acute systemic toxicity. Based on in silico results, the chemicals are being tested in high-throughput (Tier 1) and organotypic (Tier 2) in vitro assays, which will be used with IVIVE to derive an equivalent administered dose (EAD). EADs will be compared to in vivo points-of-departure (PODs) and published exposure limits to assess the utility of this process for estimating safe limits for acute operational exposures.

Evaluation of IATA-based assessment of skin sensitization potential in Air Force occupational exposures

Tags: DoD, defined approaches/IATA, skin sensitization

USAF PRT performed a study to characterize the risk of allergic contact dermatitis from occupational chemical exposures using NAMs. The goals of the study were to evaluate the sensitization potential of chemicals used by fabricators and maintainers and to develop preliminary candidate surface guidelines, i.e., guidelines for maximum surface concentrations to prevent induction of sensitization. The kinetic direct peptide reactivity and

KeratinoSens^{TM} assays were used to predict mouse local lymph node assay effective concentration values. In vitro assay results, in silico model predictions, and available human and animal data were used in an IATA to predict sensitizer status using a weight-of-evidence approach. Preliminary candidate surface guidelines were derived from predicted effective concentration values for predicted sensitizers. Findings from this study were published by DoD as a [Defense Technical Information Center](#) report. Additional data from the in vitro human cell line activation test and updated analyses will be presented at the 2024 SOT annual meeting. A manuscript describing this work is planned for peer-reviewed publication in 2024.

Strategy using in silico methods and zebrafish assays to characterize algal toxins and contaminants

Tags: DOI, cardiotoxicity, ecotoxicity, mixtures toxicity, small model organisms

As part of ongoing assessments of wildlife health, DOI is investigating potential chemical effects on the cardiovascular system and general health, with measured endpoints including pericardial area, circulation, heart rate, body length, median lethal concentration, and mode-of-action. Chemicals of interest include pesticides and pharmaceuticals detected in surface waters and fish tissues, as well as polycyclic aromatic hydrocarbons (PAHs) and oxygenated PAHs from a subsurface oil spill by assaying groundwater samples. DOI's USGS conducts high-content screening of compounds to formulate hypotheses and prioritize contaminants for further toxicity testing. This approach reduces animal use, quantity of test compound, and waste by utilizing pre-feeding zebrafish embryos in a microtiter plate format. The high-content screening is also being coupled with behavioral assays and in silico approaches to characterize toxicity of algal toxins associated with harmful algal blooms, which continue to pose health concerns to the public and natural resources. These assays can provide evidence to justify larger-scale studies to determine actual versus perceived risk of contaminants.

Use of in vitro assays to test for endocrine activity

Tags: DOI, ecotoxicity, endocrine disruptors

DOI's USGS applies and modifies microbial and eukaryotic reporter bioassays to detect the presence of bioactive chemicals in water samples. These analyses compliment traditional analytical chemistry and biological datasets, which detect specific analytes, by documenting

synergistic or antagonistic activities detected by in vitro assays. Additionally, bioreactivity can be measured by bioassays, where it might be missed by traditional methods targeting suspected, but unconfirmed, compounds. USGS is using the bioluminescent yeast estrogen screen to estimate total estrogenicity of a test substance. Relative net agonistic activity per liter is calculated based on sample concentration. Similar assays utilizing other yeast strains can be conducted to determine the presence of androgens and measure cytotoxicity of chemical compounds collected in water or other matrices.

Use of minimally invasive sampling for epidemiology studies
Tags: DOI, ecotoxicity

Virus transmission from handling of fish has not been adequately studied, but damage to a fish's external slime coat may facilitate entry of pathogens. Blotchy bass syndrome is a condition characterized by visible, variable, and discrete areas of hyperpigmentation on the external surface of black basses. This condition has received increased attention from anglers and resource managers in recent years and is a popular topic of discussion and reporting on angling websites and blogging platforms. Advances in sequencing technology and diagnostics led to the discovery that blotchy bass syndrome is associated with viruses of the family Adomaviridae. Sampling of the epidermis of affected fish is necessary to identify presence of adomaviruses. Typical sample collection for suitable molecular biology methods requires removal of fish scales or clips of fin tissue to collect DNA. Recently, USGS has begun using non-invasive skin swabs in lieu of traditional sampling methods. Skin swabbing does not require the use of anesthetics, is fast (<10 seconds), and reduces changes in behavior and physiology associated with tissue clips. It is a more refined technique for DNA and RNA collection with the potential to improve animal health and welfare. By using swabs and shelf-stable collection buffers, USGS was able to undertake a nationwide effort using both state agencies as well as live-display animals at public aquaria to collect minimally invasive samples. Such collections have been used to generate complete genomes and has resulted in the identification of at least five novel viruses.

Use of in vitro data for developing respiratory irritation points-of-departure and human risk assessments
Tags: EPA, inhalation toxicity

As part of its human health risk assessments, EPA evaluates potential health effects from different routes of exposures based on the pattern or conditions of use. Subchronic studies performed with laboratory animals (typically rats) are used to evaluate route-specific inhalation effects. However, human and animal respiratory tracts differ to an extent that may affect the ability of animal test results to accurately predict effect in humans. Furthermore, there are specific challenges associated with testing irritating and corrosive compounds. The EPA Office of Pesticide Programs previously used a refined inhalation approach that employs an in vitro inhalation model to derive a POD for [inhalation toxicity for the fungicide chlorothalonil](#), which is a direct contact irritant. The EPA Office of Pesticide Programs has since been working with staff in the Office of Research and Development and external researchers to investigate the use of in vitro data to apply similarly for other contact irritants or to provide additional information for other chemicals.

Screening of PFAS for potential developmental neurotoxicity using NAMs
Tags: EPA, defined approaches/IATA, developmental neurotoxicity, PFAS

PFAS are a distinct set of commercial chemicals widely found in humans and the environment. However, only a small number of PFAS have epidemiological or experimental data to characterize any potential hazard they might pose. Using in vitro NAMs, EPA scientists tested 160 PFAS to characterize their potential to induce developmental neurotoxicity (DNT) ([Carstens et al. 2023](#)). The [DNT NAMs battery](#) used evaluated proliferation, apoptosis, neurite outgrowth, and neural network formation. While most of the PFAS tested were inactive or equivocal in the DNT NAMs, specific chemical structures were identified that appeared to correlate with PFAS bioactivity in the NAMs. These data demonstrate that a subset of PFAS perturb neurodevelopmental processes in vitro and suggest focusing future studies of DNT on PFAS with specific structural features. Analytical quality control indicated that significant numbers of both inactive PFAS and active PFAS samples were degraded. This indicates a need for careful interpretation of test results of these substances, as some negatives may have been due to loss of the parent PFAS and some active results may have been caused by PFAS degradants.

Integration of NAMs into a weight-of-evidence analysis for pesticide DNT assessment
Tags: EPA, defined approaches/IATA, developmental neurotoxicity

Guideline in vivo DNT studies are conditionally required for pesticide registration with need determined on a case-by-case basis taking into consideration toxicological effects, biological plausibility, and an understanding of the mode-of-action. However, the guideline DNT study is time-consuming and costly both financially and in terms of animal use. While such studies have been conducted on the herbicide DL-glufosinate ammonium (DL-GLF), no such data exist for L-GLF acid and L-GLF ammonium, compounds that have the same molecular composition as DL-GLF but are structurally different. This situation presented an opportunity for EPA scientists to explore whether toxicokinetic assessments based on in vitro assay data could be used to support a decision for the need for guideline DNT studies (Dobreniecki et al. 2022). DL-GLF, L-GLF acid, and L-GLF ammonium were screened using in vitro assays for network formation and neurite outgrowth, and toxicokinetic assessments were conducted to derive administered equivalent doses for the in vitro testing concentrations. The results indicated that the available guideline study would be protective of potential DNT due to L-GLF exposure, and were thus used in a weight-of-evidence evaluation to support the decision not to require L-GLF isomer guideline DNT studies, providing a case study for a useful application of DNT screening assays. Similarly, results from in vitro DNT studies were used in a weight-of-evidence evaluation to support the need for additional DNT data for the pesticide dicloran. EPA has also continued its work to analyze data from the DNT NAM battery for use in chemical-specific weight-of-evidence analyses to evaluate the DNT potential of organophosphate pesticides with the weight-of-evidence evaluation for the organophosphate insecticide acephate released in 2023 as part of registration review.

Application of IATAs to DNT evaluation of organophosphorus flame retardants
Tags: EPA, NIEHS, defined approaches/IATA, developmental neurotoxicity

Organophosphorus flame retardants are abundant and persistent in the environment due to their extensive use in industrial processes and products. The structural similarity of these flame retardants to organophosphate pesticides has prompted concern that they could potentially cause both acute neurotoxicity and DNT.

DNT testing is traditionally done using animals, which is resource-intensive and fails to provide information on cellular processes affected by chemicals. OECD has published a series of case studies to support application of IATAs to identification of potential

developmental neurotoxicants. One of these case studies, developed by EPA and NIEHS scientists, used an in vitro testing battery to prioritize a class of organophosphorus flame retardants. The case study was published in September 2022.

Since the development of the IATAs, new data have become available that could refine the testing approach and provide a stronger case study. NIEHS is currently extracting these data and incorporating additional parameters such as exposure data, toxicokinetics, and endpoints and mechanisms relevant to DNT but not included in the original data. The revised dataset is described in an abstract (Oyetade et al.) accepted for a poster presentation at the 2024 annual meeting of the Society of Toxicology. The original case study submitted to OECD will be revised into manuscript that will include and integrate the new data and updated resources into the information generated in the first project.

Development and evaluation of integrated testing strategies for eye irritation evaluation of agrochemical products

Tags: EPA, NIEHS, defined approaches/IATA, eye irritation, mixtures toxicity

NICEATM, PETA Science Consortium International e.V., 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 DAs for agrochemical formulations testing for prediction of U.S. and international irritancy classifications. Agrochemical formulations were selected for prospective testing based on availability of historical rabbit test data, to represent common agrochemical formulation types, and to span the full range of ocular irritation hazard. Test methods were included based on their relevance to mechanisms of human eye irritation, and the results were assessed to determine which methods should advance to potential incorporation in a DA. Twenty-nine formulations were tested in up to five methods: bovine corneal opacity and permeability, EpiOcular, SkinEthic time-to-toxicity for liquids, in vitro depth of injury, and EyeIRR-IS.

In a project led by PETA Science Consortium International, two DAs were developed to predict eye irritation potential in the context of the EPA pesticide classification system (van der Zalm et al. 2023). Predictions derived using the DAs were assessed using orthogonal

validation and weight-of-evidence, rather than direct concordance analysis with the historical in vivo rabbit eye data. Both DAs were demonstrated to be as or more fit-for-purpose, reliable, and relevant than the in vivo rabbit eye test.

A separate project led by NICEATM focused on predicting eye irritation potential based on the GHS classification system. A preliminary analysis of alignment across the five in vitro methods and historical rabbit test data was conducted to determine consensus predictions for each formulation. Four methods were then used in the development of four DAs to predict GHS classifications. All four proposed DAs may have high utility for predicting eye irritation classification of agrochemical formulations, as the hazard labeling associated with the predictions are as or more protective of human health compared with the in vivo rabbit test. Furthermore, using the consensus prediction as the reference standard, some standalone in vitro methods can predict the human eye irritation hazard of agrochemical formulations as well as or better than the rabbit test. Results of the study will be described in a poster at the [2024 annual meeting of the Society of Toxicology](#) (Daniel et al., Ocular Toxicology session) and in a paper to be submitted for publication in 2024.

Validation of an in vitro human thyroid microtissue model for chemical screening
Tags: EPA, NIEHS, endocrine disruptors, MPS

Current regulatory decision-making for potential thyroid-disrupting chemicals is based on in vivo apical endpoints including alterations in thyroid hormone levels. NAMs to complement or replace traditional in vivo tests are being developed to enable higher-throughput mechanistic understanding of potential endocrine-disrupting chemicals. A key element to the acceptance of NAMs in regulatory contexts is to establish confidence through validation studies that evaluate a NAM's reliability and relevance for a specific application.

NICEATM is coordinating an interlaboratory validation study of the utility of a thyroid 3D microtissue model for evaluating chemical effects on thyroid hormone synthesis and tissue viability. The lead laboratory is within the EPA Office of Research and Development, with three agrochemical industry and commercial labs participating in the transferability phase. Goals of this effort include (1) development of the study design, (2) test method harmonization and standardization and demonstration of intralaboratory reproducibility, and (3) method transferability, reference chemical testing, and demonstration of interlaboratory

reproducibility. Refinement of standard operating procedures, tissue procurement, method standardization in the main laboratory, and an initial assessment of method transferability were completed in 2023. Method transfer and model validation are currently being completed in the additional laboratories, to be followed by method performance evaluation by all laboratories. Results of the study will be described in a study validation report.

Applying MPS to assess liver toxicity
Tags: FDA, hepatotoxicity, MPS

FDA's Center for Food Safety and Applied Nutrition (CFSAN) partnered with organ chip developer Emulate to evaluate the utility of their Beta Human Liver Emulation System for its regulatory science program ([Eckstrum et al. 2022](#)). The platform's performance was evaluated using both known hepatotoxic compounds and compounds that have no reported human cases of liver toxicity. Toxicity was assessed by albumin secretion, urea and lactate dehydrogenase release, nuclei number, mitochondrial membrane potential, and apoptosis. System/platform performance was evaluated in terms of sensitivity and specificity, power, and variability and repeatability. Chemical interactions with the chip material were also assessed. Preliminary findings suggested that for the model test compounds selected, the system accurately predicted toxicity, demonstrated high sensitivity and specificity, high power, and low variability. This evaluation of the Beta Human Liver Emulation System demonstrated that it was easily transferred to the CFSAN laboratory. However, some compounds interacted with the chip material resulting in variable exposure levels that should be accounted for when planning experimentation.

Applying MPS to assess inhalation toxicity of formaldehyde
Tags: FDA, inhalation toxicity, MPS

Formaldehyde is an irritating, highly reactive aldehyde that is widely believed to cause asthma. Additionally, it is classified as a Group 1 carcinogen by NTP and the International Agency for Research on Cancer, and airborne formaldehyde exposure is associated with nasal cancer in animals and humans. FDA-regulated products may release formaldehyde fumes and present toxicity risks to patients and healthcare workers. Airway epithelium is a key boundary between the environment and mammalian systemic circulation. To study tissue responses to formaldehyde fumes in a nonanimal system, FDA scientists used an in vitro

human ALI airway tissue model ([Ren et al. 2022](#)). In the model, exposure to formaldehyde induced functional changes in cells as well as possible squamous differentiation. Although DNA damage was not detected in a comet assay, formaldehyde exposures lowered the rate of DNA repair enzymes suggesting that it interferes with DNA repair ability. A general agreement was observed between in vitro responses to formaldehyde fumes and the reported in vivo toxicity of formaldehyde, supporting the application of the ALI airway system as a potential in vitro alternative for screening and evaluating the respiratory toxicity of inhaled substances.

Use of an in vitro/in silico quantitative systems pharmacology approach to support approval of a naloxone autoinjector

Tags: FDA, VA ORD

Naloxone was the first opioid antagonist specifically indicated for prophylaxis and treatment of overdose from highly potent opioids such as fentanyl and its analogues. Due to ethical considerations, clinical trials evaluating overdose reversal in patients are unfeasible. To support a regulatory review of a naloxone autoinjector 10 mg product under development for use by military personnel and chemical incident responders, FDA and Veterans' Administration scientists and collaborators evaluated whether an in vitro/in silico quantitative systems pharmacology model could be used to support approval ([Mann et al. 2022](#)). The model was used to evaluate the effects of this naloxone product and [contributed confirmatory evidence](#) in support of its approval. The modeling approach was also used to [support approval](#) of another opioid antagonist product, nalmefene intranasal 3 mg. This approach is described in a December 2022 [post on the FDA Regulatory Science in Action website](#). Since then, the model has been used to evaluate different dosing strategies for intramuscular and intranasal naloxone products and to support pediatric dosing. A subset of results was presented at a March 2023 FDA workshop on "[Understanding Fatal Overdoses to Inform Product Development and Public Health Interventions to Manage Overdose](#)." The model is being used to optimize intranasal naloxone repeat dosing strategies, and a paper adapting this model to pediatric patients has been accepted and will be published in 2024 ([Strauss et al. 2024](#)). In addition, the modeling is being expanded to assess the safety of opioid antagonists by simulating precipitated withdrawal, an adverse effect of concern in patients with heavier opioid dependency.

Tissue chips in space
Tags: FDA, NCI, NIH, MPS

In May 2021, the National Aeronautics and Space Administration (NASA) announced a multiagency initiative, "Extended Longevity of 3D Tissues and Microphysiological Systems for Modeling of Acute and Chronic Exposures to Stressors." The initiative is focused on adapting existing 3D tissues and MPS to extend their longevity to at least 6 months. Among the sponsors of the initiative are several ICCVAM agencies: National Institutes of Health (NIH; NCATS and National Institute of Allergy and Infectious Diseases), NCI, and FDA. Proposals for projects to be funded under the initiative were accepted through September 2021, with awardees announced in March 2022.

Fruit fly models for assessment of population variability in toxicity
Tags: NIH, AOP, small model organisms

Toxic responses are specific to cell types, and the gene expression patterns of cell types are highly conserved in evolution. The diversity in gene expression among cell types in a single individual is far greater than gene sequence diversity in populations. Thus, any cell type-restricted study using either cell lines or physiological systems such as organoids will fail to capture the full diversity of human exposure risk. Invertebrate organisms such as insects are exempt from most animal regulations and have thousands of cell types, many of which are shared between these organisms and humans. The European Union PrecisionTox project is an international consortium that explores the replacement of traditional mammalian chemical safety testing by comparative toxicology in fruit flies, nematodes, water fleas, clawed frog and zebrafish embryos, and human cell lines.

NIH is participating in a PrecisionTox study that is supplementing comparative toxicology data using more traditional terminal endpoints with data from gene expression and metabolite profiling induced by low subphenotypic doses of chemicals to map conserved AOPs. The study is leveraging fruit fly genetics to inform testing of key human genes and allelic variants, as well as counteracting chemicals that might change susceptibility in people. These data are expected to provide mechanistic insight and feed predictive models useful for regulating groups of chemicals. During 2022 and 2023, institutions participating in the study assembled information on a 250-chemical library, including chemical class, diversity in

terms of structure, physicochemical properties, toxicity modes-of-action if known, and database/literature-derived associations with disease pathology, genes, and putative metabolic biomarkers. Harmonized toxicity testing experiments were conducted on about 90 substances, with 54 substances having sufficient comparative toxicology results for a first "phylogenetic toxicity analysis" to allow cross-species extrapolation. RNA expression and metabolite profiling is in progress for the 90-substance set, and the consortium is also conducting genome-wide screenings for genetic variation in toxicity. Study data will be made available in a manner supporting FAIR (findable, accessible, interoperable, and reusable) data standards.

PrecisionTox participating institutions are also engaging with government stakeholders to advance the use of NAMs in chemical regulation. Outcomes of the studies will be applied to support the development of cost-effective NAMs to assess chemical hazard and exposure that can be applied by regulatory bodies and industry.

Evaluation of in vitro skin models for dermal absorption studies
Tags: FDA, NIH, NIEHS

Skin permeation is a major consideration in the safety assessment of cosmetics, topical drugs, and veterinary medicine products. FDA, NIH, and NIEHS scientists conducted a study to explore the usefulness of alternative skin barrier models to replace excised human skin or animal models to assess skin penetration (Salminen et al. 2023). A standardized dermal absorption testing protocol was developed to predict skin absorption in humans. Caffeine, salicylic acid, and testosterone were used in side-by-side assessments of a reconstructed human epidermis model, a synthetic barrier membrane model, and an excised human skin model. Transepidermal water loss and histology of the biological models were compared. Based on the results of this study, authors made specific recommendations about how to evaluate and use both alternative skin barrier models and excised human skin to assess skin penetration. Evaluating novel models in the manner outlined in this study has the potential to reduce the time from basic science discovery to regulatory impact.

Broadening applicability of defined approaches for identification of skin sensitizers
Tags: EPA, NIEHS, defined approaches/IATA, mixtures toxicity, skin sensitization

Skin sensitization testing is a regulatory requirement for the safety of pesticides in multiple countries. Globally harmonized test guidelines that include in chemico and in vitro methods reduce animal use, but no single assay is a complete replacement for animal tests. DAs that integrate data from multiple nonanimal methods are internationally accepted, specifically via [OECD Guideline 497](). However, these DAs were evaluated with mono-constituent substances, which may limit their applicability to multi-constituent substances such as pesticides. An analysis by NIEHS scientists and collaborators evaluated rule-based DAs for hazard and/or potency categorization of skin sensitization for agrochemical formulations ([Strickland et al. 2022a]()). The data set for the analysis included 27 formulations, each tested using the direct peptide reactivity assay, the KeratinoSens™ assay, and the human cell line activation test. The KeratinoSens assay had the highest performance for predicting in vivo hazard outcomes and performed better than any of the DAs. The analysis demonstrates that nonanimal test methods are useful for evaluating the skin sensitization potential of agrochemical formulations. Further investigation is necessary to determine whether DAs can outperform individual assays for predicting in vivo sensitization hazard of pesticide formulations in general.

In a separate study, NIEHS and EPA scientists and collaborators evaluated the use of DAs to evaluate sensitization potential of isothiazolinones. Isothiazolinones are used as preservatives in a range of consumer products but are known to cause skin sensitization and irritation. The skin sensitization potential of six isothiazolinones was evaluated using three internationally harmonized nonanimal test methods: the direct peptide reactivity assay, KeratinoSens™, and the human cell line activation test. Results from these test methods were then applied to two versions of the Shiseido Artificial Neural Network defined approach. Hazard or potency predictions showed high concordance with those produced by reference animal test data with less variability. The application of in silico models to in chemico and in vitro skin sensitization data is a promising data integration procedure for DAs to support hazard and potency classification and quantitative risk assessment.

GARD models for identification of potential skin and respiratory sensitizers
Tags: NIEHS, defined approaches/IATA, inhalation toxicity, skin sensitization

To explore broadening the number of test methods that can be used in defined approaches to identify skin sensitizers, NIEHS scientists used the GARD®skin assay (SenzaGen AB) to test 31 substances nominated by several U.S. federal agencies. GARDskin results were applied as a substitute for results from the human cell line activation test within two accepted DAs. Both assays measure the same endpoint, mobilization of dendritic cells and induction of inflammatory cytokines and surface molecules, which represents Key Event 3 in the AOP for skin sensitization. Results were evaluated both for prediction of skin sensitization hazard (i.e., sensitizer vs. nonsensitizer) and potency classification according to GHS categories. Concordance and performance of the GARDskin results were also compared to existing DAs from OECD Guideline 497 and individual test methods, including murine local lymph node assay reference data. While GARDskin tended to overpredict sensitization hazard when compared to reference data skin sensitization hazard classification, concordance against the local lymph node assay was higher for DAs that incorporated GARDskin than those that did not. In vitro testing and DAs provide a useful alternative to animal testing for skin sensitization hazard and potency classification of substances relevant to a wide range of federal agency programs.

Another SenzaGen NAM model, the GARDair assay, shows promise to assess respiratory sensitization, an endpoint for which there is no good animal model. NIEHS and collaborators are testing about 100 substances nominated by U.S federal agencies using GARDair. Results for chemicals tested thus far indicate that hazard classification using the GARDair assay shows promising concordance with hazard classifications based on existing occupational human reference data and results from skin sensitization assays. Both studies are described in an abstract accepted for a poster presentation (Johnson et al.) at the 2024 SOT annual meeting.

Development of machine learning-based approaches to develop cytotoxicity flags for Tox21/ToxCast assays

Tags: NIEHS, machine learning, Tox21

Results of in vitro assays used in the Tox21 and ToxCast HTS programs may be confounded by overt cell stress and cytotoxicity, such that a decrease in viable cells could erroneously be attributed to a chemical's mechanistic effects. Integration of cytotoxicity assessment with assay endpoints can bolster confidence in the interpretation of assay outcomes. However,

many chemicals tested in HTS assays lack directly relevant cytotoxicity data needed to ensure overt toxicity does not confound mechanistic outputs. Chemicals may also vary in their potency for eliciting cytotoxicity across cell types and time trajectories. NIEHS scientists are investigating applying multiple machine-learning algorithms to predict chemical- and cell type-specific cytotoxicity concentrations to provide context for flagging nonspecific in vitro chemical-elicited bioactivity using cytotoxicity assays included in Tox21 and ToxCast. Cell type- and time point-specific machine-learning models were developed to predict chemical concentrations likely to induce cytotoxicity to provide context for assays without concurrent cytotoxicity data. After being further refined, the predictive models will be integrated with bioactivity data to provide context and bolster confidence in assay outcome interpretation for identifying specific vs. nonspecific/cytotoxicity-confounded bioactivities. Concurrent cytotoxicity readouts from Tox21/ToxCast assays were mapped for 492 assay endpoints, allowing direct comparison of bioactivity potency against cytotoxicity. These comparisons will be integrated into future versions of concentration–response visualizations for the curated HTS data in the [Integrated Chemical Environment](#) Curve Surfer tool. This project was described in an abstract accepted for a poster presentation (Tedla et al.) at the [2024 SOT annual meeting](#).

Application of an in vitro exposure system with air-liquid interface airway tissue models for the investigation of inhalation toxicity

Tags: NIEHS, AOP, inhalation toxicity

To reduce animal use for investigating human-relevant inhalation toxicity, the Occupational and Inhalation Exposures Program in the NIEHS DTT is evaluating the use of in vitro lung models, including ALI airway tissues. Potential applications include screening-level assessments to help predict the adverse airway effects of inhaled substances and prioritize them for further testing. A proof-of-concept study was conducted to characterize and optimize a VITROCELL 48 2.0 Plus exposure system with ALI airway tissues. The chemical used was 2,3-pentandione, a highly volatile component of artificial butter flavorings that has been associated with airway injury and fibrosis via occupational inhalation exposure. NIEHS scientists first performed method development and validation of the VITROCELL system using 2,3-pentandione vapor. Then, both normal human and rat tracheaobronchial epithelial cell-derived ALI tissues were treated to evaluate 2,3-pentandione-induced airway toxicity in

vitro. These studies were designed to explore the validity of extrapolations among rat in vivo, rat in vitro, and human in vitro effects in these models. Preliminary results show that exposure of the ALI tissues to 2,3-pentandione induces concentration-dependent changes in relevant airway toxicity endpoints that are comparable between species. Specific observations included decreased transepithelial electrical resistance, cytotoxicity, and histopathologic effects characteristic of the airway injury caused by in vivo exposure to artificial butter flavorings. Results from this work were presented at the SOT annual meeting in 2022 (Gupta et al.) and further results are described in an abstract (Gwinn et al.) accepted for a poster presentation at the 2024 SOT annual meeting. Further work planned for 2024-2025 will focus on expanding the capabilities applicable to this exposure system and tissue models, including supporting transcriptomic analysis, informing on quantitative adverse outcome pathways (AOPs), supporting testing of different types of chemicals, and evaluating reproducibility in other laboratories.

Communication and Education

The 2018 ICCVAM [Strategic Roadmap](#) identified stakeholder engagement as critical to acceptance and use of new methods. To facilitate stakeholder engagement, ICCVAM and its member agencies organize public meetings and webinars. These events inform stakeholders about the availability and appropriate use of new methods and provide opportunities for stakeholders and agencies to discuss opportunities for test method development.

ICCVAM Communities of Practice webinars

Tags: FDA, ICCVAM, NIEHS, developmental neurotoxicity, hepatotoxicity, metabolism, MPS, neurotoxicity, small model organisms

In 2015, ICCVAM initiated a series of Communities of Practice webinars to provide opportunities for detailed presentations on a current topic relevant to alternative test method development.

- The 2022 webinar, presented on Jan. 25, was titled [New Approach Methodologies to Assess (Developmental) Neurotoxicity](#). In this webinar, two scientists discussed NAMs that are being considered or developed for assessing potential effects of chemicals on the nervous system. NICEATM scientist Helena Hogberg, Ph.D., discussed use of in vitro assays for developmental neurotoxicity assessment.

Jyotshnabala Kanungo, Ph.D., of the FDA National Center for Toxicological Research summarized their studies using zebrafish to assess the effects of dietary supplements on drug safety and drug–drug interactions.

- The Jan. 30, 2023, Communities of Practice webinar focused on [Emerging Approaches for Anchoring Biological Relevance of New Approach Methodologies](#). In this webinar, three scientists from the academic and private sectors discussed approaches to evaluating NAMs that focus on the biological relevance of the NAM to the species of regulatory interest. Lorna Ewart, Ph.D., of Emulate Inc. discussed their work on using MPS to predict liver toxicity. James McKim, Ph.D., IONTOX/LifeNet Health LifeSciences summarized studies using integrated organ models of absorption, distribution, metabolism, and excretion. Finally, Tamara Tal, Ph.D., Helmholtz-Centre for Environmental Research UFZ explained their approach to building confidence in larval zebrafish behavior assays.

ICCVAM Public Forums

Tags: ICCVAM, developmental neurotoxicity, genotoxicity

ICCVAM's goals include promotion of national and international partnerships between governmental and nongovernmental groups, including academia, industry, advocacy groups, and other key stakeholders. To foster these partnerships, ICCVAM holds public forums annually to share information and facilitate direct communication of ideas and suggestions from stakeholders.

- [View materials from the 2022 Public Forum](#)

- [View materials from the 2023 Public Forum](#)

ICCVAM public forums started in 2014. The 2022 meeting was held virtually on May 26-27 and expanded to two days to cover the large number of activities ongoing within member agencies. More than 100 individuals attended the meeting virtually each day. The program featured 15 presentations from eight of the 17 ICCVAM member agencies each describing activities both to advance new approaches to safety testing of chemicals and medical products and to reduce the amount of testing required. Updates were also provided on ICCVAM committee and international activities. Commenters at the meeting praised the

accomplishments of NICEATM, ICCVAM, and ICCVAM member agencies. They asked that agencies establish specific goals and timelines for acceptance of alternatives to animal testing and stressed the need for education and communication about availability and use of alternatives.

The 2023 public forum was held in person on May 18-19 at NIH in Bethesda, MD, with an option for remote viewing. The event featured 15 presentations from 12 ICCVAM member agencies on activities directed toward reducing and replacing animal use for chemical safety testing, as well as updates on ICCVAM workgroup and international activities. The meeting generated broad public interest with over 200 viewers. Presentations from both agency representatives and stakeholder groups highlighted NAMs with the potential to replace animal testing. Speakers from two test method developers described new technologies that could be used to identify chemicals that can cause neurotoxicity in developing embryos or DNA damage. Several presentations focused on regulatory agencies' initiatives to reduce animal use requirements. Commenters welcomed ICCVAM's development of an updated validation guidance document on criteria to establish confidence in new methods for regulatory application.

ICCVAM advisory committee meetings

Tags: ICCVAM, acute toxicity, developmental neurotoxicity, metrics, PFAS, population variability

The [Scientific Advisory Committee on Alternative Toxicological Methods](#) (SACATM) is a federally chartered advisory group that advises NICEATM, ICCVAM, and the Director of NIEHS about ICCVAM activities.

Traditionally held in person, the September 21-22, 2022, SACATM meeting was held virtually because of restrictions on gatherings due to COVID-19. About 300 people viewed each day of the webcast; representatives from two [ICATM participating organizations](#) presented or attended. ICCVAM asked SACATM members to specifically advise on metrics and measures of success and new approaches to validation. In response, SACATM members urged ICCVAM agencies to expand their efforts to measure progress in adoption of NAMs for chemical testing. They encouraged agencies to work with stakeholders to share data on animal use and provide incentives for reducing animal use. The committee also encouraged

ICCVAM agencies to consider relevance to human biology when evaluating a nonanimal test method for a specific purpose, rather than relying on a simple comparison to historic animal data.

The September 21-22, 2023, SACATM meeting was held in person at NIEHS in Research Triangle Park, NC. About 70 people attended in person and over 100 people viewed each day of the webcast; representatives from four participating organizations of the International Cooperation on Alternative Test Methods (ICATM) presented or attended. This event focused on detailed discussion of the status of replacement of animal testing for required acute toxicity testing and approaches to validation for new methods. Participants also considered how NAMs could be used to improve environmental health protection. SACATM members and commenters praised ICCVAM agencies on their accomplishments in reducing animal use for determining whether chemicals might cause acute effects such as oral toxicity and skin sensitization. They advised agencies on how they might address remaining challenges, such as predicting whether chemicals might cause complex effects such as developmental neurotoxicity, or characterizing effects of per- and polyfluoroalkyl substances (PFAS) and other emerging contaminants. They also emphasized the need for clear communication from agencies to stakeholders.

ICCVAM agency-sponsored workshops and webinars
Tags: CPSC, DoD, EPA, FDA, NIEHS, NIH, USDA, exposure, metabolism, MPS, population variability, skin sensitization, structure similarity
ICCVAM agencies convened workshops and webinars during 2022 and 2023, summarized in the table below, to foster collaboration and provide information about alternative testing methods.

Meeting Date	Sponsoring Agency (and partner organization)	Meeting Title (with link to page if available)	Meeting Summary
January 27, 2022- December 7, 2023	EPA	Computational Toxicology Communities of Practice Webinar Series	These monthly webinars explore topics of interest to stakeholders interested in using advances in computational toxicology and exposure science to

Meeting Date	Sponsoring Agency (and partner organization)	Meeting Title (with link to page if available)	Meeting Summary
			evaluate the safety of chemicals. The series will continue into 2024, and videos are available for many past webinars.
April 4, 2022-August 4, 2023	NIEHS	[Environmental Health Language Collaborative Webinars](#)	Six webinars and a virtual workshop were held during 2022 and 2023 to advance the goals of the [Environmental Health Language Collaborative](#). Topics discussed included developing use cases, metadata and coding, and data platforms.
May 11, 2022-January 18, 2023	EPA (PSCI, PCRM)	[Webinar Series on Use of NAMs in Risk Assessment](#)	Three webinars concluded a series that began in 2018. In a May 2022 webinar, EPA scientists discussed computational tools and analyses EPA is using to reduce animal use for ecotoxicity testing. The last two webinars discussed frameworks for establishing confidence in NAMs.
June 7-8, 2022	NIH	[NIH Workshop on 3D Tissue Models](#)	This NIH workshop covered practical approaches and best practices for developing standardized 3D cellular assays. The overall goal was to help scientists establish robust, reproducible, scalable, consistent, advanced 3D tissue models to study pandemic threat viruses.
June 14, 2022	FDA	[FDA Public Meeting of Science Board](#)	This FDA advisory committee discussed

Meeting Date	Sponsoring Agency (and partner organization)	Meeting Title (with link to page if available)	Meeting Summary
			challenges in evaluating the safety of dietary supplements and food ingredients, adoption and use of NAMs to accelerate product development and enhance emergency preparedness, and enhanced efforts to ensure optimal organization, infrastructure, and expertise for data science efforts in alignment with its regulatory scope and evidence-based decision-making, in support of FDA's public health priorities.
June 22-24, 2022	NIH/OLAW, USDA/AWIC (JHU CAAT and Dept. of Molecular and Comparative Pathobiology)	9th Annual 3Rs Symposium	Presentations focused on 3Rs methods to improve laboratory animal welfare while maintaining or improving scientific results. Topics include mixed strain housing, best animal models for disease research, adoption programs, and collaborating to solve animal welfare issues among other topics.
October 3-4, 2022	NIEHS	Clustering and Classification: Applications to Investigate Adverse Effects of Chemicals on Human Health and Environment	This workshop introduced the concept of chemical similarity and explored the uses of different classification and clustering approaches for toxicity research and risk assessment. Speakers identified best practices

Meeting Date	Sponsoring Agency (and partner organization)	Meeting Title (with link to page if available)	Meeting Summary
			and guidelines for application of these approaches.
October 12-13, 2022	EPA	EPA NAMs Conference for Chemical Safety Testing	EPA hosts regular conferences to provide updates on reducing the use of vertebrate animals in chemical testing and solicit input from interested stakeholders. The conferences highlight the state-of-the-science on the development and use of NAMs for chemical safety testing.
July 19-October 7, 2022	NIEHS (PCRM)	Webinar Series: NAMs to Address Population Variability and Susceptibility	Three webinars addressed how NAMs can incorporate population variability into assessments of skin sensitization potential, rare disease investigations, and exposure assessments.
October 26-27, 2022	NIEHS	NIEHS Symposium on Using New Approach Methodologies to Address Variability and Susceptibility Across Populations	Speakers at this symposium highlighted information needs for population variability and susceptibility and considered where NAMs could be designed or improved to fill those needs. An important goal of the event was to initiate conversations to build trust in NAMs use and stress the importance of risk assessment to protect all populations.
May 17-18, 2023	NIH/OLAW, USDA/AWIC (JHU CAAT and Dept. of	10th Annual 3Rs Symposium	Presentations focused on 3Rs methods to improve laboratory

Meeting Date	Sponsoring Agency (and partner organization)	Meeting Title (with link to page if available)	Meeting Summary
	Molecular and Comparative Pathobiology)		animal welfare while maintaining or improving scientific results. Topics include liver-chip technologies, environmental health monitoring, refinement and enrichment strategies, and regulatory insights into the 3Rs.
August 23-November 29, 2023	EPA (PSCI, Institute for In Vitro Sciences, California Department of Pesticide Regulation [CPDR])	EPIC Webinar Series on the Use of NAMs in Risk Assessment	The EPIC series covers timely topics on the use of NAMs for risk assessment within EPA and CDPR. The quarterly series will continue into 2024.
September 18-October 6, 2023	NIEHS/CPSC/DoD/EPA FDA (JHU CAAT, Unilever)	Webinar Series: Trust Your Gut: Establishing Confidence in Gastrointestinal Models	This webinar series provided background information for a workshop on the state-of-the-science for using NAMs to predict gastrointestinal absorption and toxicity. Presentations focused on (1) absorption and pharmacokinetics, (2) microbiome effects on toxicity, and (3) evaluating potential allergenicity.
October 11-12, 2023	NIEHS/CPSC/DoD/EPA FDA (JHU CAAT, Unilever)	Workshop: Trust Your Gut: Establishing Confidence in Gastrointestinal Models	This workshop considered the state-of-the-science for using NAMs to predict gastrointestinal absorption and toxicity.

Development and updates of agency webpages on alternatives development and acceptance

Tags: CPSC, DOI, EPA, FDA, USDA, ecotoxicity, exposure, metrics

During 2022 and 2023, several ICCVAM agencies published new webpages summarizing activities to advance alternative methods.

- In June 2023, CPSC updated its [Policy on Animal Testing](#) web page. It has been redesigned to be more user-friendly and have direct access to necessary information and documents. One of the resources available on this page is CPSC's "[Guidance for Industry and Test Method Developers: CPSC Staff Evaluation of Alternative Test Methods and Integrated Testing Approaches and Data Generated from Such Methods to Support FHSA Labeling Requirements](#)."

- In 2020, FDA launched "[Advancing Alternative Methods at FDA](#)," which highlights activities of its [Alternative Methods Working Group](#). A 2023 update expanded this site from a single page to a site with pages providing information on:

  - FDA partnerships to advance alternatives.

  - FDA implementation of alternative methods.

  - The [FDA alternatives webinar series](#).

  - Relevant publications and events.

- EPA maintains a number of webpages describing efforts to replace and reduce animal use for chemical safety testing. The Office of Research and Development's "[New Approach Methods Research](#)" site links to the EPA's 2021 [New Approach Methods Work Plan](#), which describes objectives and strategies to implementing NAMs. Other links from this page lead to EPA's [Catalog of NAMs Training Materials](#) and materials from [EPA NAMs Conferences](#). The EPA Office of Pesticide Programs provides a webpage describing its "[Strategic Vision for New Approach Methodologies – Metrics](#)." This page includes data compiled by the Hazard and Science Policy Council, the Chemistry and Acute Toxicology Science Advisory Council, and other groups about how EPA activities and guidance documents have reduced the number of animals used in testing and saved EPA and stakeholder resources. The Office of Pollution Prevention and Toxics "[Predictive Models and Tools for Assessing Chemicals](#)" site links to models and tools that can be used to assess hazards, exposure, and environmental fate of new chemicals.

- DOI's USGS is preparing a public webpage titled "Reducing Animal Use in Ecotoxicity Testing and Biomonitoring." It briefly describes the types of ecotoxicological work conducted by the DOI, the 6R Principles, and ongoing efforts to reduce animal use and increase use of nonanimal alternatives. It also provides some animal alternative reports and training opportunities.

- A major update of the U.S. Department of Agriculture (USDA) National Agricultural Library's [Animal Welfare Information Center](#) (AWIC) website in 2022 improved user experience and streamlined information discovery; a new introductory video gives an overview of AWIC resources.

EPA NAMs training program
Tags: EPA, ecotoxicity, metrics, structure similarity

EPA created its [New Approach Methods (NAMs) Work Plan](#) to prioritize agency efforts and resources toward activities that aim to reduce the use of vertebrate animal testing while continuing to protect human health and the environment. The Work Plan was first released in June 2020 and updated in December 2021.

To implement the Work Plan's objective of "Engage and Communicate with Stakeholders," EPA developed and implemented a [NAMs training program](#) in 2021-2023. This program provides users courses and workshop with the appropriate level of oversight to enable them to use EPA NAMs tools and approaches in their own work. During 2022-2023, EPA hosted five trainings on ECOTOX Knowledgebase, CompTox Chemicals Dashboard, ECOTOX, GenRA, and httk. EPA also posted six tool tips videos for the CompTox Chemicals Dashboard, and created a one-stop-shop [website](#) for all available training materials. In all, 321 participants attended the ECOTOX NAMs training in May 2022, 554 attended the CompTox Chemicals Dashboard training in October 2022, and 575 attended the February 2023 ECOTOX training.

FDA reports and presentations on progress in advancing alternatives
Tags: FDA, MPS

In 2021, FDA published "[Advancing New Alternative Methodologies at FDA](#)," which provided updates on activities to advance development of alternatives to animal use and their

application to regulatory decision-making. The report was developed by the [FDA Alternative Methods Working Group](#) to highlight the progress made by FDA scientists in laying the groundwork to integrate alternatives to animal testing into FDA regulatory programs. Two reports issued in 2022 expanded on topics discussed in the report:

- The 2022 "[Update to the Focus Areas of Regulatory Science (FARS) Report](#)" outlines topics FDA has identified as needing continued targeted investment. FDA reviewed each of the focus areas from the 2021 report, and this document provides important updates to the examples highlighted in the report.

- A November 2022 report on "[Successes and Opportunities in Modeling & Simulation for FDA](#)" was prepared by the FDA Modeling and Simulation Working Group. The report:

  - Elucidates how and where modeling and simulation is used across FDA, and the type and purpose of modeling and simulation used.

  - Presents a selection of modeling and simulation case studies from across nearly all FDA centers, which demonstrate how modeling and simulation is playing a tangible role in FDA fulfilling its mission.

  - Identifies opportunities for FDA to better harness modeling and simulation in upcoming years by embracing computational advances and new (and big) data streams to develop improved public health solutions.

FDA scientists also gave presentations on activities to advance alternatives at several public meetings during 2022 and 2023. Details of these presentations and presentation slides are [available on the FDA website](#).

FDA Alternatives Webinar Series

Tags: FDA, hepatotoxicity, inhalation toxicity, metrics, MPS, neurotoxicity

As part of FDA's commitment to promote novel technologies for potential regulatory use, FDA gives developers and others the opportunity to present their new methods and methodologies directly to FDA scientists. Educational opportunities to hear more about new predictive in vitro, in vivo, and in silico methods is vital to ensuring that FDA regulators and researchers have a broad skill set and remain current with cutting-edge science and

technology. Developers presented 29 webinars during 2022 and 2023; presentations described in silico models and in vitro platforms such as microphysiological systems, organoids, and engineered tissues relevant to contexts including drug-induced liver injury, neurotoxicity, inhalation toxicity, and veterinary medicine. Information for prospective presenters is available on the FDA website.

Animal Welfare Information Center resources
Tags: USDA, metrics

The 1985 amendments to the Animal Welfare Act established the Animal Welfare Information Center (AWIC), an information service at the USDA National Agricultural Library. AWIC's mission is to provide information pertinent to employee training that could prevent unintended duplication of animal experimentation and on improved research methods that could reduce or replace animal use and minimize pain and distress to animals.

Within the National Agricultural Library website, AWIC provides information to the toxicology community related to the development and use of alternatives to toxicity testing, as well as information and guidelines on mandatory and regulatory testing set by various agencies. These web pages highlight peer-reviewed publications and other online resources that discuss ways to replace, reduce, and refine animal use in toxicity testing. A major update of the website in 2022 improved user experience and streamlined information discovery; a new introductory video gives an overview of AWIC resources.

During 2022 and 2023, 340,587 people visited the AWIC website. These visits generated a total of 580,863 page views. AWIC also provides information on animal use alternatives through outlets such as presentations and posters, workshops, webinars, and conference exhibits. Through these outlets, AWIC presented to over 4,348 participants at 78 different outreach events in 2022 and 2023, collectively. At these outreach events, AWIC described best practices for searching and finding current literature on methods to reduce, refine, or replace animal use in testing and experimentation.

AWIC offers workshops on meeting the requirements of the Animal Welfare Act. These workshops provide guidance on where to find 3Rs literature and resources, instruction on database literature searching techniques (e.g., search syntax), and demonstration of alternatives literature searches. They are free and open to the public. During 2022 and 2023

AWIC presented six of these workshops to a total of 789 attendees. [Information about upcoming workshops](#) is available on the AWIC website. This page also provides a link to online on-demand self-paced training for users unable to participate in a live workshop.

Development of topic-specific searches for NAMs applications

Tags: NIEHS, NLM, USDA, developmental neurotoxicity, inhalation toxicity, IVIVE, MPS, QSAR

The National Library of Medicine developed ALTBIB, the Bibliography on Alternatives to the Use of Live Vertebrates in Biomedical Research and Testing, to provide users with a predeveloped strategy to search PubMed citations information on alternatives to animal testing. In early 2021, responsibility for hosting and maintenance of ALTBIB transitioned to NICEATM, and [ALTBIB now resides on the NTP website](#). In addition to the basic search strategy, the National Library of Medicine's ALTBIB contained a page with a list of topics focused on nonanimal testing platforms or endpoints for which users might be interested in searching for alternatives to animal testing. This page was not included in the NICEATM ALTBIB launch because the topics and search strategies provided were out of date, and resources were not immediately available to update them.

Over the past couple of years, USDA AWIC has been developing search strategies, known as "hedges", for topics specific to alternatives. The focus of AWIC's activity has been refinement of animal use, and their hedges tend to be focused on animal species. In 2023, NICEATM and AWIC collaborated to develop hedges on topics focused on replacement of animal use. Topic under development include microphysiological systems, QSAR models for hazard identification, nonanimal tests for developmental neurotoxicity and acute inhalation toxicity, and IVIVE. The hedges are currently being refined by an AWIC-sponsored working group with input from NICEATM subject matter experts and will be made available on a [new webpage on the ALTBIB site](#) in spring 2024.

Computational Tools Applications

ICCVAM and its member agencies are exploring how computational approaches can be applied to reduce animal use for toxicity testing. These approaches have potential application for acute oral toxicity and eye irritation testing, and for predicting whether chemicals could cause cardiotoxicity, developmental toxicity, or neurotoxicity.

Review of applications of in vitro to in vivo extrapolation within federal agencies
Tags: ATSDR, CPSC, DoD, EPA, FDA, ICCVAM, NIEHS, NIST, NLM, IVIVE

In vitro toxicity assays are being applied to transform toxicology from an observational to a predictive science, improve throughput, and reduce costs. The qualitative linkage between in vitro and in vivo toxicity endpoints can be strengthened via application of IVIVE of dosimetry, which relates an in vitro concentration associated with bioactivity to an equivalent external exposure level. In some contexts, applications of IVIVE have advanced past the exploratory research stage and are beginning to gain acceptance for risk assessment of chemicals. The ICCVAM IVIVE Workgroup requested information from ICCVAM member agencies regarding the extent and context of their use of IVIVE. Surveyed agencies were also asked about programmatic needs, data gaps, and agency-specific guidance documents or publications related to IVIVE, as well as for information about modeling tools or software they had used or may use for facilitating IVIVE analysis and decision-making. This information was compiled into a review summarizing the workgroup's findings, current challenges, and future needs (Chang et al. 2022b). The review also proposes operational definitions for IVIVE, presents literature examples for several common toxicity endpoints, and highlights implications of IVIVE use in decision-making processes. This well-received paper was recognized by the journal Toxics as an "Annual Recommended Review for 2022."

Using in vitro data and PBPK models to predict inhalation toxicity
Tags: ATSDR, NIEHS, genotoxicity, inhalation toxicity, IVIVE, metabolism

In vitro assay data can provide valuable information about the effects of substances on biological systems. However, to determine safe exposure levels in real-life situations, it is necessary to consider the translation of in vitro results to the in vivo context. IVIVE uses pharmacokinetic models to relate concentrations of substances eliciting responses in an in vitro assay to an in vivo EAD. NICEATM applied IVIVE to a group of volatile organic compounds with abundant pharmacokinetic and in vivo toxicity data. For each chemical, EADs that would result in internal concentrations (e.g., plasma or target tissue) equivalent to in vitro activity concentrations were estimated. The in vitro activity concentrations were obtained from in vitro assays measuring different endpoints (e.g., cytochrome p450 activation, transcriptome analysis, and genotoxicity) from public resources. EAD estimates

were compared to published in vivo PODs or [minimal risk levels](#) provided by the Agency for Toxic Substances and Disease Registry (ATSDR) covering multiple target organs toxicities via inhalation exposure. The impact of mechanistic relevance of in vitro assays, target organs chosen for analysis, and the concordance between in vitro and in vivo exposure regimens on IVIVE outcomes will be evaluated and discussed in a presentation (Chang et al.) at the [2024 annual meeting of the Society of Toxicology](#). For most chemicals tested, close agreements between EAD estimates and rat in vivo PODs were observed, but most EADs were at least 10-fold higher than minimum risk levels, suggesting that a "modifying factor" may need to be established to approximate minimum risk levels based on in vitro assay data. This study provides proof-of-concept case examples to illustrate the utility NAMs in informing human risk following exposure to inhaled substances.

Expanding httk to model military population and exposures
Tags: DoD, IVIVE

The modern battlefield is consistently evolving, and the USAF Force Health Protection program must account for new technologies and chemical threats. While much progress has been made in the development of computational methods for predicting public health risk, the warfighter is expected to experience exposures under conditions that are substantially different from those experienced by the general U.S. population. Thus, USAF PRT is evaluating the utility of existing models for operational exposure scenarios and modifying existing models when necessary.

The Air Force population differs from the general U.S. population with respect to age and gender distribution. PRT constructed an approach to use published demographic data ([Mullenger and Zehner, 2020](#)) to customize the httk pipeline to adequately model Air Force population. A Monte Carlo simulation was performed to generate 48 anthropometric characteristics that closely resemble the USAF population. Upon completion, an analysis was compiled to compare previous oral-equivalent doses for the general population to those derived for the military. This pipeline is being used as a foundation to perform a high-throughput risk analysis on military-relevant chemicals and will continue to be expanded as necessary as the population changes in the future. Additional areas for

development in 2024 include consideration of occupational and acute dosing scenarios and physiological changes due to extreme environments.

Applying httk and in vitro data to estimate acute neurotoxicity risk
Tags: DoD, IVIVE, neurotoxicity

Neurotoxicity is of particular concern for the military due to the potential for cognitive, behavioral, and physiological effects. With an ever-evolving operational landscape, de novo generation of in vivo data for all potential neurotoxicants is not feasible. USAF PRT evaluated the utility of high-throughput NAMs for rapid risk assessment of 220 potentially neurotoxic chemicals. Chemical-specific PODs were derived from novel in vitro neuronal assay data and ToxCast bioactivity concentrations. The AC_{50} (concentration with 50% maximum activity) was derived for each chemical/assay combination and PODs were calculated from the 5th percentile of the AC_{50} values. IVIVE was performed using the EPA httk model to estimate the EAD for each POD. Monte Carlo calculations from httk were modified to reflect USAF active-duty demographics. Neurotoxicity-associated endpoints produced higher PODs compared to all CompTox in vitro endpoints combined. In vitro-derived EADs were lower than in vivo-derived PODs for most of the chemicals in both the U.S. and USAF populations. In vitro-derived EADs were also lower than 45% of provisional reference doses calculated using one-year Military Exposure Guideline procedures. An uncertainty factor of 1000 results in conservative estimated exposure limits (vs. Military Exposure Guidelines) for almost all the chemicals. This case study supports the use of NAMs to derive conservative PODs for risk assessment. A manuscript is currently being prepared for publication in 2024.

QSAR comparison to predict mammalian maximal rates of metabolism and Michaelis constants
Tags: DoD, metabolism, QSAR

Warfighters are subject to a variety of chemical exposures, and PBK models can be used to predict chemical metabolism and potential toxicity given a specific exposure concentration. However, most in silico techniques for estimation of metabolism are only suitable for low exposure concentrations. Saturable metabolism is a key component for extending current high-throughput PBK models beyond environmental exposures to occupationally relevant

concentrations. To adequately support human health decision-making, USAF PRT scientists identified a need for in silico approaches to estimate chemical metabolism parameters suitable for describing the rate of compound biotransformation over a wide range of exposure concentrations. Sweeney and Sterner (2022) describe the reconstruction of published, insufficiently validated quantitative structure–activity relationships (QSARs) and the application of these QSARs to jet fuel components. A subsequent publication (Sweeney 2022) used these QSAR estimates, along with other techniques, to demonstrate the value of smaller families of highly similar compounds for generating reliable QSAR-derived metabolism estimates and their application to internal dosimetry-based risk assessment for both low dose (chronic) and higher dose (acute) risk assessment activities. PRT scientists are coordinating a newly established DoD QSAR interest group and are exploring future collaborations that will expand the application of these tools. Additionally, the team is assessing the feasibility of adding saturable metabolism into the EPA httk model.

Application of QSARs and in vitro methods to evaluate novel PFAS-free firefighting agents
Tags: DoD, genotoxicity, mixtures toxicity, QSAR, PFAS

PFAS are organofluorine chemicals manufactured and used for decades in products and applications such as firefighting foams. PFAS are highly persistent and bioaccumulative in the environment, biota, and humans, and concern about their health effects has driven efforts to identify and evaluate possible substitutes. Scientists at the Defense Centers for Public Health used in silico and in vitro approaches to predict toxicity of six fluorine-free firefighting formulations were assessed for toxicity. Ingredient information provided by the manufacturers missed up to 70% of the constituents per formulation. QSARs were completed for some of the known ingredients, and in vitro assays (the Ames test for genotoxicity, the Microtox test for cytotoxicity, and the EpiDerm assay for skin irritation) were conducted to fill data gaps to help rank the toxicity of these formulations before progression to in vivo tests. Direct comparisons of these products through these QSARs and in vitro screens provided early data to prevent potential regrettable substitutions when selecting alternative PFAS-free firefighting foams.

Use of IVIVE to characterize fish toxicants
Tags: DOI, acute toxicity, ecotoxicity, IVIVE

The use of pesticides to control invasive species is a key component of integrated pest management plans. Traditionally, the development of new chemical controls required testing on numerous animals. In vitro cytotoxicity testing, which reduces animal use, is becoming more common to prioritize candidate toxicants and permits high-throughput testing. However, it remains unknown whether in vitro cytotoxicity values (i.e., effective concentrations or EC50 values) are representative of in vivo toxicity values (i.e., lethal concentration for 50% of organisms or LC50). DOI's USGS has begun proof-of-concept studies on the use of fish cytotoxicity screening assays to prioritize new candidate pesticides to control nuisance fishes. Recent studies have quantified the cytotoxicity EC50 values of the fish toxicant Antimycin-A using a commercially available gill cell line (RTgill-W1) from rainbow trout (*Oncorhynchus mykiss*). This study utilized the procedure described in OECD Test Guideline 249 and the CellTiter-Glo 2.0 (Promega) cell viability assay for assessing RTgill-W1 cytotoxicity. IVIVE was applied to assay results and indicated that, for most valuable metrics, the ratio of in vitro to in vivo toxicity was approximately 1. These results demonstrate that toxicity as measured in rainbow trout gill cell lines is predictive of whole-animal toxicity and shows promise for the development of additional fish gill cell lines for screening of pesticide candidates.

Evaluation of CATMoS models for estimating pesticide ecotoxicity
Tags: EPA, NIEHS, acute toxicity, ecotoxicity, QSAR

EPA uses the in vivo acute rat oral LD50 to assign hazard classifications for acute oral toxicity for pesticides before they are approved for marketing. These classifications determine the precautionary statements placed on the pesticide label for acute human exposure. The in vivo acute rat oral LD50 is also used by EPA as a surrogate for acute oral toxicity to all mammalian wildlife. The rat LD50 is predicted by the Collaborative Acute Toxicity Modeling Suite (CATMoS), an in silico predictive tool for estimating acute oral toxicity based on molecular structure. EPA and NICEATM scientists and collaborators evaluated how well CATMoS predicted acute oral toxicity LD50 values of pesticides with available in vivo acute rat oral LD50 data (Bishop et al. 2024). The evaluation included 177 pesticides registered for use in the United States, mostly fungicides, herbicides, and insecticides. Most of the evaluated pesticides fell into the lower-toxicity Categories III and IV, and for these CATMoS predictions were found to correlate well with in vivo data,

although it was felt that in some cases CATMoS estimates for toxicity might result in a more stringent label warning than animal tests would require. There were too few chemicals evaluated from the higher-toxicity Categories I and II to support a conclusion about CATMoS performance for more toxic substances. This analysis will help inform whether CATMoS can be used to estimate acute oral toxicity from pesticides to identify toxicity categories and assess risk to wildlife.

Use of SeqAPASS to extrapolate honeybee data to non-Apis bees
Tags: EPA, AOP, ecotoxicity, structure similarity

An AOP is a model that identifies the sequence of molecular and cellular events required to produce a toxic effect when an organism is exposed to a substance. As most AOPs are defined using a single or small number of species, they have a narrow taxonomic domain of applicability (tDOA). Defining the tDOA of an AOP is critical for use in regulatory decision-making for ecotoxicity, particularly when considering protection of untested species. Structural and functional conservation are two elements that can be considered when defining the tDOA. Publicly accessible bioinformatics approaches, such as the Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool, take advantage of existing and growing databases of protein sequence and structural information to provide lines of evidence toward structural conservation of key events and key event relationships of an AOP. It is anticipated that SeqAPASS results could readily be combined with data derived from empirical toxicity studies to provide evidence of both structural and functional conservation to define the tDOA for AOPs and elements of AOPs. Such data could be incorporated into resources such as the AOP-Wiki as lines of evidence toward biological plausibility for the tDOA. EPA scientists developed a case study describing the process of using bioinformatics to define the tDOA of an AOP using an AOP linking the activation of the nicotinic acetylcholine receptor to colony death/failure in the honeybee (*Apis mellifera*). Although the AOP was developed to gain a particular biological understanding relative to honeybee health, applicability to other Apis bees, as well as non-Apis bees, has yet to be defined. The EPA study demonstrates how bioinformatics can be utilized to rapidly take advantage of existing protein sequence and structural knowledge to enhance and inform the tDOA and elements of AOPs, focusing on providing evidence of structural conservation across species.

Use of Web-ICE to predict acute toxicity values for aquatic vertebrate and invertebrate species in TSCA risk evaluations

Tags: EPA, acute toxicity, ecotoxicity

Chemical risk evaluations under the Toxic Substances Control Act (TSCA) are often conducted with limited test data, as TSCA does not have minimum data requirements. EPA is evaluating how this limitation can be addressed using Web-based Interspecies Correlation Estimation (Web-ICE), a model that can predict toxicity values for environmental species absent from a dataset and provide a more robust dataset to estimate toxicity thresholds. Web-ICE is an online tool that estimates acute toxicity values for aquatic and terrestrial species using surrogate species data and least-squared regressions. Each Web-ICE model represents the relationship of inherent sensitivity between a surrogate species and a predicted taxon (species, genus, or family). Web-ICE models use surrogate species sensitivity as an input to estimate the sensitivity of all available taxa. For the industrial chemicals examined so far under TSCA, Web-ICE predictions increased the number of aquatic species represented in the dataset and provided more species representation. Additionally, the use of Web-ICE predictions with empirical data to create species sensitivity distributions provided a data-driven way of accounting for uncertainties in aquatic hazard characterization. A poster describing the project (Koehrn et al.) was presented at the 2022 meeting of SETAC North America.

Development of QSAR models to predict acute toxicity of pesticides to fish

Tags: EPA, acute toxicity, ecotoxicity, QSAR

QSAR models may be used to assess the potential acute toxicity of chemicals in fish for pesticides or pesticide degradation products for which there are little or no toxicity data available. However, data used to develop QSARs often do not represent the broad range of pesticidal structures and modes-of-action that contribute to pesticide toxicity. EPA Office of Pesticide Programs and Office of Research and Development scientists compared the performance of three existing QSAR models with a random forest model newly developed for this purpose. The new random forest model predicted toxicity better than the existing models, likely because it was trained using only pesticide data and a targeted predictor set, and because its algorithm accounts for predictor interactions and non-linear relationships.

The new random forest model is being incorporated into a graphical user interface tool with the goal of making it publicly available to web users.

Computational approaches to cross-species risk assessment for potential endocrine-disrupting chemicals

Tags: EPA, AOP, ecotoxicity, endocrine disruptors

EPA's EDSP is responsible for determining the potential for certain chemicals to cause adverse effects in humans and wildlife via endocrine pathways. One goal of the EDSP is to evaluate how broadly results can be concluded across taxa. Two EPA projects are using computational approaches to assess how potential endocrine disruptors might affect a diversity of mammalian and nonmammalian species.

EPA scientists and collaborators assessed the cross-species conservation of androgen receptor-modulated pathways by using computational analyses and systematic literature review approaches to conduct a comprehensive analysis of existing in silico, in vitro, and in vivo data (Vliet et al. 2023). Results indicate that androgen receptors are conserved across vertebrate species, which could thus be predicted to share similarly susceptibility to chemicals that interact with the human androgen receptor. This study demonstrated a framework for utilizing bioinformatics and existing data to build weight-of-evidence for cross-species extrapolation and provides a technical basis for extrapolating human androgen receptor-based data to prioritize hazard in nonmammalian vertebrate species.

Thyroid hormone system-disrupting compounds are considered potential threats for human and environmental health. Multiple AOPs for thyroid hormone system disruption are being developed in different taxa. Combining these AOPs results in a cross-species AOP network for thyroid hormone system disruption which may provide an evidence-based foundation for extrapolating these data across vertebrate species and bridging the gap between human and environmental health. A review by EPA scientists and collaborators (Haigis et al. 2023) aimed to advance the description of the taxonomic domain of applicability in the network to improve its utility for cross-species extrapolation. The study focused on molecular initiating events and adverse outcomes, evaluating both the taxa they are likely applicable to and where evidence for applicability to various taxa exists in the context of thyroid system disruption. The evaluation showed that all molecular initiating events in the AOP network are applicable

to mammals. There was some evidence of structural conservation across vertebrate taxa, especially for fish and amphibians and to a lesser extent for birds. The results of this evaluation are summarized in a conceptual AOP network that helps prioritize specific parts of AOPs for a more detailed evaluation.

Quantitative IVIVE for developmental toxicity

Tags: EPA, FDA, NIEHS, developmental toxicity, IVIVE, stem cells

The Stemina devTOX quickPredict (devTOXqP) assay, an in vitro human induced pluripotent stem cell assay, assesses a chemical's potential to induce developmental toxicity. Assessments are based on a chemical's developmental toxicity potential (dTP) concentration in the assay. A study conducted by EPA, FDA, and NIEHS scientists and collaborators applied IVIVE approaches to see if the devTOXqP assay could quantitatively predict in vivo developmental toxicity lowest-effect levels for the prototypical teratogen valproic acid and a group of comparable structures (Chang et al. 2022a). EADs that would lead to plasma concentrations equivalent to the in vitro dTP concentrations for valproic acid and its analogues were quantitatively similar to in vivo data from both rats and humans, where available, and the derived rank order of the chemicals was consistent with observed in vivo developmental toxicity. This study highlighted the importance of pharmacokinetic considerations when using in vitro assays and demonstrates the usefulness of the devTOXqP assay to quantitatively assess a chemical's developmental toxicity potency.

Computational models to predict penetration of the blood-brain barrier by e-cigarette chemicals

Tags: FDA, inhalation toxicity, neurotoxicity, structure similarity

Seizures have been reported among e-cigarette users, in particular youth or young adults. FDA scientists used chemoinformatic computational models to compare chemicals documented to be present in e-cigarettes with known neuroactive compounds, with the goals of predicting blood-brain barrier penetration potential, central nervous system activity, and structural similarities (Stratford et al. 2024). The e-cigarette chemicals identified showed structural similarity to neuroactive compounds based on chemical fingerprint similarity analyses. Most chemicals studied were predicted to cross the blood-brain barrier and were also predicted to have central nervous system activity. This study showed that computer-

based models can be useful to screen e-cigarette chemicals, allowing for prioritization for further possible in vitro and in vivo testing and potential early identification of central nervous system toxicity.

Literature analysis to develop environmental hazard assessments from waterpipe wastewater data

Tags: FDA, ecotoxicity

FDA is required to assess the environmental impact of its tobacco regulatory actions per the National Environmental Policy Act. Increases in use of waterpipe tobacco products raises concerns about environmental impacts from waterpipe waste disposal. To characterize the scope of this issue, FDA scientists compiled a comprehensive list of waterpipe wastewater chemical concentrations from literature (Termeh-Zonoozi et al. 2023). Chemicals were then selected for risk assessment by estimating persistence, bioaccumulation, and aquatic toxicity characteristics and hazardous concentration values. Of 38 chemicals with concentration data, 20 were found to be listed as harmful or potentially harmful constituents in tobacco smoke and tobacco products by FDA, and 15 are EPA hazardous waste substances. Six metals on both lists were selected for future risk assessments, as were three non-metals because of their persistence and/or toxicity. The presence of multiple hazardous compounds in waterpipe wastewater highlights the importance of awareness on the proper disposal of waterpipe wastewater in residential and retail settings. Future studies can build on the hazard characterization provided in this study through fate and transport modeling, exposure characterization and risk assessments of waterpipe wastewater chemicals.

Data analyses and predictive models to develop environmental hazard characterizations for e-cigarette chemicals

Tags: FDA, ecotoxicity, QSAR

FDA is required to assess the environmental impact of its tobacco regulatory actions per the National Environmental Policy Act. Increased use and sales of e-cigarettes raises concerns about the potential environmental impacts throughout their life cycle. However, few available research studies focus on the environmental impacts and ecotoxicity of e-cigarettes. Using a combination of available laboratory data and structure–activity relationship models, FDA scientists compiled a list of e-liquid chemicals to be considered for future environmental impact and risk assessments (Venugopal et al. 2023). Characteristics

considered included environmental persistence, bioaccumulation, and aquatic toxicity. Of the 421 unique e-liquid chemicals considered, 35 are considered hazardous constituents by EPA, 42 are considered by FDA to be harmful or potentially harmful constituents in tobacco products and smoke, and 20 were included on both lists. The study ultimately identified 81 chemicals that should be considered for future environmental impact and risk assessments, including tobacco-specific compounds, polycyclic aromatic hydrocarbons, flavors, metals, phthalates, and flame retardants. This study underscores the importance of awareness and education when handling or disposing of e-liquids/e-cigarettes and aim to inform strategies to prevent and reduce hazards from e-cigarettes.

Computational approach for respiratory hazard identification of flavor chemicals in tobacco products
Tags: FDA, inhalation toxicity, structure similarity

Flavor chemicals contribute to the appeal and toxicity of e-cigarettes and other tobacco products, and the assortment of flavor chemicals available for use in tobacco products is extensive. FDA scientists used a chemistry-driven computational approach to evaluate flavor chemicals based on intrinsic hazardous structures and reactivity of chemicals ([Goel et al. 2022](#)). A library of 3,012 unique flavor chemicals was evaluated based on physicochemical properties, GHS health hazard classification, structural alerts linked to the chemical's reactivity, instability, or toxicity, and substructures shared with known respiratory toxicants. Computational analysis of the constructed flavor library flagged 638 chemicals with GHS classified respiratory health hazards, 1,079 chemicals with at least one structural alert, and 2,297 chemicals with substructures of concern. From further analysis of a subset of 173 chemicals, four general structures with an increased potential for respiratory toxicity were identified. This study indicated that computational methods are efficient tools for hazard identification and understanding structure-toxicity relationship. With appropriate context of use and interpretation, in silico methods may provide scientific evidence to support toxicological evaluations of chemicals in or emitted from tobacco products.

Applying IVIVE to determine margins of exposure for potentially cardiotoxic chemicals
Tags: NIEHS, cardiotoxicity, exposure, IVIVE

Cardiovascular disease is the leading cause of death for people of most ethnicities in the United States. In a pioneering effort to minimize animal testing to evaluate chemicals for potential cardiotoxicity, NIEHS scientists applied NAMs that blend in vitro, in chemico, and in silico methods to investigate potential cardiotoxicity of over 800 substances. These substances, characterized by widespread human exposure, included personal care product ingredients, flame retardants, herbicides, pesticides, pharmaceuticals, and industrial byproducts. A systems-based modeling workflow including PBPK models was used to transform data from molecular and cellular assays relevant to cardiovascular endpoints into human daily EADs. The study compared these in vitro-derived EADs against both human exposure predictions and in vivo toxicological data to gauge human-relevant risks. It also integrated geospatial analyses to evaluate the compounded risks across diverse U.S. populations, spotlighting communities at disproportionate risk. Through this comprehensive approach, which merged HTS assays, PBPK modeling, and exposure data, the project aimed to refine human health risk assessments for chemicals posing cardiovascular hazards. This endeavor marks a significant stride in transitioning from molecular insights to actionable public health interventions, striving to replace animal testing with human biology-based strategies for chemical safety evaluation. A paper describing this work will be submitted for publication in 2024.

Enhancing seizure liability assessment: integrating target-based data and addressing knowledge gaps

Tags: NIEHS, AOP, neurotoxicity

Animal models are currently used to predict whether a chemical might cause human neurotoxicity, which can lead to adverse effects such as seizures. However, research suggests that animal models have limited reliability, particularly in predicting drug safety for the central nervous system. The prediction of whether a drug might cause seizures, specifically, is unreliable due to a significant failure rate of novel drugs in human clinical trials caused by unforeseen toxicity, revealing the inadequacy of these models. To address this issue, a collaboration between NIEHS and industry was initiated to identify potential biological targets associated with seizures. By combining targets from established AOPs and drug discovery databases, a seizure-specific AOP network was generated. Resources including NICEATM's [Integrated Chemical Environment](#) and the European Molecular Biology

Laboratory's [ChEMBL](#) database were employed to identify NAMs that could measure identified targets. The search identified both compounds likely to induce seizure and compounds that have tested negative for seizure effects. A proof-of-concept evaluation to assess in vitro mechanistic assay data availability across the seizure-relevant targets was conducted. Through this comprehensive approach, the current landscape of seizure risk-informative testing assay availability was assessed, laying the groundwork for future predictive modeling efforts with the ultimate goal of enhancing the ability to anticipate and mitigate seizure-related adverse effects. This project was presented at the 2023 annual meeting of the American College of Toxicology (Behl et al.), and a manuscript is in preparation to be submitted in 2024.

Utilization

Federal agencies need to take an active role in facilitating the successful adoption and use of NAMs , both within the government and internationally. ICCVAM agencies strive to provide clear guidance on the use and acceptance of data from new methods. Where possible, they implement approaches to reduce and replace animal use.

Assessments of Agency Needs and Practices

The 2018 ICCVAM [Strategic Roadmap](#) identified the definition of agency information needs as a key step in its implementation. ICCVAM member agencies are collaborating both within ICCVAM workgroups and independently to define agency information needs and make those needs known to stakeholders. Areas considered in 2022 and 2023 included acute toxicity, ecotoxicity, and PFAS toxicity.

Review of current ecotoxicity testing needs among selected U.S. federal agencies
Tags: DoD, DOI, EPA, FDA, ICCVAM, NIEHS, NIST, USDA, ecotoxicity

U.S. regulatory and research agencies use ecotoxicity test data to assess the hazards and risks associated with substances that may be released into the environment, including but not limited to industrial chemicals, pharmaceuticals, pesticides, food additives, and cosmetics. These data are used to conduct hazard assessments and evaluate potential risks to non-target animals and plants. To identify opportunities for regulatory uses of nonanimal replacements for ecotoxicity tests, the needs and uses for these types of test data must first be clarified. The ICCVAM Ecotoxicology Workgroup prepared a review ([Ceger et al. 2022](#)) that identifies the ecotoxicity test data relied upon by U.S. federal agencies. The review describes the standards, test guidelines, guidance documents, and endpoints that are used to address each of the responding agencies' regulatory and research needs regarding ecotoxicity testing in the context of their application to decision-making. This information will be useful for coordinating efforts to develop and implement alternative test methods to reduce, refine, or replace animal use in chemical safety evaluations.

Prioritization of application of alternatives for acute fish toxicity testing
Tags: ICCVAM, acute toxicity, ecotoxicity

Regulatory agencies use data from the in vivo fish acute toxicity test to assess potential risk of substances to aquatic vertebrates. The ICCVAM Ecotoxicology Workgroup is preparing a review, to be submitted for publication in 2024, to help guide prioritizing development and application of alternatives to the fish acute toxicity test, focusing on regulatory use of fish acute toxicity data and alternatives. The Ecotoxicology Workgroup asked each agency:

- What does your agency do with acute fish toxicity data?

- What is your flexibility to use NAMs?

- What should the data submitter know about your agency's process?

- Are there legal or regulatory impediments to the adoption of NAMs? For example, are live animal data specifically called for in your agency's regulations?

Responses were included in the draft manuscript, which will also address performance of two alternative test methods described in OECD test guidelines, the fish embryo acute toxicity test and the RTgill-W1 cell line assay.

Federal agency needs and requirements for nanomaterials toxicity testing
Tags: CPSC, DoD, EPA, FDA, ICCVAM, NIEHS, NIOSH, NIST

Engineered nanomaterials (ENMs) come in a wide array of shapes, sizes, surface coatings, and compositions, and often possess novel or enhanced properties compared to larger-sized particles of the same elemental composition. To ensure the safe commercialization of products containing ENMs, it is important to thoroughly understand their potential risks. Given that ENMs can be created in an almost infinite number of variations, it is not feasible to conduct in vivo testing on each type of ENM. Instead, NAMs such as in vitro or in chemico test methods may be needed, given their capacity for high-throughput testing, lower cost, and ability to provide information on toxicological mechanisms. However, the different behaviors of ENMs compared to dissolved chemicals may challenge safety testing of these substances using NAMs. The ICCVAM Nanomaterials Workgroup queried ICCVAM member agencies about what types of ENMs are of agency interest and whether there is agency-specific guidance for ENMs toxicity testing (Petersen et al. 2022). To support the ability of NAMs to provide robust results in ENM testing, two key issues in the usage of NAMs, namely dosimetry and interference/bias controls, are thoroughly discussed.

ICCVAM PFAS Workgroup

Tags: ICCVAM, PFAS

PFAS are organofluorine chemicals manufactured and used for decades in products and applications such as firefighting foams, food packaging, and stain- and water-resistant fabrics and carpeting. PFAS have very specific properties that make them resistant to oil, water, and high temperatures. They are highly persistent and bioaccumulate in the environment, biota, and humans. While there is great national and international pressure to understand PFAS hazard, doing so effectively and efficiently presents many challenges. There is no consensus definition of PFAS, and their large variety of chemical structures, physiochemical characteristics, and toxicological properties makes PFAS hazard prediction extremely challenging.

ICCVAM established a workgroup in 2023 to provide expertise in identifying and evaluating NAMs to predict PFAS toxicity. The group's charges include:

- Evaluate current PFAS definitions and groupings.

- Assess the utility of NAMs currently being used by national and international regulatory agencies for testing and risk assessment of specific PFAS based on case examples of successful application of certain NAMs.

- Identify research challenges and data gaps for the use of specific NAMs for testing and risk assessment of different PFAS.

- Identify NAMs that have not been used for PFAS and explore opportunities for expanding their application for their testing and risk assessment.

The planned activities of the group were discussed at the [September 2023 SACATM meeting](). The workgroup is currently drafting a white paper that will propose definitions and groupings of PFAS, outline current applications of NAMs for PFAS hazard identification, and identify challenges and data gaps that need to be addressed for further applications of NAMs. It will then convene a workshop to address issues identified in the white paper, with findings from the workshop presented in a published manuscript.

Retrospective analysis of dog study data from food and color additive petitions

Tags: FDA

The use of animals in toxicity testing and in scientific research more broadly has been a subject of increasing discussion in recent years. Objections to the use of dogs for this purpose focus both on ethical and humanitarian grounds and practical concerns, which note both the expense and time needed for these studies and the value and relevance of dog studies in human risk assessment. To explore alternatives to animal testing for food and color additives, scientists within the FDA Center for Food Safety and Applied Nutrition analyzed a sample of food additive and color additive petitions submitted to FDA (Flannery et al. 2023). The analysis indicated that most safety evaluations of food and color additives did not rely upon dog studies to set an acceptable daily intake, although dog study data were used in making safety decisions in roughly one-third of petitions. Future research should include the development and use of qualified alternative studies to replace the use of animal testing for food and color additive safety assessment while ensuring human safety.

Review of international regulatory uses of acute systemic toxicity data and integration of NAMs

Tags: NIEHS, acute toxicity, inhalation toxicity

Chemical regulatory authorities around the world require systemic toxicity data from acute exposures via the oral, dermal, and inhalation routes for human health risk assessment. To identify opportunities for regulatory uses of nonanimal replacements for these tests, NICEATM scientists and collaborators reviewed acute systemic toxicity testing requirements for jurisdictions that participate in the International Cooperation on Alternative Test Methods (ICATM): Brazil, Canada, China, the European Union, Japan, South Korea, Taiwan, and the United States (Strickland et al. 2023). The chemical sectors included in review of each jurisdiction were cosmetics, consumer products, industrial chemicals, pharmaceuticals, medical devices, and pesticides. The study demonstrated acute systemic toxicity data were most often required for hazard assessment, classification, and labeling, and to a lesser extent quantitative risk assessment. Where animal methods were required, animal reduction methods were typically recommended. For many jurisdictions and chemical sectors, nonanimal alternatives are not accepted, but several jurisdictions provide guidance to support the use of test waivers to reduce animal use for specific applications. An understanding of international regulatory requirements for acute systemic toxicity testing will inform

ICATM's strategy for the development, acceptance, and implementation of nonanimal alternatives to assess the health hazards and risks associated with acute toxicity.

Initiatives to Replace or Reduce Animal Use

Reducing or eliminating animal testing is a key goal of the 2018 ICCVAM [Strategic Roadmap](#). To this end, ICCVAM agencies that conduct chemical safety testing develop and use approaches that reduce or replace animal use. Likewise, ICCVAM agencies that require their stakeholders to conduct testing are reducing required animal use by developing criteria for waiving tests.

Activities of the ICCVAM Consideration of Alternative Methods Workgroup
Tags: ICCVAM, metrics

In the United States, Animal Welfare Act regulations require investigators to consider alternative methods prior to using animals for research, teaching, or testing whenever proposed procedures involve more than slight or momentary pain or distress [9 C.F.R. § 2.31 (d)(ii)(2022)]. However, there is little incentive for investigators that have long used specific in vivo models and well-established protocols to replace those models with NAMs. ICCVAM established the Consideration of Alternative Methods Workgroup to explore opportunities for encouraging investigators using animal-based models to not only consider, but actively pursue, potential NAMs that could contribute to replacing, reducing, or refining the use of laboratory animals. Virtual stakeholder meetings were held between May 2022 and May 2023 with workgroup members and stakeholder group representatives from industry, academic researchers, and academic Institutional Animal Care and Use Committee members. A common set of discussion questions was formulated and used to collect stakeholders' perspectives on how alternatives to traditional animal tests are considered when developing their respective organization's toxicology testing programs. These interactions revealed that many stakeholders already use NAMs for mechanistic research, justification in animal protocols, internal company decision-making, and various regulatory processes. However, they also identified barriers to using NAMs, including limited funding available for NAM development, scientific and technical challenges, regulatory acceptance and harmonization issues, as well as limited knowledge and training on NAMs. Recommendations for overcoming these barriers included ensuring fit-for-purpose use of NAMs, developing

national and global harmonized acceptance criteria, identifying funding sources, and increasing awareness about the strengths and limitations of NAMs. Regulators, NAMs developers, and end-users need to increase dialogue on priorities and opportunities for NAMs. To foster increased consideration of NAMs, targeted funding opportunities are needed to develop and incorporate NAMs in research and regulatory applications. An abstract describing the activities of the workgroup (Marko et al.) has been accepted for a poster presentation at the 2024 SOT annual meeting, and the workgroup is preparing a white paper for submission to a peer-reviewed journal in 2024.

Integrating "one health" concepts into phased approaches to toxicology data collection
Tags: DoD, exposure, QSAR, structure similarity

Development of military-specific substances can include substances used in new weapons systems and platforms, medications for countermeasures, insect repellants, fire extinguishing agents, warfighter gear, propellants, smokes, simulators, and specialized coatings. All of these must be shown to be safe for warfighter use and be sustainable for manufacture, use, and demilitarization. DoD has developed an effective approach to providing environmental, safety, and occupational health information early in the research, development, testing, and evaluation stages for new and emerging materials (Johnson and Adams 2023). Research begins with modeling promising chemical structures in silico. These quantum mechanical models can also provide information useful for understanding environmental fate and transport and assist in understanding relevant exposure pathways. Once chemical structure information is available, toxicologists provide interpretation on toxicity using in silico models (e.g., QSARs) and read-across techniques. These data are provided to chemists selecting the optimal chemical solutions. Prospective substances are then tested using a variety of in vitro assays useful for suspected toxicity targets based on structural read-across or in silico predictions. Data from these studies are used as the basis for recommendations on progression to in vivo studies focusing on identifying toxic thresholds to protect human health and the environment, and subsequent studies for specific endpoints as needed.

Use of human data for the classification of skin sensitization hazard and potency
Tags: CPSC, FDA, NIEHS, skin sensitization

Regulatory entities worldwide use skin sensitization test data to classify substances for their likelihood and potency for inducing allergic contact dermatitis. One hazard classification system for this purpose is defined by the GHS. As humans are the primary species of interest for these classifications, and high-quality human data sets exist for this endpoint, it is appropriate to consider how human data might be applied to defining these classifications. Scientists with the German Federal Institute of Risk Assessment and CPSC, FDA, and NIEHS assessed the value of human predictive patch test (HPPT) data for classification of chemicals as skin sensitizers under the GHS (Herzler et al. 2024). They proposed that the dose per skin area (DSA) used for classification by the GHS to be replaced by or complemented with a dose descriptor that may better reflect sensitization incidence. Two such descriptors described in the paper are the DSA causing induction of sensitization in one individual or the DSA leading to an incidence of induction in 5% of the tested individuals. The paper also proposed standardized concepts and workflows for assessing individual HPPT results, as well as for integrating multiple HPPT results and for using them in concert with local lymph node assay data in a weight-of-evidence assessment. The study determined that HPPT results are often not sufficient for deriving unambiguous classifications on their own. However, where they are, the resulting classifications are reliable and reproducible and can be integrated well with those from other skin sensitization data.

Rethinking Carcinogenicity Assessment for Agrochemicals Project
Tags: EPA, carcinogenicity, exposure, metabolism, mixtures toxicity

Rodent cancer bioassays have long been required for regulatory assessment of human cancer hazard and risk. However, decades of information from chronic and carcinogenicity studies have revealed some limitations of these studies. For example, certain aspects of the assay may lack human relevance. In addition, long-term rodent bioassays are costly, time-consuming, require large numbers of animals, and can be difficult to reproduce. Human-relevant NAMs based on an improved understanding of human cancer biology may provide a less resource-intensive and more rapid method to assess chronic toxicity and carcinogenicity when making health-protective decisions. The use of NAMs in chemical safety assessment can be achieved by way of regulatory flexibilities, including a weight-of-evidence-based approach in which studies can be waived when alternative methods or existing data are sufficient. To streamline and support approval of new technologies,

scientists from government, academia, nongovernmental organizations, and industry stakeholders convened the Rethinking Carcinogenicity Assessment for Agrochemicals Project. The project developed a reporting framework ([Hilton et al. 2022](#)) to support a chronic toxicity and carcinogenicity study waiver rationale, which includes information on use pattern(s), exposure scenario(s), pesticidal mode-of-action, physicochemical properties, metabolism, toxicokinetics, toxicological data including mechanistic data, and chemical read-across from similar registered pesticides. The framework could also be applied to endpoints other than chronic toxicity and carcinogenicity, and for chemicals other than agrochemicals.

Retrospective analyses to reduce number of species needed for acute fish toxicity assessment for conventional pesticides

Tags: EPA, NIEHS, acute toxicity, ecotoxicity

EPA uses data from the in vivo fish acute toxicity test to assess potential risk of substances to aquatic vertebrates. The test is typically conducted on a cold and a warm freshwater species and a saltwater species for a conventional pesticide registration, potentially requiring upwards of 200 or more fish. EPA and NIEHS scientists conducted a retrospective data evaluation to explore the potential for using fewer fish species to support conventional pesticide risk assessments ([Ceger et al. 2023](#)). LC50 values and experimental details were extracted and curated from 718 studies on fish acute toxicity submitted to EPA. The LC50 data were analyzed to determine, when possible, the relative sensitivity of the tested species to each pesticide. In 85% of those cases, one of the tested freshwater species was identified as the most sensitive, with the cold freshwater species being the most sensitive overall among cases with established relative sensitivity. The results support potentially using fewer than three fish species to conduct ecological risk assessments for the registration of conventional pesticides.

Retrospective analyses to reduce number of species needed for avian reproductive toxicity assessment for conventional pesticides

Tags: EPA, ecotoxicity

EPA considers chronic risk to birds when evaluating ecological risk to wildlife from the application of pesticides. These risk assessments are supported by ecotoxicity data required for pesticide registration, including data on avian reproductive toxicity ([OCSPP 850.2300](#)) in two birds: an upland game species (typically bobwhite quail) and a waterfowl species

(typically mallard duck). EPA is collaborating with nongovernmental organizations and industry stakeholders on a retrospective analysis to assess species differences in sensitivity to conventional pesticides and determine the impact of the two avian test species on the ecological risk assessment. The analysis examines patterns in species sensitivity across groups of chemicals. When species differences are identified, an additional analysis is being conducted to determine if differences in species sensitivities could be reliably accounted for without testing of a second species. The goal of this project is to determine if there are factors that can be considered when determining testing requirements for conventional pesticides and if chronic risk to birds can be confidently assessed using a reduced number of test species. Results will be described in a paper to be submitted to a peer-reviewed journal in 2024.

Retrospective analyses to reduce number of tests needed to assess honeybee toxicity for conventional pesticides

Tags: EPA, ecotoxicity

EPA ecological risk assessments for pesticide registration include a summary of potential risks to bees. Toxicity data are submitted to support these assessments, and EPA maintains a database containing acute and chronic toxicity data on adult and larval honeybees (Apis mellifera), which EPA considers a surrogate for Apis and non-Apis bees. EPA compared these toxicity data to explore possible trends and assess whether efficiencies can be gained in targeting the specific studies needed for risk assessment (Farruggia et al. 2022). The analysis provided evidence that compounds with a low acute (single exposure) toxicity for adults through contact and oral exposure pathways may still be toxic for adults and larvae following chronic (repeat) oral exposure. For example, the acute toxicity of herbicides and fungicides was lower for adult versus larval honeybees, and chronic toxicity studies resulted in more sensitive endpoints in larvae than adults, indicating increased sensitivity of larvae.

Value-of-information framework to inform an EPA toxicity testing strategy

Tags: EPA

Value-of-information analysis is a systematic approach to determine the "value of information" in economic terms. It allows comparison of "what we already know" and "what we will know" to determine which data generation methodologies are most valuable for

decision-making. While the potential application of value-of-information analysis for toxicology has been discussed for a number of years, practical applications of such an analysis in toxicology to real-world problems are lacking. EPA and collaborators developed a value-of-information framework for assessing the trade-offs associated with uncertainty, duration, and cost of chemical toxicity testing ([Hagiwara et al. 2023](#)). A novel feature of this framework was the inclusion of a time dimension that permits incorporation of the cost of delay in incorporating additional information. A case study applied this framework to compare a short-term in vivo transcriptomic assay approach to developing protective reference doses with the traditional chronic rodent bioassay and human health assessment process. A panel of the EPA Board of Scientific Counselors was convened to assess the scientific rigor of this case study and the resulting conclusions. The case study was presented at a [public meeting](#) of the panel in July 2023, and [the panel's report and recommendations](#) will be published in early 2024.

Availability of NAMs in the Endocrine Disruptor Screening Program
Tags: EPA, endocrine disruptors

The EPA's EDSP employs a two-tiered approach. Tier 1 uses 11 screening assays to identify the potential of a chemical substance to interact with the estrogen, androgen, or thyroid pathways. Tier 2 testing is designed to identify any adverse endocrine-related effects caused by the substance and establish a dose-response relationship for any adverse estrogen, androgen, or thyroid effect. Given the large number of chemical substances covered by the EDSP, research efforts have focused on the development and evaluation of NAMs for use as alternatives to the current suite of assays in the EDSP Tier 1 battery to accelerate the pace of screening, add efficiencies, decrease costs, and reduce animal testing.

In January 2023, [EPA released a draft whitepaper](#) for public comment on the availability of NAMs in the EDSP that describes validated NAMs, including those incorporated into the estrogen receptor and androgen receptor pathway models, that EPA may now accept as alternatives for certain EDSP tests. Additional NAMs were identified for potential use in prioritization or for consideration as Other Scientifically Related Information in weight-of-evidence evaluations. After considering public comments, EPA will issue a finalized document.

Additionally, in October 2023, EPA announced the [availability of near-term strategies](#) to help the agency meet its obligations and commitments relating to the EDSP. As part of these strategies, results from the estrogen receptor and androgen receptor pathway models were utilized as part of a prioritization framework.

Incorporation of tiered testing into TSCA Section 4 test orders on PFAS
Tags: EPA, genotoxicity, inhalation toxicity, metrics, PFAS

The Frank R. Lautenberg Chemical Safety in the 21st Century Act, enacted in 2016, amended the Toxic Substances Control Act of 1976. Section 4 of the updated law authorizes EPA to issue test orders to chemical manufacturers (including importers) and processors to develop information on chemical toxicity, subject to specific requirements to reduce vertebrate animal use. One such requirement, described in Section 4(a)(4), is to employ a tiered-testing strategy. Other requirements, described in Section 4(h), directs EPA to review all reasonably available toxicity information to avoid unnecessary or duplicative testing, and to consider non-vertebrate methods where appropriate.

As of the end of 2023, EPA has issued three test orders on PFAS and plans to issue more in the near future. The PFAS test orders include in vitro tests in the tiered-testing strategy, including a respiratory tract epithelial cell culture and genotoxicity tests. One of the test orders, on a chemical known as HFPO-DAF (a precursor to GenX), contains no vertebrate testing at all. Vertebrate testing is included in Tier 2 tests for the other two PFAS test orders, but the tiered testing provides for waiving of these tests based on Tier 1 test results. Taken together, the orders demonstrate EPA's commitment to employ non-vertebrate testing to the extent practicable and scientifically justified.

Reduction of use of dog testing for agrochemical registration
Tags: EPA, NIEHS, AOP, metabolism, mixtures toxicity

Progress in developing new tools, assays, and approaches to assess human hazard and health risk provides an opportunity to re-evaluate the need of dog studies for the safety evaluation of agrochemicals. EPA and NIEHS scientists and a diverse group of international collaborators met at a 2021 workshop to discuss the strengths and limitations of past use of dogs for pesticide evaluations and registrations ([Bishop et al. 2023](#)). Opportunities were identified to support alternatives to the 90-day dog study for answering human safety

questions. Participants proposed developing a decision tree for determining when the dog study might not be necessary to inform pesticide safety and risk assessment. Such a process will require global regulatory authority participation to lead to its acceptance. The identification of unique effects in dogs that are not identified in rodents will need further evaluation and determination of their relevance to humans. The establishment of in vitro and in silico approaches that can provide critical data on relative species sensitivity and human relevance will be important in the decision. Novel tools including in vitro comparative metabolism studies, in silico models, and high-throughput assays able to identify metabolites and mechanisms of action could potentially support development of AOPs; such tools show promise but will need further development. To replace or eliminate the 90-day dog study, a collaborative, multidisciplinary, international effort that goes beyond organizations and regulatory agencies will be needed in order to develop guidance on when the study would not be necessary for human safety and risk assessment.

FDA regulatory science tools to help assess new medical devices
Tags: FDA, cardiotoxicity, neurotoxicity

FDA provides a [Catalog of Regulatory Science Tools to Help Assess New Medical Devices](#). This peer-reviewed resource assists medical device companies to evaluate new medical devices in contexts where standards and qualified medical device development tools do not yet exist. The catalog collates a variety of regulatory science tools developed by the FDA's Center for Devices and Radiological Health's Office of Science and Engineering Labs. The tools use the most innovative science to support medical device development and patient access to safe and effective medical devices. Tools categories include lab methods, computer models, and data sets, and they are applicable to program areas including cardiovascular, neurology, and biocompatibility and toxicology. An update of the catalog is planned for early 2024.

A recent example of a qualified alternative method is the [Chemical Risk Calculator (CHRIS) - Color Additives](#) tool, which was qualified by the Center for Devices and Radiological Health in November 2022. CHRIS – Color Additives is a nonclinical assessment model to conduct screening-level risk assessments to aid in the biocompatibility evaluation of polymeric medical device components that contain color additives.

Policies and Guidance for Implementation of Alternative Methods

To encourage adoption and use of NAMs the 2018 ICCVAM [Strategic Roadmap](#) called on agencies to provide clear guidance on use and acceptance of data from these approaches. During 2022 and 2023, agencies issued guidance for use of NAMs in the areas of testing for carcinogenicity, DNT, eye irritation, and immunotoxicity.

CPSC Guidance on New Approach Methodologies
Tags: CPSC

In June 2023, CPSC updated its [Policy on Animal Testing webpage](#). It has been redesigned to be more user-friendly and have direct access to necessary information and documents.

One of the resources available on the [CPSC webpage](#) is CPSC's "[Guidance for Industry and Test Method Developers: CPSC Staff Evaluation of Alternative Test Methods and Integrated Testing Approaches and Data Generated from Such Methods to Support FHSA Labeling Requirements](#)." CPSC developed this guidance, building on its Animal Testing Policy, to assist stakeholders in determining what test methods are deemed reliable for determining compliance with the labeling requirements under the Federal Hazardous Substances Act. This includes clarification of CPSC informational requirements and process for evaluating NAMs and integrated approaches to testing and assessment (IATAs).

Amendments to the Hazardous Materials Regulations to expand options for in vitro testing for classifying corrosives
Tags: DOT

The U.S. Department of Transportation (DOT) classifies substances to establish precautions that may need to be taken when materials are handled or transported. Two rulemaking activities during 2022 and 2023 brought DOT regulations into alignment with international regulations that enable reduction of animal use for this purpose. These resulted from ongoing collaborations with the United Nations to expand options for nonanimal testing for classifying hazardous materials for transportation.

- The Final Rule for HM-215P ([87 FR 44944](#)) was published in July 2022. It incorporates the 2016 version of [OECD Test No. 431](#), "In Vitro Skin Corrosion: Reconstructed Human Epidermis (RHE) Test Method." This test method is used to assign a packing group to Class 8 (corrosive) materials under [49 CFR § 173.137](#).

Since the RHE test method does not distinguish between packing groups II ("medium danger") and III ("low danger"), DOT added language to allow corrosive materials classified using this method to be assigned to the more conservative packing group without further testing.

- A Notice of Proposed Rulemaking for HM-215Q ([88 FR 34568](#)) was published in May 2023. This Notice proposed incorporating a new in vitro test method, [OECD Test No. 439](#), "In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method," to allow a material to be ruled into or out of Class 8. DOT also proposed revising 49 CFR 173.137 to allow corrosive materials to be assigned to the most conservative applicable packing group when tested using test methods that cannot distinguish between each packing group. As of early 2024, DOT is finishing the Final Rule that will incorporate these changes.

Decision framework to evaluate new chemicals for eye and skin irritation and corrosion hazards

Tags: EPA, NIEHS, eye irritation

EPA, in collaboration with NICEATM and nongovernmental organizations, is developing a [new framework for identifying eye irritation and corrosion hazards](#) for new chemicals reviewed under TSCA. With this new framework, EPA will prioritize data from nonanimal test methods that are more reproducible and provide results more relevant to humans than the corresponding in vivo animal model test. Under the framework, information is collected and evaluated in the following order: (1) data from human cell/tissue-based test methods that have been demonstrated to be reproducible and relevant to eye irritation; (2) data from in chemico or non-human in vitro and/or ex vivo test methods that have been demonstrated to be reproducible and provide information on the mechanisms of toxicity relevant to eye irritation in humans; and (3) data from in vivo animal studies. If no acceptable information on eye irritation is available, the framework allows for consideration of skin irritation data that predict irritating or corrosive properties to make inferences about eye irritation hazard of the new chemical substance. Evaluation of skin irritation and corrosion data follows the same prioritization order as presented above for eye irritation information. This framework will streamline the decision-making process and increase efficiency through a standard process for EPA to use each time it evaluates eye irritation or corrosion hazards test data. The new

framework supports EPA's mandate under TSCA to promote the development and implementation of alternative test methods and strategies that can provide information on chemical hazards without vertebrate animal testing. This framework also supports EPA's ongoing efforts to reduce the use of animal testing and make the Agency's review of new chemicals more efficient, helping to bring new chemicals to market more quickly while protecting human health.

EPA report on use of NAMs for human health risk assessment
Tags: EPA, developmental neurotoxicity

NAMs can provide human-relevant information that may be challenging to obtain from whole-animal tests. EPA's Office of Pesticide Programs is interested in using NAMs to reduce the reliance on default assumptions for risk assessment, including the application of 10X default uncertainty factors each for interspecies and intraspecies extrapolations. To this end, the Office of Pesticide Programs collaborated with EPA's Office of Research and Development, academia, and industry to use NAM data to inform extrapolation/uncertainty and safety factors. As a part of this effort, EPA held a public meeting of the Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel in September 2020 to consider and review the use of NAMs to derive extrapolation factors and evaluate developmental neurotoxicity (DNT) for human health risk assessment. The panel was supportive of using the DNT NAM battery in weight-of-evidence evaluations of DNT potential, while recognizing that the battery should be a "living and evolving process." The panel also supported the use of chemical-specific in vitro data to inform interspecies extrapolation factors for organophosphate pesticides, but provided recommendations to reanalyze the data in a manner more consistent with the EPA's "[Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors](.)." EPA responded to the panel's recommendations in a [March 2023 memorandum](), [available with related documents on the Regulations.gov website]().

Update to FDA guidance on qualification of medical device development tools
Tags: FDA

"[Qualification of Medical Device Development Tools: Guidance for Industry, Tool Developers, and FDA Staff]()," was issued in July 2023. This document, an update of guidance

issued is 2017, provides guidance on a voluntary program for qualification of medical device development tools for use in evaluating devices subject to regulation by FDA's Center for Devices and Radiological Health. Medical device development tools are methods, materials, or measurements used to assess the safety, effectiveness, or performance of medical devices. They can include NAMs, biomarker tests, and clinical outcome assessments.

FDA ISTAND program for new drug development tools
Tags: FDA, metrics, MPS

Drug development tools are methods, materials, or measures that have the potential to facilitate drug development. FDA established the Innovative Science and Technology Approaches for New Drugs (ISTAND) pilot program in 2020 to encourage innovation of drug development tools that are out-of-scope for existing qualification programs but may still be useful for drug development. Approaches that could be considered under the pilot program include:

- Using MPS to assess safety or efficacy questions.

- Developing novel nonclinical pharmacology or toxicology assays.

- Using artificial intelligence-based algorithms to evaluate patients, develop novel endpoints, or inform study design.

In the pilot phase, FDA anticipates accepting two to four submissions into the ISTAND program each year with a triage and selection process that focuses on public health impact and feasibility of implementation. There were three projects accepted into ISTAND during 2022 and 2023, including one representing both the first artificial intelligence-based and digital health technology-based project and the first project in neuroscience to be accepted into the program. The Drug Development Tools Research Grant Cycle will be open through May 13, 2024 for Fiscal Year 2024, and through May 13, 2025 for Fiscal Year 2025.

FDA guidance on computational modeling for medical device submissions
Tags: FDA

In November 2023 FDA published guidance on "Assessing the Credibility of Computational Modeling and Simulation in Medical Device Submissions." Computational modeling and simulation can be used in a variety of ways in medical device applications, including to

perform in silico device testing or as part of software embedded in a device. This guidance provides a risk-informed framework for credibility assessment of computational modeling and simulation used in medical device regulatory submissions. The guidance is intended to promote consistency and facilitate efficient review of medical device submissions, to increase confidence in the use of computational modeling and simulation in regulatory submissions, and to facilitate improved interpretation of computational modeling and simulation credibility evidence submitted in regulatory submissions. The guidance will be discussed in a presentation at the January 2024 ICCVAM Communities of Practice webinar.

FDA guidance on nonclinical evaluation of immunotoxicity
Tags: FDA, skin sensitization

In June 2023 FDA finalized the guidance document, "Nonclinical Evaluation of the Immunotoxic Potential of Pharmaceuticals." The purpose of this guidance is to assist sponsors in the nonclinical evaluation of the immunotoxic potential of drug products and biopharmaceuticals.

The guidance addresses evaluation of topical pharmaceuticals for skin sensitization potential. Specifically, the guidance states that, for individual chemicals, "FDA will consider a battery of studies (e.g., in silico, in chemico, in vitro) that have been shown to adequately predict human skin sensitization with an accuracy similar to existing in vivo methods."

FDA guidance on carcinogenicity testing
Tags: FDA, carcinogenicity

In a November 2022 Federal Register notice (87 FR 66195), FDA announced availability of a final guidance for industry, "S1B(R1) Addendum to S1B Testing for Carcinogenicity of Pharmaceuticals." The guidance was prepared under the auspices of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, and offers an integrative approach that provides specific weight-of-evidence criteria that inform whether a 2-year rat study is likely to add value in completing a human carcinogenicity risk assessment. This final guidance considered comments received from the public in response to release of the draft guidance in October 2021.

Leadership

As an interagency committee of the U.S. federal government, ICCVAM takes a leadership role in promoting the use of new approach methodologies (NAMs). ICCVAM agencies promote new testing approaches through providing funding to support development of new methods, as well as through international interactions and collaborations.

Resources for Alternative Methods Development

To support developers of new testing approaches, ICCVAM is drafting a new document describing criteria and processes for validation and regulatory acceptance of toxicological testing methods. During 2022 and 2023, NIEHS and other ICCVAM member agencies also supported alternative methods development through grants to small businesses and academic institutions. These grants supported development of new testing approaches for ecotoxicity and platforms such as in vitro organoids and tissue chips.

Updated criteria and processes for validation, qualification, and regulatory acceptance of toxicological test methods

Tags: ATSDR, CPSC, DoD, EPA, FDA, ICCVAM, NIEHS, NIH, NIST, OSHA, VA ORD

In 1997, ICCVAM published "Validation and Regulatory Acceptance of Toxicological Test Methods," which recommended criteria and processes for validation and regulatory acceptance of toxicological testing methods that would be useful to federal agencies and the scientific community. Approaches to validating new methods have evolved considerably since the publication of this document. New concepts have emerged related to the development of NAMs and the evaluation of their utility for regulatory uses, such as the qualification of a NAM for a particular context of use. However, guidance is needed on specific processes to put these concepts into practice.

In 2021, ICCVAM established a Validation Workgroup to consider this issue. The workgroup, which has members from ten ICCVAM agencies, applied the advances of the last two decades to a new document describing criteria and processes for validation and regulatory acceptance of toxicological testing methods. The new document provides more specific insight on establishing confidence in NAMs building upon the principles outlined in the ICCVAM 2018 Roadmap. In addition to supporting development of flexible validation practices that consider context of use (as described, for example, in van der Zalm et al. 2022),

the new document addresses the need to align validation approaches in a manner that encourages international harmonization and incorporates best practices for quality and quality systems development ([Petersen et al. 2023](#)). The document draws upon recent well-established validation publications to ensure alignment of approaches.

A draft of the new document was made public in August 2023. Comments were requested from the public via the Federal Register ([88 FR 54342](#)), and the document was discussed at the September 2023 meeting of the [Scientific Advisory Committee on Alternative Toxicological Methods](#). The new document is being finalized to address comments received and [will be published in early 2024](#).

Ecotox Challenge to develop approaches to measure gene transcriptional responses to chemicals in ecotox target species
Tags: DoD, EPA, ecotoxicity

In 2020, EPA announced a partnership with DoD, international governments, and industry to sponsor the [EcoTox TARGET innovation challenge](#). The challenge awarded $300,000 to the applicant who successfully developed a low-cost high-throughput technology for measuring global gene expression in samples from four common aquatic toxicity test organisms. The winner, BioSpyder Technologies, Inc., was announced in spring 2022. An abstract describing the challenge and outcome (Villeneuve et al.) has been accepted for a poster presentation at the Advances in Genome Biology and Technology general meeting in February 2024. There are no plans to repeat the challenge in the foreseeable future but research to apply the technology is ongoing.

FDA and NCATS grants to develop tissue chips for botulinum toxin testing
Tags: FDA, NIH, MPS, neurotoxicity

Tissue chips have emerged as an in vitro alternative to animal use with the potential to be more predictive of human response in the safety and efficacy assessment of leading therapeutics. In 2022 and 2023, NCATS and FDA offered [small business innovation research](#) and [business technology transfer](#) grants for the development of neuromuscular junction tissue chips to replace the mouse lethality bioassay as a potency assay for botulinum toxin. A main objective for this funding opportunity was to position these tissue chips as an alternative test method as a standalone replacement for mouse lethality bioassays.

Grants to support development of biomimetic tissue-engineered technology for cancer research

Tags: NCI, carcinogenicity

To support participation in its [Cancer Tissue Engineering Collaborative Research Program](#), NCI offers grants to support the development and characterization of state-of-the-art biomimetic tissue-engineered technologies for cancer research. The goals of the program are to (1) catalyze the advancement of innovative, well-characterized in vitro and ex vivo systems available for cancer research, (2) expand the breadth of these systems to several cancer types, and (3) promote the exploration of cancer phenomena with biomimetic tissue-engineered systems. The program awards grants of up to $400,000 to fund projects that can continue up to five years. Eligibility for these grants is open to for-profit and nonprofit institutions within and outside the United States. NCI began accepting applications for funding in May 2017; applications will be accepted and considered through February 2025.

Small business grants to support alternative methods development

Tags: NIEHS, developmental neurotoxicity, population variability

Throughout 2022 and 2023, NIEHS provided [funding for small businesses](#) developing technologies of interest to the Tox21 program and other Institute goals. The funding was offered as part of the 2022 and 2023 Omnibus Solicitations of NIH, Centers for Disease Control, and FDA for small business grant applications to support development and commercialization of innovative technologies. Technologies supported by NIEHS included improved or expanded testing methods for toxicity screening, computational approaches for predictive toxicology, and other technologies such as alternative or improved methods for fixing and preserving tissues.

In addition to funding offered via the Omnibus Solicitations, NIEHS offers grants to support development of specific types of technologies targeting endpoints of interest, as well as resources useful to the methods developer community. Funding offered in 2022 and 2023 supported:

- Development of resources, new methods, and approaches that can be applied in testing strategies to better understand the role of environmental chemicals in the etiology of neurodevelopmental disorders.

- Projects to enable environmental health sciences communities to openly develop, extend, adapt, or refine data and metadata standards as well as associated tools to implement standards.

- Development of resources and approaches that reflect the variability in responses to chemical exposures based on genetic diversity in the human population.

International Interactions

Efforts by individual countries to develop NAMs will have little impact without international adoption of the new methods. To advance international adoption, ICCVAM and its member agencies interact frequently with international partners to facilitate harmonization and regulatory acceptance.

Contributions to OECD activities

Tags: ICCVAM, defined approaches/IATA, developmental neurotoxicity, endocrine disruptors, eye irritation, skin sensitization

ICCVAM member agencies participate in the development and review of chemical testing guidelines issued by the [OECD Test Guidelines Programme](). OECD test guidelines are used by government, industry, and independent laboratories of the 38 OECD member countries to assess chemical safety. The U.S. National Coordinators for the OECD Test Guidelines Programme, who are members of ICCVAM, solicit and collate U.S. comments on draft test guidelines, guidance documents, project proposals, workshop reports, and other documents of the Test Guidelines Programme. The National Coordinators represent the United States at the annual meeting of the Working Group of National Coordinators and in other test guideline development activities. One or more ICCVAM subject matter experts may join the U.S. National Coordinators at this meeting.

In 2022 and 2023, ICCVAM agencies commented on draft OECD documents through the U.S. National Coordinators. ICCVAM members and/or NICEATM staff also supported the Test Guidelines Programme during 2022 and 2023 by:

- Supporting OECD activities to revise Test Guidelines Programme resources and processes to better support uptake of emerging technologies. Workshops held in December 2022 and December 2023 focused on how to prepare for emerging technologies and practical and financial aspects of validation studies, respectively.

ICCVAM members assisted in organizing these meetings and provided expert participants.

- Co-leading a project and participating in an OECD expert group coordinating revision of Guidance Document 34 on validation and acceptance of new or updated test methods for hazard assessment. Revision of this document was also a key topic of discussion at the 2023 ICATM coordination meeting.

- Leading development of the OECD Omics Reporting Framework. This modular framework released in 2023 facilitates data sharing for toxicology experiments using various types omics approaches (i.e. transcriptomics, metabolomics), increases transparency of omics data processing and analysis approaches, enables quality assessments, promotes reproducibility in omics experiments, and fosters the uptake of omics data for use in regulatory processes (Harrill et al. 2021).

- Contributing to a proposal to update OECD Test Guideline 496 for in vitro test methods to identify eye irritants to add the OptiSafe test method to the guideline.

- Serving on an expert group developing a guideline for defined approaches for skin sensitization. This group supported the development of Guideline 497, Defined Approaches for Skin Sensitisation, issued in 2021. Guideline 497 is the first internationally harmonized guideline to describe a nonanimal defined approach (DA) that can be used to replace an animal test to identify skin sensitizers. NICEATM and ICCVAM scientists are currently contributing to two proposals to update Guideline 497 to include new information sources for existing DAs, and new DAs for quantitative risk assessment.

- Participating in a group developing a Case Study on the Use of an IATA for Identifying Androgen Receptor Active Chemicals, which has been submitted to the OECD Working Party on Hazard Assessment.

- Serving on the Validation Management Group – Non-animal, which focuses on evaluation of new methods for identifying endocrine disruptors.

- Serving on an expert group considering test batteries for developmental neurotoxicity (DNT). NIEHS and EPA scientists developed a case study on application of IATAs

for DNT, which was published by OECD in 2022. Expert group members also [contributed to a 2023 guidance document on evaluating data from in vitro DNT test batteries](#).

- Participating on an expert group evaluating methods to assess thyroid disruption.

- Participating on an advisory group on emerging science in chemicals assessment.

Participation in the International Cooperation on Alternative Test Methods

Tags: ICCVAM, acute toxicity, IVIVE, skin sensitization, structure similarity

The [International Cooperation on Alternative Test Methods](#) (ICATM) was created to foster dialogue among national validation organizations. This dialogue facilitates international cooperation in the critical areas of validation studies, independent peer review, and development of harmonized recommendations. ICATM includes member organizations from the European Union, United States, Japan, Canada, and South Korea. In recent years Brazil, China, Singapore, and Taiwan have participated in ICATM as observers.

ICATM Participant Organizations

- **[ICCVAM](#)** is an interagency committee of the U.S. government that coordinates technical reviews of alternative test methods and cross-agency activities relating to validation, acceptance, and harmonization of test methods. [NICEATM](#) administers ICCVAM and provides scientific support for its activities.

- **[EURL ECVAM](#)** (European Union Reference Laboratory for Alternatives to Animal Testing) is a unit within the Institute of Health and Consumer Protection in the European Union's Joint Research Centre. EURL ECVAM coordinates the validation of alternative test methods in the European Union.

- **[JaCVAM](#)** (Japanese Center for the Validation of Alternative Methods) coordinates the evaluation of alternative test methods for the Japanese National Institute of Health Sciences, its parent organization.

- **[Health Canada](#)'s** Environmental Health Science and Research Bureau coordinates the evaluation of alternative test methods in Canada. The [Canadian Centre for Alternatives to Animal Methods](#) and its subsidiary, the Canadian Centre for the

Validation of Alternative Methods, participate as partners with Health Canada in ICATM activities.

- **KoCVAM** (Korean Center for the Validation of Alternative Methods) is part of the National Institute of Food and Drug Safety Evaluation of the South Korean Food and Drug Administration.

- **BraCVAM** (Brazilian Center for the Validation of Alternative Methods) functions as the focal point within Brazil to identify or receive requests for test method validation. It works to implement appropriate validation studies with the National Network of Alternative Methods to the Use of Animals within the Brazilian Ministry of Science, Technology, Innovations, and Communications.

In most years, ICATM member organizations hold at least one coordination meeting. The most recent coordination meeting was convened in August 2023 in Niagara Falls, Canada, in conjunction with the 12th World Congress on Alternatives and Animal Use in the Life Sciences. Representatives from the United States, European Union, Canada, Japan, South Korea, Taiwan, Brazil, and the Organisation for Economic Co-operation and Development (OECD) Test Guidelines Programme met to discuss ongoing efforts to update OECD's "Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment," informally known as "Guidance Document 34."

Participants in the August 2023 ICATM coordination meeting felt that the traditional validation approach of conducting multi-laboratory ring trials to assess transferability and performance may not be practical for many new technologies upon which NAMs are based. Elements identified as key to successful validation included well-defined protocols that incorporate quality management principles; a diverse set of reference chemicals; clear acceptance criteria that consider standards other than prediction of in vivo animal data (e.g., human biological relevance); and coordinated peer review that minimizes duplication of effort.

ICATM representatives also attend or participate in meetings of the Scientific Advisory Committee on Alternative Toxicological Methods.

NICEATM participated in a peer review of a JaCVAM-coordinated validation study of the EpiSensA skin sensitization test method. The method utilizes a reconstructed human epidermis model to evaluate changes in gene expression of four markers of keratinocyte response to skin sensitizers, the second key event of the adverse outcome pathway for skin sensitization. A poster presented at the 2023 annual meeting of the Society of Toxicology (Reinke et al., Alternatives to Mammalian Models II session) summarized the validation study. The method will be under evaluation at OECD for inclusion in TG 442D in the 2024 review cycle. NICEATM and JaCVAM scientists and members of the EURL ECVAM advisory committee also participated in a peer review of the SENS-IS test method for skin sensitization.

The following ICCVAM workgroups had ICATM member liaison representatives during 2022 and 2023.

ICCVAM Workgroup	ICATM Organizations with Liaison Members
Acute Toxicity Workgroup	EURL ECVAM
In Vitro to In Vivo Extrapolation Workgroup	JaCVAM
Read Across Workgroup	JaCVAM

Participation on ICH

Tags: FDA, carcinogenicity

The FDA Center for Drug Evaluation and Research pursues international harmonization of nonclinical recommendations for pharmaceutical development through its engagement with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). ICH brings together regulatory authorities and the pharmaceutical industry to discuss scientific and technical aspects of drug registration. ICH's mission is to achieve greater harmonization worldwide to ensure that safe, effective, and high-quality medicines are developed and registered in the most resource-efficient manner. These activities prevent repetition of studies and reduce and refine animal use in overall drug development. New guidance recently approved will continue to contribute to the 3Rs as will future guidance currently being developed or revised.

FDA published guidance aligned with ICH documents on carcinogenicity testing of pharmaceuticals as discussed elsewhere in this report.

Review of animal testing requirements in World Health Organization guidelines and recommendations for biologics

Tags: FDA, NIEHS

Biologics such as vaccines, cytokines, enzymes, and hormones are tested routinely after approval to ensure the safety and potency of products. Many of these tests use animals, and thus a large number of animals are used for biologics testing each year.

FDA and NIEHS are supporting a project led by the World Health Organization and NC3Rs to carry out an independent and comprehensive review of animal use requirements within World Health Organization guidelines for biologics and to make recommendations for where nonanimal approaches can be adopted. The project began with a survey of biologics manufacturers to better understand the opportunities and barriers to adoption of replacement, reduction, and refinement (3Rs) alternatives and use of nonanimal approaches in quality control, batch, and lot release testing of biologics. A similar survey of national regulatory authorities and national control laboratories was conducted in early 2022. A September 2022 virtual workshop focused on requirements in North and South America. Biologicals regulatory agencies and manufacturers from the region discussed their perspectives on current testing strategies and how changes to WHO guidelines could be implemented to support wider adoption of 3Rs and nonanimal approaches. The findings from this project were presented to the World Health Organization Expert Committee on Biological Standardization in October 2023.

GHS nonanimal testing working group

Tags: OSHA, skin sensitization

The Occupational Safety and Health Administration (OSHA) is leading the United States' effort to update and expand the use of nonanimal test data for GHS hazard classification. OSHA participated in meetings convened by the United Nations Subcommittee of Experts on the GHS in 2022 and 2023 to harmonize international standards on safe handling of chemicals. OSHA held multiple public meetings to receive input from U.S. stakeholders in advance of the international meetings. The update of GHS chapters on skin and eye

corrosion/irritation was finalized in 2021 to reflect new in vitro test methods for classifying hazards as well as updating guidance on the use of data from in silico methods. Updates to the chapter on eye irritation included updated and expanded guidance on the use of in vitro and in silico test data for classification as well as introducing the use of DAs for use of data from multiple test methods. The work continued in 2022 with an update of skin sensitization, which also includes new in vitro test methods for classifying hazards as well as updating guidance on the use of data from in silico methods and use of DAs. OSHA anticipates the GHS will adopt the changes to the skin sensitization chapter by July 2024.

Collaborations

The 2018 ICCVAM [Strategic Roadmap](#) identified stakeholder engagement as critically important to advancing development and use of alternative methods. The articles below and others throughout this document describe collaborations to advance alternatives within agencies, among agencies, and between agencies and stakeholders.

National Emerging Contaminants Research Initiative
Tags: DoD, DOE, DOT, DOI, EPA, NIH, NIST, USDA, exposure

A contaminant of emerging concern is a material for which there is no regulatory standard, that may have toxicity at lower levels of exposure than previously characterized, is challenging to address across jurisdictions, and for which it is difficult to share information with stakeholders in a timely manner. Many contaminants of emerging concern are of interest to multiple federal agencies, which has given rise to efforts to coordinate addressing them. Initiatives by the White House Office of Science and Technology Policy and directives of the 2020 omnibus National Defense Authorization Act led to establishment of the National Emerging Contaminants Research Initiative to improve the identification, analysis, monitoring, and treatment methods for contaminants of emerging concern. The Contaminants of Emerging Concern Interagency Working Group coordinates federal programs and activities to address contaminants of emerging concern; 18 agencies are involved including eight ICCVAM member agencies. The [working group's 2022 report](#) summarizes current government activities and future needs. Specifically, the report articulated a vision to provide access to clean and plentiful drinking water for everyone in the U.S. and specified five goals to achieve this. Three coordination teams will focus on (1) non-targeted analysis and effects-

based monitoring to discover and screen contaminants of emerging concern; (2) characterizing risk by assessing the potential hazards and exposure; and (3) formulating joint solicitations across agencies. This last activity will involve making a plan for how to work together to craft solicitations and manage them. A draft implementation plan, which was under agency review in fall 2023, outlines a series of short- and long-term activities that are anchored to success metrics. NAMs are seen as a critical tool for potential hazard characterization.

Toxic Exposure Research Working Group
Tags: ATSDR, DoD, DOE, EPA, VA ORD, NIEHS, NIST, AOP, exposure

DoD and the Veterans' Administration have a responsibility to care for its service members who bravely put themselves in harm's way. The Promise to Address Comprehensive Toxics (PACT) Act of 2022 provided resources and intent to improve and expand healthcare to veterans who were exposed to toxic substances during their military service. Understanding past exposure events and their potential influence on health is a requirement for providing informed care and to reduce the probability of future adverse events. This need drove the establishment of an interagency group to outline a strategy to fill research and data gaps associated with toxic exposures during military service. The [Toxic Exposures Research Working Group](), which includes representatives of seven ICCVAM agencies, was initiated to help scope the problem, outline goals, objectives, and tasks that could be reasonably accomplished in five years to help address some of these gaps. The working group's goals include:

1. Improving understanding of the military exposome.

2. Prioritizing and linking military exposures to toxicity and adverse health outcomes, including understanding modes-of-action and AOPs.

3. Investigating associations and interplay between priority military toxic exposures, toxicological endpoints, and adverse health outcomes.

4. Preventing and mitigating military adverse health outcomes from military exposures.

5. Communicate toxic exposure risk and adverse health outcomes to relevant stakeholders to enhance exposure-informed care.

Objectives and tasks were developed with the intention of developing interagency cooperation to establish a federal infrastructure framework for coordinating implementation of this strategic plan. At the end of 2023, the working group is focusing on its final report, which includes contributions from all federal agency partners. The report will be issued in 2024 and address requirements of the PACT Act to outline goals in this area over the next five years.

MPSCoRe Working Group activities
Tags: DoD, NIEHS, NIH, MPS

In 2021, NICEATM, the National Institute of Allergy and Infectious Diseases, the U.S. Army Combat Capabilities Development Command Chemical Biological Center, and NCATS established the [MPS for COVID Research (MPSCoRe) Working Group](#) in collaboration with NC3Rs. The MPSCoRe Working Group facilitates open communication among stakeholders to maximize the impact of MPS technologies in understanding disease mechanisms and treatments and reducing animal use while improving human health. In this way, the group aims to promote adoption of MPS for studying COVID-19 and future emerging infectious diseases ([Kleinstreuer and Holmes 2021](#)). These efforts will accelerate the development and adoption of MPS in infectious disease research and may reduce the reliance on animal models in future studies.

The MPSCoRe Working Group held a [virtual workshop in May-June 2023](#) to facilitate discussion and collaboration about current regulatory approaches and to raise awareness of opportunities for accelerating the integration of MPS models for infectious diseases in the regulatory framework. The working group is planning to organize future webinars on topics of interest.

Workshop on Wildlife Risk Assessment in the 21st Century
Tags: DOI, ecotoxicity

Model species have been used for decades in guideline tests to generate survival, growth, and reproductive data for prospective ecological risk assessments. While such endpoints are central to hazard assessment, nonstandard measures of toxicity at multiple levels of biological organization (e.g., molecular, cellular, tissue, organ, organism, population, community, ecosystem) may enhance the relevance of prospective and retrospective wildlife

risk assessments. The incorporation of NAMs into ecological risk assessment frameworks to reduce or replace animal tests require robust validations against in vivo responses. To address these issues and develop a plan for applying NAMs to ecological risk assessments, the Society of Environmental Toxicology and Chemistry convened a 2021 virtual workshop, "Wildlife Risk Assessment in the 21st Century: Integrating Advancements in Ecology, Toxicology, and Conservation," for which the DOI's USGS was among the sponsors. Two reports from that workshop describe many of the activities that are needed to support application of NAMs to ecological risk assessment. Bean et al. (2023) explores how existing test guidelines for in vivo studies could be improved to provide better data to support NAMs validation. Rattner et al. (2023) discusses how laboratory- and field-derived data along with modeling approaches will likely need to be combined to apply NAMs to ecological risk assessment.

International Consortium to Advance Cross-Species Extrapolation
Tags: EPA, ecotoxicity

There are currently several peer-reviewed and publicly available methods and tools, such as EPA's SeqAPASS, to facilitate cross-species extrapolation. However, because no individual method can advance the application of these types of data in regulatory decision-making, collaboration is needed to effectively advance these approaches. EPA participates in the International Consortium to Advance Cross-Species Extrapolation (LaLone et al. 2021), which was founded to advance cross-species extrapolation and uphold regulatory goals for assessing human and ecological health without animal testing. This is a global, cross-sector consortium that includes researchers, regulators, and other advocates working to integrate bioinformatics approaches. The consortium aims to review and bring the available bioinformatic techniques together to advance the science of cross-species extrapolation using to inform regulatory needs.

EPA and Unilever cooperative research and development agreement
Tags: EPA, ecotoxicity, exposure, metabolism

EPA computational toxicology researchers are developing and using new approaches to evaluate the potential health effects of chemicals. As part of this effort, EPA's Center for Computational and Exposure and Unilever's Safety and Environmental Assurance Centre

have established a cooperative research and development agreement aimed at development and evaluation of NAMs for use in next-generation risk assessment. Currently 40 case study chemicals representing common natural ingredients found in consumer products are being used to explore the utility of a battery of NAMs for evaluating the safety and potential hazards. Research topics in this project include (1) exploring the use of combinations of approaches for determining molecular PODs, (2) comparing molecular PODs to hazardous and non-hazardous human exposure scenarios, (3) evaluating the impact of in vitro metabolism on bioactivity assay readouts, (4) exploring methods for metabolite determination, and (5) piloting the use of fish-derived cell lines to evaluate ecotoxicity hazard. The cooperative research and development agreement was initiated in 2021 and has recently been extended to May of 2027. The project's current status will be discussed in a [webinar planned for April 2024](#).

National Academies Panel on variability and relevance of mammalian toxicity tests
Tags: EPA, NIEHS, NIST

ICCVAM members from NIEHS and NIST served on a committee convened by the National Academies of Sciences, Engineering, and Medicine for the consensus study, "[Variability and Relevance of Current Laboratory Mammalian Toxicity Tests and Expectations for New Approach Methods (NAMs) for use in Human Health Risk Assessment](#)." This study was undertaken by the National Academies in response to a request from EPA. It reviewed the variability and relevance of existing mammalian toxicity tests, specifically in the context of addressing challenges to applying NAMs to human health risk assessment. The committee held two public meetings in 2021. [Proceedings from the second of these](#), a workshop to address the potential utility and expectations for the future use of NAMs in risk assessment and to reflect on the challenges to their implementation, were published in 2022.

While the promise and need for NAMs is clear, many barriers to their use remain. [The final report of the study](#), issued in 2023, aims to bridge the gap between the potential of NAMs and their practical application in human health risk assessment. Lessons learned from laboratory mammalian toxicity tests are used as a basis to help inform approaches for building scientific confidence in NAMs and for incorporating such data into risk assessment and decision-making. Overall, the report recommendations aim to ensure a seamless handoff

from the evaluation of NAM-based testing strategies in the laboratory to the incorporation of NAM data into modern, systematic-review-based risk assessments.

National Academies workshops to support EPA development of human health assessment
Tags: EPA, NCI, NIEHS

At the request of EPA, an ad hoc planning committee of the National Academies of Sciences, Engineering, and Medicine organized two workshops on topics pertinent to their assessment of human health effects. The workshops were designed to assist EPA with increasing the quality, transparency, and confidence in its chemical assessments by addressing scientific issues related to systematic review, hazard identification, and dose-response analysis. Scientists from NCI and NIEHS served on the committee. Topics included:

- Evaluating elements of the systematic review process (e.g., development and application of specialized tools, methods for evaluating mechanistic data).

- Hazard identification approaches (e.g., techniques for integrating evidence).

- Incorporating NAMs for testing chemicals into chemical assessments.

- Research on quantitative analyses for evidence integration and dose-response analyses.

The first workshop, "[Triangulation of Evidence in Environmental Epidemiology](#)," considered approaches to integrating results from a variety of data streams to inform and strengthen causal inferences. The second workshop, "[Artificial Intelligence and Open Data Practices in Chemical Hazard Assessment](#)," focused on approaches to automating and streamlining data extraction and evidence synthesis for systematic reviews. Videos of the workshops and meeting materials are available on each of the meeting webpages.

New Chemicals Collaborative Research Program for risk assessments of new chemical substances
Tags: EPA, exposure, metabolism, structure similarity

In February 2022, EPA launched a new program under the Toxic Substances Control Act (TSCA) to modernize the process and bring innovative science to the review of new chemicals before they can enter the marketplace. Under the [New Chemicals Collaborative](#)

[Research Program](#), EPA's Office of Research and Development is working with the Office of Chemical Safety and Pollution Prevention to advance five key research areas:

- Updates and refinements to chemical analogue and category approaches.

- Development and expansion of databases containing TSCA chemical information.

- Development and refinement of predictive models for physicochemical properties, environmental fate/transport, hazard, exposure, and toxicokinetics.

- Integration and application of in vitro NAMs.

- Development of a TSCA new chemicals decision support tool to modernize the process.

Each of these five research areas represents translation and extension of computational toxicology research that has been in development under the vision of the CompTox Blueprint ([Thomas et al. 2019](#)) and the 2021 [EPA NAMs Work Plan](#), which together form a strategic roadmap for developing and integrating NAMs to fill information gaps, establishing scientific confidence of NAM application, and engaging with stakeholders.

EPA announced the program in February 2022. A public meeting in April 2022 provided an overview of the program and gave individual stakeholders an opportunity to provide input. Subsequently a 2023-2026 research plan was [reviewed by the Board of Scientific Counselors in October 2022](#) with a [final report](#) posted in early 2023. In 2023, the program advanced multiple research objectives, including publications of research evaluation of chemical and analogue approaches, cheminformatics-driven selection of chemicals to include in an in vitro NAM proof of concept, and the beginning of screening work on that proof-of-concept.

Collaborations on adverse outcome pathways
Tags: EPA, NIEHS, AOP

An adverse outcome pathway (AOP) is a conceptual framework for organizing information and evidence on toxicity. It defines a causal relationship between perturbation of a biological pathway at the molecular, biochemical, or cellular level and one or more resulting adverse outcomes to human or ecosystem health. AOPs are useful in supporting both the

development and application of NAMs and DAs in chemical safety assessment because they can:

- Organize information about biological interactions and toxicity mechanisms into sequential steps that describe how exposure to a substance might cause illness or injury.

- Suggest cell- or biochemical-based tests for pathway elements that could be used to develop testing strategies for targeted toxicity.

- Identify steps in a toxicity mechanism that need improved characterization.

EPA is an active partner in the [OECD adverse outcome pathways development workplan](#), managed under OECD's Working Party for Hazard Assessment and Working Party of the National Test Guidelines program. EPA is also an active participant in the Society for the Advancement of Adverse Outcome Pathways, which promotes and advances scientific research that fosters the development and use of AOPs. The Society hosts the [AOP-Wiki](#), which is one component of a larger OECD-coordinated [AOP Knowledgebase](#), also supported by EPA. EPA scientists were among the speakers at a [2023 workshop on AOP-Wiki 3.0](#), which was presented in two sessions in July and August 2023.

EPA and NIEHS also participate with international partners in the Methods2AOP Collaboration, which seeks to develop an infrastructure to accommodate the integration of defined method details with key events in AOPs, including molecular initiating events. This connection between specific test methods/assays and key events in AOPs can facilitate improved interpretation of likely chemical hazards of substances shown to elicit a given assay or endpoint response. A poster describing the Methods2AOP Collaboration (Wittwehr et al.) was presented at the [2023 annual meeting of the Society of Toxicology](#), and collaboration leader Clemens Wittwehr was profiled in an [article in the September 2022 NIEHS Environmental Factor newsletter](#). Ongoing activities of the collaboration include development of a prototype for the next version of the AOP-Wiki to demonstrate functionalities and data model improvements that better reflect the importance of test method information in the AOP Framework. This includes an increased focus on method validation and readiness status, better communication of various stakeholder roles, and the introduction and implementation of ontologies to tag methods in a way that makes them better identifiable

in neighboring initiatives (e.g., the OECD test guidelines program). An outreach event for regulators is planned April 2024 and a second version of the M2AOP prototype is under development. The collaboration will provide recommendations to OECD beyond information and communications technology aspects, as well as a publication summarizing activities and outcomes to be submitted to a peer-reviewed journal.

Collaborations with HESI
Tags: EPA, NIEHS, carcinogenicity, genotoxicity, hepatotoxicity, IVIVE

ICCVAM agency scientists participate in programs coordinated by the [Health and Environmental Sciences Institute](#) (HESI). HESI collaboratively identifies and helps to resolve global health and environmental challenges through the engagement of scientists from academia, government, industry, nongovernmental organizations, and other strategic partners. During 2022 and 2023, ICCVAM agency scientists participated in these [HESI projects](#).

- The Emerging Systems Toxicology for the Assessment of Risk (eSTAR) Committee was established to develop and deliver innovative systems toxicology approaches for risk assessment. In 2023, this committee launched the Omics for Assessing Signatures for Integrated Safety Consortium to increase confidence in the use of techniques such as imaging-based phenomics, transcriptomics, and proteomics for chemical safety assessment. The consortium has assembled a list of over 1000 candidate molecules for testing in Cell Painting, high-throughput transcriptomics and high-throughput proteomics assays. These molecules have liver toxicity data from traditional animal studies and/or human clinical studies. Data generation from in vitro screening studies is scheduled to begin in summer of 2024.

- The eSTAR Committee is also coordinating an effort to develop and qualify biomarker gene expression panels that measure widely accepted molecular pathways linked to tumorigenesis and their activation levels to predict tumorigenic doses of chemicals from short-term exposures ([Corton et al. 2022](#)). Success from these efforts will facilitate the transition from current heavy reliance on conventional two-year rodent carcinogenicity studies to more rapid animal- and resource-sparing approaches

for mechanism-based carcinogenicity evaluation supporting internal and regulatory decision-making.

- The HESI eSTAR Molecular Point-of-Departure workgroup published a paper that establishes the scientific principles underpinning the genomic dose-response approach being investigated by the group (Johnson et al. 2022). Ongoing efforts are focused on a manuscript that describes the current standards for data analysis and identifies points of uncertainty in relation to analysis pipeline parameter selections and definition of terms (e.g., concerted molecular change). The data analysis standards paper will provide background information and a starting point for a workshop, tentatively planned for late 2025, with the goal of codifying best practices in genomic dose-response analysis and molecular POD determination.

- The HESI eSTAR microRNA biomarkers workgroup continued to develop biomarkers based on extracellular microRNA released by region-specific nephron cells that are indicative of toxicity due to chemical or drug exposure. The current project builds on foundational work in a rat model that established kidney microRNA release into urine corresponding to subregion-specific expression that indicated nephrotoxicity due to drug exposure (Chorley et al. 2021). A manuscript currently in review describes a project started in 2022 studying temporal modulation of primate urinary microRNA biomarkers after mild nephrotoxicity. NAMs development work has focused on proximal tubule microRNA release in a human primary cell culture media after nephrotoxicant exposure.

- The Genetic Toxicology Technical Committee is working to improve the scientific basis of the interpretation of results from genetic toxicology tests for more accurate hazard identification and assessment of human risk and develop follow-up strategies for determining the relevance of test results to human health. The committee's In Vitro Working Group is critically evaluating NAMs for in vitro genotoxicity testing and envisioning how NAMs could expand current in vitro genetic toxicology testing strategies. A recent publication reports on a case study on 31 reference chemicals to evaluate the performance of IVIVE application to genotoxicity data (Beal et al. 2023). The group will make recommendations for creating an "in vitro only" approach for

genetic toxicology testing that would meet the needs of various regulatory decision-makers.

- The Transforming the Evaluation of Agrochemicals Committee seeks to develop fit-for-purpose safety evaluation for agrochemicals that is applicable to changing global, as well as local, needs for evaluation and regulatory decisions that can incorporate relevant evolving science inputs. The group has been assessing the use of various test guidelines across different regulatory agencies in the context of agrochemical safety assessment and compiling information from industry members on the types of NAMs typically used.

- The HESI PBPK Models Committee aims to address key needs related to physiologically based pharmacokinetic (PBPK) modeling practices and applications that could facilitate use of PBPK models more consistently within the risk assessment context. The group has been working to develop PBPK templates and frameworks, as well as evaluate and address commonly raised technical and scientific issues related to the use of toxicokinetic data for dose selection and/or interpretation of results in toxicity testing. The PBPK Models Committee is also collaborating with the Transforming the Evaluation of Agrochemicals Committee to evaluate the utility of the subchronic dog study for agrochemical safety evaluation.

FDA Alternative Methods Working Group
Tags: FDA

For over four years, FDA has maintained an [Alternative Methods Working Group](#) with representatives from all of FDA. The goals of this working group are to:

- Strengthen FDA's long commitment to promoting the development and use of new technologies and to reduce animal testing.

- Discuss new alternative in vitro/in silico/in vivo methods across FDA.

- Interact with federal government partners and other global stakeholders to facilitate discussion and development of draft performance criteria for such assays.

During 2022 and 2023, the working group continued to coordinate an internal [webinar series](#) on alternative methods that provided test method developers the opportunity to present their

new methods to FDA scientists. The group also updated [FDA webpages on alternative methods](#) to provide a more comprehensive and user-friendly resource for this information. Two 2022 reports are available from these pages:

- The [Focus Areas of Regulatory Science (FARS) report](#), updated in 2022, outlines areas FDA has identified as needing continued targeted investment in regulatory science research to foster the development of innovative products, provide data and methods to inform regulatory decision-making, and improve guidance to sponsors. One focus area is [novel technologies](#) to improve predictivity of nonclinical studies and replace, reduce, and refine animal testing.

- The November 2022 report, [Successes and Opportunities in Modeling & Simulation for FDA](#), elucidates in part how and where modeling and simulation are used across FDA.

Workshop on implementing virtual control groups for preclinical safety studies
Tags: FDA, NIEHS

Animal safety studies are usually performed with three groups of animals where increasing amounts of the test chemical are given to the animals and one control group where the animals do not receive the test chemical. The design of such studies, the characteristics of the animals, and the measured parameters are often very similar from study to study. Therefore, it has been suggested that measurement data from the control groups could be reused from study to study to lower the total number of animals per study. This could reduce animal use by up to 25% for such standardized studies. FDA and NIEHS scientists joined a broad range of stakeholder groups at a March 2023 workshop to discuss the pros and cons of such a concept and what would have to be done to implement it without threatening the reliability of the study outcome or the resulting human risk assessment ([Golden et al. 2023](#)). Workshop participants concluded that implementation of virtual control groups could support a better understanding of nonclinical data, increased study power, and reduced animal use, making such an approach an attractive alternative to running concurrent controls. However, before use of virtual control groups can be fully implemented, there is work to be done to increase confidence in their utility. In particular, any implementation must be fully validated in a manner that does not compromise study interpretation. It became evident during the

workshop that overcoming the identified hurdles and challenges will require a joint effort of all stakeholders, ideally in a consortium approach.

FDA and pharmaceutical industry collaboration on MPS applications
Tags: FDA, metabolism, MPS

Complex in vitro models such as MPS offer the potential to improve pharmaceutical clinical drug attrition due to safety and/or efficacy concerns. For this technology to have an impact, robust characterization and qualification plans constructed around specific contexts of use need to be establish. To address this need, FDA and the pharmaceutical industry Innovation and Quality Microphysiological Systems (IQ MPS) Affiliate convened a 2020 workshop. The workshop considered through various case studies both how these technologies are currently being applied by pharmaceutical companies during drug development and how they are being tested at the FDA. The goal was to identify hurdles (real or perceived) to the adoption of MPS technologies and to address evaluation/qualification pathways for these technologies. Output from the workshop ([Baran et al. 2022](#)) included a working definition of MPS, a detailed description of 11 case studies, and in-depth analysis. The report also includes key take aways from breakout sessions on ADME (absorption, distribution, metabolism, and excretion), pharmacology, and safety that covered topics such as qualification and performance criteria, species differences and concordance, and how industry can overcome barriers to regulatory submission of data from complex in vitro models. IQ MPS Affiliate and FDA scientists reached a general consensus on the need for animal models for preclinical species to better determine species concordance. Furthermore, there was acceptance that technologies for use in ADME, pharmacology and safety assessment will require qualification, which will vary depending on the specific context of use.

Botanical Safety Consortium
Tags: FDA, NIEHS, hepatotoxicity, mixtures toxicity

In 2019, FDA, NIEHS, and HESI signed a memorandum of understanding establishing the [Botanical Safety Consortium](#). The Consortium includes participants from industry, academia, and government. It considers how to use cutting-edge toxicology tools, including alternatives to animal testing, to promote scientific advances in evaluating the safety of botanical ingredients and mixtures in dietary supplements.

The Botanical Safety Consortium held virtual annual meetings in 2022 and 2023. The 2022 meeting considered topics including supplement-induced liver injury, approaches to screening complex mixtures, and the role of analytical methods. In addition to another look at screening approaches, speakers at the 2023 meeting discussed global research and regulatory activities. The Consortium also presented webinars in January and November 2022, and participants gave presentations at meetings of SOT, Environmental Genomics and Mutagen Society, and other toxicology organizations.

Establishment of the International MPS Society
Tags: FDA, NIEHS, NIH, MPS, stem cells

MPS comprise several bioengineering breakthroughs that reproduce organ architecture and function in vitro. Fueled by stem cell technologies, a broad variety of human models and test systems have emerged. Convening of global conferences on MPS was identified by opinion leaders in the field as a key step forward in the maturation and harmonization of the area. In response, international companies and organizations teamed up in 2021 to initiate [annual MPS World Summits](#) to present the latest scientific achievements, discuss advances and challenges, and enable communication between young and newly interested scientists and pioneers of the MPS field. Scientists from FDA, NIEHS, and NIH spoke at the 2022 and 2023 MPS World Summits.

The 2021 and 2022 MPS World Summits also laid the groundwork for establishment in 2023 of the [International MPS Society](#). The Society's mission is to connect, exchange, and educate to promote international standardization and harmonization of MPS, establish a global training environment, and maximize the potential of MPS to advance the life sciences. ICCVAM co-chair Suzanne Fitzpatrick (FDA) serves on the Society's governing board, as does Danilo Tagle of NCATS (NIH). ICCVAM member Donna Mendrick (FDA) and FDA scientist Carolina Romero serve on the Society's scientific advisory committee.

Environmental Health Language Collaborative
Tags: EPA, NIEHS

NIEHS and EPA are fostering a community-driven initiative, the [Environmental Health Language Collaborative](#), to advance integrative environmental health research by developing and promoting adoption of a harmonized language. This initiative will facilitate answering

large-scale complex research questions that require integration of multiple disparate data sources by developing language standards for describing data and biomedical knowledge. The collaborative's website has resources including a glossary, bibliography, links to ontologies, and overview videos.

The collaborative presented six webinars during 2022 and 2023 and held a three-day workshop in early 2023 on "Sharing Your Environmental Health Sciences Data: Metadata, Standards, and Tools." Videos and materials from the webinars and workshops are [available on the collaborative's website](). The collaborative also established use-case working groups to explore language solutions that will enhance the findability, reuse, and/or operability of environmental health and safety data. Current working groups are focused on the use cases of "Biomarkers and Biological Processes of Exposure," "Data Discovery," and "Data Harmonization." All working groups meet periodically and welcome new members.

Reference Pages

Agency Representatives in 2022 and 2023

Tags: ICCVAM

The individuals listed on this page served as representatives from ICCVAM member agencies in 2022 and 2023. ICCVAM includes three types of representatives:

- The **Principal Agency Representative** is the primary source of communication from his or her agency to the committee and NICEATM. He or she regularly attends all ICCVAM meetings and teleconferences, coordinates participation of other representatives from the agency, and casts the agency's vote on occasions when formal voting is required.

- **Alternate Agency Representatives** participate in ICCVAM committee activities in place of the principal when necessary.

- An agency may designate one or more **Other Agency Representatives** to participate in ICCVAM to provide broader representation or expertise.

Agency (Office)	Representative	Representative Type (as of December 2023)	Service Continuing into 2024
Agency for Toxic Substances and Disease Registry	Moiz Mumtaz, M.Sc., Ph.D.	Principal	Yes
Agency for Toxic Substances and Disease Registry	Patricia Ruiz, Ph.D..	Alternate	Yes
National Cancer Institute	Brian Cholewa, Ph.D.	Principal	Yes
National Cancer Institute	Ron Johnson, Ph.D.	Alternate	Yes
National Institute for Occupational Safety and Health	Richard Probst, D.V.M., M.P.H., DACLAM, DACPVM	Principal	-
National Institute of Environmental Health Sciences	Brian Berridge, D.V.M., Ph.D., DACVP	Principal	-
National Institute of Environmental Health Sciences	Stephen Ferguson, Ph.D.	Alternate	Yes
National Institute of Environmental Health Sciences	Warren Casey, Ph.D., DABT	Principal	Yes

Agency (Office)	Representative	Representative Type (as of December 2023)	Service Continuing into 2024
National Institute of Environmental Health Sciences	Nicole Kleinstreuer, Ph.D.	Other	Yes
National Institute of Environmental Health Sciences	David M. Reif, Ph.D.	Other	Yes
National Institute of Standards and Technology	John Elliott, Ph.D.	Principal	Yes
National Institute of Standards and Technology	Elijah Petersen, Ph.D.	Alternate	Yes
National Institutes of Health	Carol Clarke, D.V.M., DACLAM	Other	Yes
National Institutes of Health	Michael Eichner, D.V.M., DACLAM	Other	Yes
National Institutes of Health	Nicolette Petervary, V.M.D., M.S. DACAW	Principal	Yes
National Library of Medicine	Jeanne Goshorn, M.S.	Principal	-
National Library of Medicine	In-Hye Cho, M.S.	Principal	Yes
Occupational Safety and Health Administration	Deana Holmes, M.T.	Principal	Yes
Occupational Safety and Health Administration	Janet Carter, M.S.	Alternate	Yes
U.S. Consumer Product Safety Commission	John Gordon, Ph.D. (ICCVAM Co-chair)	Principal	Yes
U.S. Consumer Product Safety Commission	Kristina Hatlelid, Ph.D.	Alternate	Yes
U.S. Consumer Product Safety Commission	Eric Hooker, M.S.	Alternate	Yes
U.S. Consumer Product Safety Commission	Joanna Matheson, Ph.D.	Alternate	Yes
U.S. Department of Agriculture	Carol Clarke, D.V.M., DACLAM	Principal	-
U.S. Department of Agriculture	Kristina Adams, M.S.	Alternate	-
U.S. Department of Agriculture	Jessie Carder (Kull), M.S.	Principal	Yes
U.S. Department of Agriculture	Louis DiVincenti, M.S., DVM, DACLAM, DACAW	Other	Yes
U.S. Department of Agriculture	Erika Edwards, M.S.	Other	Yes

Agency (Office)	Representative	Representative Type (as of December 2023)	Service Continuing into 2024
U.S. Department of Agriculture	Aisha Ellis, D.V.M.	Other	-
U.S. Department of Agriculture	Ben Green, Ph.D.	Other	Yes
U.S. Department of Agriculture	Katherine Horak, Ph.D.	Other	Yes
U.S. Department of Agriculture	Patrice Klein, M.S., V.M.D., DACPV, DACVPM	Alternate	Yes
U.S. Department of Defense	Matthew Johnson, D.V.M., DACLAM	Alternate (acting Principal)	-
U.S. Department of Defense	Emily N. Reinke, Ph.D., DABT (ICCVAM Co-chair to March 2022)	Alternate	-
U.S. Department of Defense	Donald Cronce, Ph.D.	Other	Yes
U.S. Department of Defense	Natalia Garcia-Reyero Vinas, Ph.D.	Other	Yes
U.S. Department of Defense	Saber Hussain, Ph.D., ATS Fellow, AFRL Fellow	Other	Yes
U.S. Department of Defense	Shannon Marko, D.V.M.	Principal	Yes
U.S. Department of Defense	Elaine Merrill, Ph.D.	Other	Yes
U.S. Department of the Interior	Barnett A. Rattner, Ph.D.	Principal	Yes
U.S. Department of the Interior	Jessica Leet, Ph.D.	Alternate	Yes
U.S. Department of the Interior	Tim Bargar, Ph.D.	Other	-
U.S. Department of the Interior	Paula F.P. Henry, Ph.D.	Other	-
U.S. Department of the Interior	Luke R. Iwanowicz, Ph.D.	Other	-
U.S. Department of Transportation	Steve Hwang, Ph.D.	Principal	Yes
U.S. Department of Transportation	Ryan Vierling, Ph.D.	Alternate	Yes
U.S. Department of Transportation	Rebecca Rothhaas, M.S.	Other	Yes

Agency (Office)	Representative	Representative Type (as of December 2023)	Service Continuing into 2024
U.S. Department of Veterans Affairs Office of Research and Development	Christopher Bever, M.D., MBA	Principal	-
U.S. Department of Veterans Affairs Office of Research and Development	Holly Krull, Ph.D.	Principal	Yes
U.S. Department of Veterans Affairs Office of Research and Development	George Lathrop, D.V.M., M.S., DACLAM	Alternate	Yes
U.S. Environmental Protection Agency (Office of Pesticide Programs)	William (Bill) Eckel, Ph.D.	Other	Yes
U.S. Environmental Protection Agency (Office of Pollution Prevention and Toxics)	Anna Lowit, Ph.D. (ICCVAM Co-chair)	Principal	Yes
U.S. Environmental Protection Agency (Office of Pollution Prevention and Toxics)	Louis (Gino) Scarano, Ph.D.	Other	Yes
U.S. Environmental Protection Agency (Office of Research and Development)	Alison Harrill, Ph.D.	Alternate	Yes
U.S. Environmental Protection Agency (Office of Research and Development)	Pamela Noyes, Ph.D.	Alternate	-
U.S. Environmental Protection Agency (Office of Research and Development)	Stephanie Padilla, Ph.D.	Alternate	-
U.S. Environmental Protection Agency (Office of Research and Development)	Kelly Carstens, Ph.D.	Other	Yes
U.S. Environmental Protection Agency (Office of Research and Development)	Grace Patlewicz, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Biologics Evaluation and Research)	Robin Levis, Ph.D.	Other	-
U.S. Food and Drug Administration (Center for Biologics Evaluation and Research)	Leslie Wagner, B.S.	Other	Yes

Agency (Office)	Representative	Representative Type (as of December 2023)	Service Continuing into 2024
U.S. Food and Drug Administration (Center for Biologics Evaluation and Research)	Allen Wensky, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Devices and Radiological Health)	Jennifer Goode, B.S.	Alternate	Yes
U.S. Food and Drug Administration (Center for Devices and Radiological Health)	Simona Bancos, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Devices and Radiological Health)	Rakhi Dalal-Panguluri, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Drug Evaluation and Research)	Paul C. Brown, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Drug Evaluation and Research)	Jill Merrill, Ph.D.	Other	-
U.S. Food and Drug Administration (Center for Drug Evaluation and Research)	Nakissa Sadrieh, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Food Safety and Applied Nutrition)	Omari J. Bandele, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Food Safety and Applied Nutrition)	Patrick Crittenden, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Food Safety and Applied Nutrition)	Suzanne Fitzpatrick, Ph.D., DABT	Principal	Yes
U.S. Food and Drug Administration (Center for Food Safety and Applied Nutrition)	Shruti Kabadi, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Tobacco Products)	Jueichuan (Connie) Kang, Ph.D.	Other	Yes

Agency (Office)	Representative	Representative Type (as of December 2023)	Service Continuing into 2024
U.S. Food and Drug Administration (Center for Veterinary Medicine)	M. Cecilia Aguila, D.V.M.	Other	Yes
U.S. Food and Drug Administration (Center for Veterinary Medicine)	Li You, Ph.D.	Other	Yes
U.S. Food and Drug Administration (National Center for Toxicological Research)	Mugimane (Manju) Manjanatha, Ph.D.	Other	Yes
U.S. Food and Drug Administration (National Center for Toxicological Research)	Donna Mendrick, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Office of the Chief Scientist)	Tracy Chen, Ph.D., DABT	Other	Yes
U.S. Food and Drug Administration (Office of the Chief Scientist)	Chad P. Nelson, Ph.D., M.S.P.H.	Other	Yes
U.S. National Coordinator for OECD Test Guidelines Programme	Charles Kovatch, M.S.	Other	Yes

NICEATM and ICCVAM Publications, 2022-2023

This page lists publications issued in 2022 and 2023 that describe NICEATM and ICCVAM activities.

NICEATM and ICCVAM reports

ICCVAM. 2022. Biennial Progress Report 2020-2021 [Internet]. Research Triangle Park (NC): National Institute of Environmental Health Sciences [cited 17 February 2022]. Available: https://ntp.niehs.nih.gov/iccvamreport/2021/index.html.

Federal Register notices

All Federal Register notices issued by NICEATM can be found on the NTP website at http://ntp.niehs.nih.gov/go/frn.

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NIEHS. 2022. ICCVAM Biennial Progress Report: 2020-2021; availability of report. Federal Register. 87(185): 58363-58364.

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Journal articles

A full list of NICEATM and ICCVAM publications is [available on the NTP website](#).

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Glossary of Key Terms

3Rs: the principles of replacement, reduction, or refinement of animal use for scientific research or product safety testing.

Accuracy: in the context of a test method validation study, the closeness of agreement between a test method result and an accepted reference value, or the test method's proportion of correct outcomes.

Acute systemic toxicity: the immediate or near-immediate effect of a toxic substance after it is absorbed and distributed throughout the body. Different acute systemic toxicities are distinguished by the route of exposure: by ingestion (oral), through the skin (dermal), or by inhalation.

Adverse outcome pathway (AOP): a conceptual framework constructed from existing knowledge that relates exposure of a type of toxic substance to subsequent steps that result in illness or injury.

Agonist: a substance that increases activity of the target receptor.

Algorithm: a set of steps that are followed to complete a computational process.

Allergic contact dermatitis: an allergic reaction that results from repeated direct skin contact with a skin sensitizer. Clinical signs of allergic contact dermatitis include redness, swelling, blistering, and itching.

Alternative methods: testing methods or approaches that replace, reduce, or refine animal use; the term new approach methodologies is also used and is becoming more prevalent.

Androgen: a class of hormones, produced largely by the testes, that serve as the primary male hormones.

Androgen receptor: a protein molecule to which an androgen or androgen-like substance can attach. This interaction produces a chemical signal or triggers a cellular response.

Antagonist: a substance that decreases activity of the target receptor.

Apoptosis: cell death that occurs as a normal and controlled part of an organism's growth or development.

Applicability domain: a range of chemicals and properties for which a test method has been proven useful.

Bioactivity: the manner in which a chemical affects or interacts with living tissue.

Biomarker: an objective measure that captures what is happening in a cell or an organism at a given moment.

Bioprinting: a process that combines 3D printing with biomaterials to replicate parts that imitate natural tissues, bones, and blood vessels in the body.

Cardiotoxicity: toxicity to the heart.

Chemokines: signaling proteins secreted by cells of the immune system that stimulate the movement of other cells.

Concentration–response curve: a curve on a graph that shows the relationship between the concentration of a chemical being tested in an assay and its measured effect on the assay endpoint.

Cytokine: a signaling protein that modulates the activity and development of immune cells.

Cytotoxic: the ability of a substance to kill or harm cells.

Defined approach (DA): a testing strategy that consists of input data generated from identified methods and a data interpretation procedure, such as a machine-learning model, flowchart, or decision tree, through which the data are evaluated.

Developmental toxicity: effects observed in offspring that occur as a result of chemical exposures of the pregnant mother.

Dosimetry: measurement or calculation of a dose of substance delivered to a target tissue.

Ecotoxicity testing: refers both to the assessment of chemical effects on invertebrates, fish, birds, plants, and other wild organisms and testing of soil, sediment, or effluents for the presence of toxic compounds.

Endocrine disruptor: a natural or man-made substance that may interfere with the endocrine system and produce adverse health effects.

Environmental fate: characterization of how a chemical will behave if released into the environment; it is affected by properties such as biodegradability and soil adsorption.

Epithelial cells: any of the cells forming the cellular sheets that cover surfaces, both inside and outside the body.

Equivalent administered dose (EAD): the output of in vitro to in vivo extrapolation; the predicted in vivo dose that would result in a plasma concentration of a chemical equal to the concentration of that chemical that induces an effect in an in vitro assay.

Estrogen: a class of hormones, produced largely by the ovaries, that serve as the primary female hormones.

Estrogen receptor: a protein molecule to which an estrogen or estrogen-like substance can attach. This interaction produces a chemical signal or triggers a cellular response.

Ex vivo: refers to an assay using tissue that has been removed from a multicellular organism and conducted while the tissue is still viable.

Formulation: a mixture of chemicals prepared according to a specific procedure to ensure a desired effect when used, improve handling properties, or achieve other desired product goals.

G protein-coupled receptor: receptors located in the cell membrane involved in cell signaling. G protein-coupled receptors are important drug targets and involved in many diseases.

Genotoxic: capable of damaging DNA.

Harmonization: the act of making systems or laws similar among different companies, countries, etc., so the organizations using those systems or laws can operate more easily within the different venues.

Hazard classification: assignment of a substance to a category according to results of toxicity testing, often for labeling purposes.

Hepatic: referring to the liver.

Hepatocyte: the main functional cell of the liver.

Hepatotoxicity: toxicity to the liver.

High-content screening: an approach that uses fluorescent tagging and automated imaging to assess changes in the structure and composition of individual cells in a high-throughput manner.

High-throughput screening (HTS): a testing approach that uses robotics, liquid-handling devices, detectors, and associated software to quickly conduct a large number of chemical or biochemical tests.

Hydrophobic: tending not to mix with water.

In chemico: refers to assays that are carried out in an artificial system, such as a test tube or assay plate, using only chemical components rather than cells.

In silico: refers to analyses that are carried out on a computer or via computer simulation.

In vitro: refers to assays that are carried out in an artificial system, such as a test tube or assay plate, using small single-cell or multicellular organisms, cultured cells, or cellular components.

In vitro to in vivo extrapolation (IVIVE): an analysis conducted to relate the test chemical concentration causing a response in an in vitro system to an external dose or exposure that results in human or animal (in vivo) illness or injury at the target tissue.

In vivo: refers to assays carried out using multicellular organisms, typically rodents or other mammals.

Induced pluripotent stem cells (iPSCs): stem cells derived from mature non-sex cells that have the potential to differentiate into various types of cells.

Integrated approach to testing and assessment (IATA): an approach that considers all available relevant information about a substance in a weight-of-evidence assessment to inform a regulatory decision regarding hazard or risk, or to indicate that specific additional tests are needed.

Keratinocyte: the major cell type of the skin.

LC50: in traditional animal tests for acute inhalation or aquatic toxicity, the concentration estimated to cause death in 50 percent of the animals tested; a value used to categorize toxic substances and determine the hazard phrases used on product labels.

LD50: in traditional animal tests for acute systemic oral or dermal toxicity, the dose estimated to cause death in 50 percent of the animals tested; a value used to categorize toxic substances and determine the hazard phrases used on product labels.

Leukocyte: any of the colorless blood cells of the immune system.

Machine learning: the study and construction of computer algorithms that, once trained on a set of data, can make predictions or decisions about a different set of data.

Metabolism: the sum of the processes by which a particular substance is handled in a living organism, such as assimilation and incorporation or detoxification and excretion.

Microphysiological systems (MPS): in vitro models of organs composed of cells and structural materials that are designed to reproduce the function of living organs; also referred to as organs-on-a-chip or tissue chips.

Microtiter plate: a flat plate with multiple wells used as small test tubes.

Nanomaterial: a substance made up of particles that measure no more than 100 nanometers in at least one dimension.

Neurotoxicity: toxicity to the brain or other parts of the nervous system.

New approach methodologies (NAMs): testing methods or approaches that replace, reduce, or refine animal use; the term alternative methods has also been widely used.

Nonclinical testing: evaluation of candidate drugs conducted in animals or in vitro assays, typically before testing in humans to assess toxicity and determine safe doses; the term preclinical testing is also used.

Ontologies: standardized nomenclature systems**.**

Organophosphorus compounds: organic compounds that contains phosphorus; many are used as pesticides and flame retardants.

Per- and polyfluoroalkyl substances (PFAS): a class of manufactured chemicals that are widely used to make various types of common products. While their potential health impacts are mostly uncharacterized, they are of concern because they resist degradation, accumulate in an organism's body over its lifetime, and are widespread in the environment.

Pharmacokinetics: an evaluation of the rate at which a chemical is absorbed, distributed, metabolized, and excreted once it enters the body, as a means to determine the relationship between exposure and toxicity (see also toxicokinetics).

Pharmacokinetic (PK) model: a mathematical model created to describe the process of absorption, distribution, metabolism, and excretion of a chemical through the body. One-compartment models treat all organs as a single unit, whereas physiologically based (PBPK) models are usually multicompartment models with separate compartments corresponding to individual or combined organs that are interconnected by blood flows.

Phenomics: the systematic study or evaluation of the traits that make up a phenotype, often in a high-throughput manner.

Phenotype: observable characteristics of an organism resulting from the interaction of its genetic makeup with the environment.

Physicochemical properties: referring to the physical or chemical properties of a substance.

Point-of-departure (POD): the lowest dose or concentration at which a treatment-related response is observed.

Population variability: the potential for genetic or nongenetic factors to impact susceptibility to toxic effects of chemicals, resulting in observed differences in both effects and severity of effects of chemical exposure to individuals within a population.

Proteomics: comprehensive evaluation of the function and structure of proteins undertaken to understand the effects of a chemical on a tissue or organism.

Quantitative structure–activity relationship (QSAR) models: classification models that predict the activities of chemicals with unknown properties by relating them to properties of known chemicals.

Read-across: a computational technique that uses toxicity data from one or more known (source) chemicals to predict toxicity for another (target) chemical, usually but not always on the basis of structural similarity.

Reference chemical: a chemical that causes a specific well-characterized biological effect, and therefore, can be used to assess the performance of a test method designed to measure that effect. Reference chemicals should represent the classes of chemicals for which a test method is proposed to be used and cover the range of expected responses.

Reference data: data from an accepted test method that can be used to assess the performance of a new test method designed to measure an analogous effect.

Refinement alternative: a test method that modifies procedures to enhance animal well-being and that lessens or avoids pain and distress in animals.

Relevance: the extent to which a test method accurately measures a biological effect of interest in a species of interest.

Reliability: the extent to which a test method provides reproducible results over time and in different laboratories.

Replacement alternative: a test method that replaces animals with a nonanimal system or one animal species with a phylogenetically lower one.

Reproductive toxicity: chemical effects on the reproductive system that interfere with an organism's sexual function or fertility.

Risk assessment: the process of characterizing the potential risk posed by a chemical, taking into consideration the hazards posed by the chemical, the dose of the chemical needed to cause health problems, and the probability of exposure at that dose.

Sensitivity: in the context of test method validation, the proportion of all positive substances that are correctly classified as positive by the test method under evaluation.

Skin irritation: minor injury or inflammation to the skin; irritation differs from an allergic reaction in that there is no coordinated response from the immune system.

Skin sensitization: a hypersensitivity reaction that occurs when a susceptible person comes in direct skin contact with an allergen, termed a skin sensitizer.

Skin sensitization potency: the relative amount of a substance that produces a skin sensitization reaction.

Specificity: in the context of test method validation, the proportion of all negative substances that are correctly classified as negative by the test method under evaluation.

Stem cells: undifferentiated cells of a multicellular organism that can indefinitely produce more cells of the same type and can also be induced to differentiate into other types of cells (see also induced pluripotent stem cells).

Subchronic: animal experiment designed to study effects produced by the test substance when administered either in repeated doses or continually in food, drinking water, or air over a period of up to about 90 days.

Sublethal: a dose or concentration of a substance that is not high enough to cause death.

Synbiotics: mixtures of probiotics (helpful gut bacteria) and prebiotics (non-digestible fibers that help these bacteria grow) that work together synergistically in the digestive tract.

Tox21: collaborative effort among four U.S. federal government agencies to develop more efficient approaches to predict how chemicals may affect human health.

Toxicant: a toxic or poisonous substance.

Toxicokinetics: an evaluation of the rate at which a chemical is absorbed, distributed, metabolized, and excreted once it enters the body, as a means to determine the relationship between exposure and toxicity (see also pharmacokinetics).

Transcriptomics: the analysis of overall gene expression in a cell or tissue to assess cell function or response to toxicity.

Validation: a process by which the reliability and relevance of a test method are established for its intended application.

Xenobiotic: a chemical found within an organism that is not naturally produced or expected to be present within the organism.

Acronyms and Abbreviations

3Rs	principles of replacement, reduction, or refinement of animal use for scientific research or product safety testing
ADME	absorption, distribution, metabolism, and excretion
AERO-TOX	aerosol exposure of respiratory organ-on-a-chip for toxicology
AIME	alginate immobilization of metabolic enzymes
ALI	air-liquid interface
ALTBIB	Bibliography on Alternatives on the Use of Live Vertebrates in Biomedical Research and Testing

AOP	adverse outcome pathway
API	application programming interface
ATSDR	Agency for Toxic Substances and Disease Registry
AWIC	Animal Welfare Information Center (U.S. Department of Agriculture)
CAR	chimeric antigen receptor
CATMoS	Collaborative Acute Toxicity Modeling Suite
CBC	Chemical Biological Center (U.S. Army, U.S. Department of Defense)
CCTE	Center for Computational Toxicology and Exposure (U.S. Environmental Protection Agency)
CDER	Center for Drug Evaluation and Research (U.S. Food and Drug Administration)
CEBS	Chemical Effects in Biological Systems (National Institute of Environmental Health Sciences)
CFSAN	Center for Food Safety and Applied Nutrition (U.S. Food and Drug Administration)
CHRIS	Chemical Risk Calculator Tool (U.S. Food and Drug Administration)
cHTS	curated high-throughput screening
CPDat	Chemicals and Products Database (U.S. Environmental Protection Agency)
CPSC	U.S. Consumer Product Safety Commission
CRADA	cooperative research and development agreement
CYP450	cytochrome P450
devTOXqP	devTOX quickPredict assay (Stemina, Inc.)
DL-GLF	DL-glufosinate ammonium
DNT	developmental neurotoxicity
DNT-DIVER	Developmental NeuroToxicity Data Integration and Visualization Enabling Resource (National Institute of Environmental Health Sciences)
DoD	U.S. Department of Defense

DOE	U.S. Department of Energy
DOI	U.S. Department of the Interior
DOT	U.S. Department of Transportation
DSA	dose per skin area
DSSTox	Distributed Structure-Searchable Toxicity (U.S. Environmental Protection Agency)
dTP	developmental toxicity potential
DTT	Division of Translational Toxicology (National Institute of Environmental Health Sciences)
EAD	equivalent administered dose
EASA	electrophilic allergen screening assay
ECOSAR	Ecological Structure Activity Relationships (U.S. Environmental Protection Agency)
eDNA	environmental DNA
EDSP	Endocrine Disruptor Screening Program (U.S. Environmental Protection Agency)
ENM	engineered nanomaterials
EPA	U.S. Environmental Protection Agency
eSTAR	Emerging Systems Toxicology for the Assessment of Risk (Health and Environmental Sciences Institute)
EURL ECVAM	European Union Reference Laboratory for Alternatives to Animal Testing
FAIR	findability, accessibility, interoperability, and reusability (of data)
FDA	U.S. Food and Drug Administration
GenRA	Generalized Read-across (U.S. Environmental Protection Agency)
GHS	United Nations Globally Harmonized System of Classification and Labelling of Chemicals
hERG	human ether-a-go-go related gene

HESI   Health and Environmental Sciences Institute

HPPT   human predictive patch test

HTS   high-throughput screening

httk   High-throughput Toxicokinetics (software package, U.S. Environmental Protection Agency)

HTTr   high-throughput transcriptomics

IATA   integrated approach to testing and assessment

ICATM   International Cooperation on Alternative Test Methods

ICCVAM   Interagency Coordinating Committee on the Validation of Alternative Methods

ICE   Integrated Chemical Environment (National Institute of Environmental Health Sciences)

ICH   International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

iPSC   induced pluripotent stem cell

IQ MPS   Innovation and Quality Microphysiological Systems

ISTAND   Innovative Science and Technology Approaches for New Drugs (U.S Food and Drug Administration)

IVIVE   in vitro to in vivo extrapolation

JaCVAM   Japanese Center for the Validation of Alternative Methods

JRC   Joint Research Centre (European Commission)

LC50   in traditional acute inhalation or aquatic toxicity tests, the concentration that produces lethality in 50% of the animals tested

LD50   in traditional acute dermal or oral systemic toxicity tests, the dose that produces lethality in 50% of the animals tested

LEL   lowest-effect level

LOAEL   lowest observable adverse effect level

MPS   microphysiological system

MPSCoRe	MPS for COVID Research working group
NAM	new approach methodology
NASA	National Aeronautics and Space Administration
NC3Rs	National Centre for the Replacement Refinement & Reduction of Animals in Research (United Kingdom)
NCATS	National Center for Advancing Translational Sciences (National Institutes of Health)
NCI	National Cancer Institute (National Institutes of Health)
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences (National Institutes of Health)
NIH	National Institutes of Health
NIOSH	National Institute for Occupational Safety and Health
NIST	National Institute of Standards and Technology
NLM	National Library of Medicine (National Institutes of Health)
NRF2	nuclear factor erythroid 2-related factor 2
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OHT201	OECD Harmonized Template 201
OPERA	Open (Quantitative) Structure–activity/property Relationship App
OSHA	Occupational Safety and Health Administration
PACT	Promise to Address Comprehensive Toxics [Act]
PAH	polycyclic aromatic hydrocarbon
PBA	pharmaceutical-based agent
PBK	physiologically based kinetic
PBPK	physiologically based pharmacokinetic

PFAS	per- and polyfluoroalkyl substances
PK	pharmacokinetic
POD	point-of-departure
PPARg	peroxisome proliferator-activated receptor gamma
PRT	Predictive Risk Team (U.S. Air Force, U.S. Department of Defense)
QSAR	quantitative structure–activity relationship
REST APIs	representational state transfer architectural style application programming interfaces
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
SARA	Skin Allergy Risk Assessment model (Unilever)
SARA-ICE	Skin Allergy Risk Assessment-Integrated Chemical Environment model
SEAZIT	Systematic Evaluation of the Application of Zebrafish in Toxicology (National Toxicology Program)
SEEM3	Systematic Empirical Evaluation of Models (U.S. Environmental Protection Agency)
SeqAPASS	Sequence Alignment to Predict Across Species Susceptibility (U.S. Environmental Protection Agency)
SOT	Society of Toxicology
STopTox	Systemic and Topical chemical Toxicity
tcpl	ToxCast data pipeline (U.S. Environmental Protection Agency)
tDOA	taxonomic domain of applicability
ToxCast	Toxicity Forecaster (U.S. Environmental Protection Agency)
ToxRefDB	Toxicity Reference Database (U.S. Environmental Protection Agency)
TSCA	Toxic Substances Control Act (U.S. Environmental Protection Agency)
USAF	U.S Air Force (U.S. Department of Defense)
USDA	U.S. Department of Agriculture
USGS	U.S. Geological Survey (U.S. Department of the Interior)

VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
Vmax	maximal capacity for metabolism
VOC	volatile organic chemical
Web-ICE	Web-based Interspecies Correlation (U.S. Environmental Protection Agency)