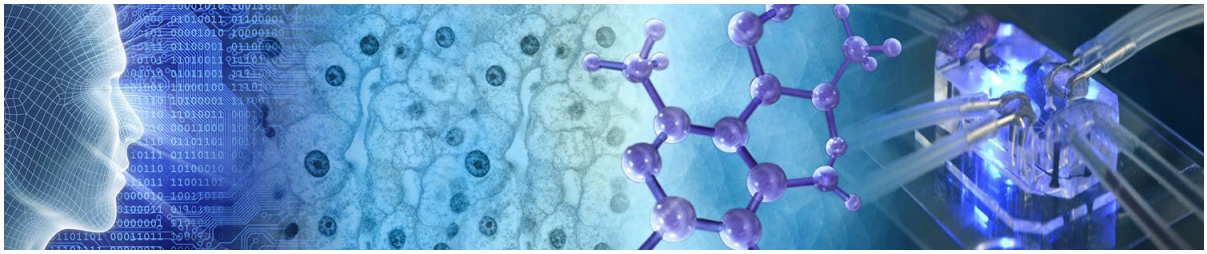
Biennial Progress Report 2024–2025

Interagency Coordinating Committee on the Validation of Alternative Methods



October 2026

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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 2024–2025 Biennial Report summarizes progress by ICCVAM agencies toward encouraging the development and utilization of and building 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 ICCVAM 2024-2025 Biennial Progress Report was revised prior to publication to conform with Executive Order 14434, "Inaugurating the Era of Super Intelligence," issued on September 29, 2026. The Executive Order directs U.S. executive departments and agencies to use the term "Super Intelligence" in place of "Artificial Intelligence" in official correspondence, public communications, websites, reports, policy documents, and other non-statutory documents within the executive branch.

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The ICCVAM Biennial Report was prepared by D-NICEATM, which is within the National Institutes of Health (NIH) Office of Research Innovation, Validation, and Application. Preparation of this report was supported by the Division of Program Coordination, Planning, and Strategic Initiatives within the NIH Office of the Director. Work was conducted under contract GS-35F-393CA-75N94025F00103.

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 18 ICCVAM agencies in the 2024-2025 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."

We congratulate the ICCVAM Validation Workgroup on the March 2024 publication of "Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies." This update of the original ICCVAM criteria for new approach methodologies (NAMs) validation reflects the advancement of alternative toxicological testing tools. It places less emphasis on replacement of in vivo tests with a single alternative method and instead emphasizes integrating evidence from multiple in vitro and in chemico assays and in silico approaches. This document is already proving to be a key resource in U.S. and international initiatives to bring state-of-the-science approaches to bear on development and validation of test methods.

The period covered by this report has been remarkable in the number and visibility of federal government initiatives to advance alternative methods to complement or replace animal use in testing and research. Policy shifts announced in 2025 by the U.S. Food and Drug Administration and the National Institutes of Health (NIH) will prioritize the development of predictive systems that use computer modeling and in vitro testing in these areas. Initiatives by the U.S. Environmental Protection Agency (EPA), the U.S. Department of the Interior, the U.S. Department of Transportation, and other ICCVAM member agencies to complement, reduce, or replace animal use are also described in this report.

ICCVAM and NICEATM also continue to be at the forefront of developing new approaches to address specific chemical safety testing needs. We congratulate NICEATM on the acceptance of the Skin Allergy Risk Assessment – Integrated Chemical Environment (SARA-ICE) defined approach for skin sensitization (Reinke et al. 2025) by the Organisation

for Economic Co-operation and Development (OECD) in an update to its Guideline 497 for defined approaches to identify skin sensitizers. SARA-ICE, developed by NICEATM in collaboration with the consumer products company Unilever, is the first defined approach that provides a numerical estimate of potency to be accepted as an OECD guideline test. ICCVAM also sponsored an EPA-led interlaboratory validation study of a thyroid 3D microtissue model for evaluating chemical effects on thyroid hormone synthesis and tissue viability (Deisenroth et al. 2026). This model is an important advance in assessing thyroid hormone interference, filling a notable gap in determining the potential of chemicals to affect the endocrine system.

A key area of the Strategic Roadmap is communication. During 2024-2025, NICEATM and ICCVAM continued to effectively connect with stakeholders, as demonstrated by the high attendance and strong participation at ICCVAM-sponsored virtual public events such as the Communities of Practice webinars and the Public Forums. In response to stakeholder requests, NICEATM and ICCVAM member agencies such as EPA and the U.S. Department of Agriculture are increasing efforts to provide training on computational tools and data resources and highlight available information on NAMs. Details and outcomes of many of these outreach efforts are described herein.

The National Institute of Environmental Health Sciences (NIEHS) and NTP have been proud to be the home of ICCVAM and NICEATM since their inception over 20 years ago and to support their important work to advance NAMs. Reflecting the increasingly central role of ICCVAM and NICEATM to NIH efforts to develop, validate, and scale the use of NAMs, we announced in 2026 plans for NICEATM to move to the newly created Office of Research Innovation, Validation, and Application (ORIVA) within the Division of Program Coordination, Planning, and Strategic Initiatives in the NIH Office of the Director. Within ORIVA, NICEATM would become the Division of the National Interagency Center for the Evaluation of Alternative Test Methods (D-NICEATM) and work alongside ORIVA's Division for Accelerating Innovation in Biomedical Research. NIEHS and NTP are actively supporting this planned transition and look forward to continued fruitful collaborations with ORIVA in the future.

The activities in this 2024-2025 Biennial Report illustrate the outstanding contributions of ICCVAM to advance alternative methodologies and the replacement, reduction, and refinement of animal use. We invite you to read this report to learn about all that ICCVAM has accomplished in the past two years.

Kyle Walsh, 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 Interagency Center for the Evaluation of Alternative Test Methods (NICEATM), we are pleased to present the 2024-2025 ICCVAM Biennial Progress Report.

Appropriate acceptance and application of new approach methodologies (NAMs) can reduce and replace animal use while improving public health protection. During 2024 and 2025, ICCVAM was central to the multifaceted efforts in scientific innovation, development of new approaches to validation, and critical evaluation of current test methods required to accomplish these goals.

The ICCVAM publication "Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies," has become a key resource for the evaluation of NAMs since it was published in March 2024. Specifically, the principles articulated in this document are informing validation activities under the National Institutes of Health (NIH) Complement Animal Research in Experimentation (Complement-ARIE) program. Complement-ARIE will accelerate the development, standardization, validation, and use of human-based NAMs, and significantly advance the understanding of human health and disease by providing a range of ready and standardized biomedical research methods. To further encourage the application of these principles to toxicity testing, ICCVAM held its Method Developers Forum. This event was held in August 2024 and focused on approaches for identification of potential carcinogens.

While validation of new methods is key to their adoption, potential users have other needs that ICCVAM is actively addressing. The ICCVAM Consideration of Alternative Methods Workgroup gathered input to identify barriers to awareness and adoption of NAMs. Their review (Oyetade et al. 2025) identified key barriers to NAMs use and provides a roadmap for advancing NAMs adoption. One key need identified by the workgroup's review, the creation of centralized repositories for NAM-related information, will be addressed by the Collection of Alternative Methods for Regulatory Application (CAMERA). Launched in 2025, this NICEATM resource is an interactive database and user interface that provides online access to information about methods that have been validated for specific regulatory purposes.

In addition to CAMERA, ICCVAM agencies continue to develop resources that provide high-quality data for method development and validation, as well as tools that help users from diverse backgrounds understand and use those data. This report describes data resources and computational tools developed by nine ICCVAM agencies that facilitate access and application of data to advance NAMs development and use.

ICCVAM also engages in collaborations to advance acceptance and harmonization of NAMs for chemical safety testing. ICCVAM members supported a wide range of activities within the Test Guidelines Programme of the Organisation for Economic Co-operation and Development, including co-leading the effort to revise Guidance Document 34 on validation and acceptance of new or updated test methods for hazard assessment. Other important collaborations over the last two years have occurred within working groups of the United Nations Globally Harmonized System for Classification and Labelling of Chemicals, the Health and Environmental Sciences Institute, the International Microphysicological Systems Society, and the International Liaison Group on Methods for Risk Assessment of Chemicals in Food.

We would like to acknowledge the contributions of the representatives and interagency workgroup members from the 18 ICCVAM member agencies, NICEATM and its contract staff, members of ICCVAM's advisory committee, and our many other stakeholders. The current reporting period has been notable for the number of representatives that have left federal service to retire or pursue other opportunities. We thank them all for their service, in particular longtime agency representatives Moiz Mumtaz, Ph.D. (Agency for Toxic Substances and Disease Registry) and Joanna Matheson, Ph.D. (U.S. Consumer Product Safety Commission). We welcome the agency representatives that joined ICCVAM as new members during 2024 and 2025, and look forward to their active participation and new ideas.

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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Key NICEATM and ICCVAM Accomplishments and Impact

Tags: ICCVAM, EPA, FDA, NIEHS, NIH, carcinogenicity, data sharing, defined approaches/IATA, endocrine disruptors, skin sensitization

Key accomplishments of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), ICCVAM member agencies, and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) in support of the ICCVAM mission during 2024 and 2025 include:

- **Updated validation guidance by ICCVAM and ICCVAM Method Developers Forum.** "Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies: A Report of the ICCVAM Validation Workgroup," developed by ICCVAM with input from 17 federal agencies, offers recommendations on how to establish confidence in new approach methodologies (NAMs). Publication of the report was followed by the August 2024 ICCVAM Method Developers Forum, which provided an opportunity for NAMs developers to present new methods to identify potential carcinogens to relevant stakeholders.
- **ICCVAM review proposing approaches to addressing barriers to adoption of NAMs.** A review by the ICCVAM Consideration of Alternative Methods Workgroup (Oyetade et al. 2025) identifies five key barriers to the adoption of NAMs encountered by a variety of stakeholder groups and proposes approaches to address these barriers.
- **Launch of the National Institutes of Health (NIH) Complement Animal Research in Experimentation (Complement-ARIE) program.** Complement-ARIE will accelerate the development, standardization, validation, and use of human-based NAMs and significantly advance the understanding of human health and disease by providing a range of ready and standardized biomedical research models.
- **Shift toward adoption of alternatives by the U.S. Food and Drug Administration (FDA) and NIH.** Policy shifts by the U.S. Food and Drug Administration and the National Institutes of Health (Scheufen Tieghi et al. 2025) will prioritize the

development of predictive systems that use computer modeling and in vitro organ and cell testing.

- **International acceptance of a defined approach to estimate skin sensitization potency.** The Skin Allergy Risk Assessment – Integrated Chemical Environment (SARA-ICE) defined approach for skin sensitization (Reinke et al. 2025) was accepted by the Organisation for Economic Co-operation and Development (OECD) in an update to its Guideline 497 for defined approaches to identify skin sensitizers. SARA-ICE is the first defined approach that provides a numerical estimate of potency to be accepted as an OECD guideline test.
- **Validation of a thyroid microtissue model.** NICEATM coordinated an interlaboratory validation study led by the U.S. Environmental Protection Agency of the utility of a thyroid 3D microtissue model for evaluating chemical effects on thyroid hormone synthesis and tissue viability (Deisenroth et al. 2026). The study established standard operating procedures that were successfully applied in multiple laboratories, and consistent results were generated among the testing laboratories.
- **Launch of the Collection of Alternative Methods for Regulatory Application (CAMERA) resource.** CAMERA is an interactive database and user interface that provides online access to validated alternative methods for U.S. regulatory and other applications. It is being developed by NICEATM, with an ICCVAM steering committee.

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 Interagency Center for the Evaluation of Alternative Test 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 about NICEATM and ICCVAM.

ICCVAM Establishment and Purpose

Tags: ICCVAM, NIEHS

United States (U.S.) regulatory agencies are charged with protecting human and animal health and the environment. To do this, agencies must determine hazards presented by substances such as pesticides, consumer products, and workplace chemicals. Evaluating 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 support the 3Rs and can be used to obtain information about chemical hazards and risk assessments.

The ICCVAM Authorization Act of 2000 (42 U.S.C. 285*l*-3) established 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

The ICCVAM Authorization Act established ICCVAM as 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 Center for Advancing Translational Sciences (NCATS)
- 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 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. Department of War (DoW)
- 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
- Ensure ICCVAM's final test recommendations are available to the public
- Prepare reports on ICCVAM progress and accomplishments under the Act and ensure they are available to the public

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ICCVAM Workgroups

Tags: ICCVAM, acute toxicity, ecotoxicity, PFAS

ICCVAM establishes ad hoc workgroups to perform specific tasks important for the development or validation of alternatives to animal models. 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 2024 and 2025, ICCVAM had active workgroups focused on the following areas:

- Acute toxicity: co-chairs Kristen Fulbright, M.S. (DoW) and Grace Patlewicz, Ph.D. (EPA)
- Consideration of alternative methods: transitioned to expert group status in 2025; co-chairs Jessie Carder, M.S. (USDA) and Shannon Marko, D.V.M. (DoW)
- Ecotoxicology: co-chairs William Eckel, Ph.D. (EPA) and Natalia Garcia-Reyero Vinas, Ph.D. (EPA)
- PFAS: co-chairs Natalia Garcia-Reyero Vinas, Ph.D. (EPA), Kelly Carstens, Ph.D. (EPA), and Shruti Kabadi, Ph.D. (FDA)
- Validation: transitioned to expert group status in 2024; co-chairs Suzanne Fitzpatrick, Ph.D. (FDA), John Gordon, Ph.D. (CPSC), and Elijah Petersen, Ph.D. (NIST)

How NICEATM Supports ICCVAM

Tags: ICCVAM, NIEHS

During 2024 and 2025, NICEATM, an office within the NIEHS Division of Translational Toxicology, provided 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:

- Supported activities of the NIEHS Division of Translational Toxicology, especially those contributing to the U.S. government's interagency Tox21 initiative
- Conducted analyses and evaluations and coordinated independent validation studies on novel and high-priority alternative testing approaches
- Provided information, data, and tools to test method developers, regulators, and regulated industry through its website and workshops on topics of interest

NICEATM staff (as of November 2025)

NIEHS

Helena Hogberg, Ph.D., Acting Director

Kamel Mansouri, Ph.D., Computational Chemist

NICEATM Contract Staff (Inotiv, Inc.)

Emily Reinke, Ph.D., DABT, Principal Investigator
Steven Morefield, M.D., Project Manager
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Under a proposal announced in late 2025, in 2026 NICEATM will become a division of the Office of Research Innovation, Validation, and Application within the Division of Program Coordination, Planning, and Strategic Initiatives, National Institutes of Health Office of the

Director.

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 2024 and September 2025.

Roster of SACATM Members 2024–2025

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
Kambez H. Benam, D.Phil., ATSF	Associate Professor, Departments of Medicine and Bioengineering	University of Pittsburgh, Pittsburgh, PA	2025
Ellen Berg, Ph.D.	Principal	Alto Predict LLC, Palo Alto, CA	2025
Corie Anthony Ellison, Ph.D.	Principal Scientist	Procter and Gamble, Cincinnati, OH	2028
Sue Leary, M.S.	President	Alternatives Research and Development Foundation, Jenkintown, PA	2026
M. Sue Marty, Ph.D., M.P.H. (Chair 2025)	Associate Director Toxicology Science Leader	The Dow Chemical Company, Midland, MI	2026
Kristini K. Miles, Ph.D., DABT	Chief Executive Officer	Miles Consulting LLC, Atlanta, GA	2026
Adrian Nañez, Ph.D.	Senior Medical Science Liaison	Servier, Inc., San Antonio, TX	2025
Kathryn E. Page, Ph.D., DABT (Chair 2024)	Product Safety Toxicologist (Senior Scientist), Global Stewardship	The Clorox Company, Pleasanton, CA	2024

Nathan D. Price, Ph.D.	Chief Scientific Officer	Thorne Health Tech, New York, NY	2026
Priyanka Sura, D.V.M., M.S., DABT	Senior Occupational Toxicologist, Nonclinical Safety and Pathobiology	Gilead Sciences, Inc., Foster City, CA	2024
Patricia Silveyra, M.S., Ph.D.	Associate Professor, Department of Environmental and Occupational Health	School of Public Health, Indiana University Bloomington, Bloomington, IN	2026
Sally Thompson-Irritani, D.V.M., Ph.D.	Assistant Vice Provost and Clinical Associate Professor, Animal Care, Outreach, and 3Rs	School of Public Health, University of Washington, Seattle, WA	2026
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 in chemical safety testing. These include new platforms such as microphysiological systems (MPS), data resources to support the development of predictive and quantitative structure–activity relationship (QSAR) models, 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 in chemical safety testing. These include technologies to address important endpoints such as carcinogenicity, developmental toxicity, immunotoxicity, inhalation toxicity, neurotoxicity, and ecotoxicity testing.

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 disrupt biological systems. The Attagene cis-FACTORIAL™ assay uses a reporter system to detect transactivation of 46 transcription factors, providing a quantitative assessment of chemical effects. A later version of this assay, CYP-FACTORIAL™, added nine key cytochrome P450 (CYP) 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 altered bioactivity profiles between parent and metabolite compounds.

To explore the application of this system to toxicity prediction, NIEHS and Attagene collaborators tested 24 chemicals of regulatory concern using both systems. Profiling across all 46 transcription factors produced chemical toxicity signatures, and applying a biological read-across approach identified chemicals with similar effects. Additionally, comparing the profiles of “toxic” vs. “nontoxic” chemicals yielded insights into the potential biological mechanisms underlying adverse outcomes. Dose–response modeling using the tcplfit2 R package (Sheffield et al. 2022) further revealed how the addition of CYP enzymes altered

bioactivity and response sensitivity across chemicals and transcription factors. This study exemplifies how integrating metabolism into a multiplexed in vitro assay system can provide additional mechanistic insights needed to understand chemical-elicited bioactivity, thereby facilitating the development of human-relevant test systems. A manuscript is in preparation and is planned for submission in 2026.

SEAZIT interlaboratory study on the use of zebrafish for developmental toxicity evaluation

Tags: NIEHS, developmental toxicity, small model organisms

Scientists at NIEHS are using zebrafish (Danio rerio) embryos as a test model to study how chemical exposure might impact development. Zebrafish are useful for developmental studies because their embryos are transparent, develop quickly, and respond similarly to humans. However, different laboratories use different testing methods, and these discrepancies make it difficult to compare results, limiting the wider use of zebrafish embryos for regulatory chemical safety testing. To address the discrepancies in testing methods, the Systematic Evaluation of the Application of Zebrafish in Toxicology (SEAZIT) initiative was created. This project aimed to improve understanding of what aspects of experimental protocols, data analysis, and reporting causes variability among zebrafish testing across laboratories and help identify ways to make methods more consistent.

In a study involving three laboratories (Hamm et al. 2024), 42 chemicals were tested under different conditions; for example, using embryos with the chorion intact versus removed or using static versus static renewal chemical exposure. The study compared how these methods influenced observed developmental toxicity. The results showed good agreement across laboratories, with 33 of the 42 chemicals (~79%) producing the same activity outcomes. However, differences in chemical potency linked to varying laboratory protocols underscored the need for method standardization. The findings of this study provide valuable insights into the application of standardized ontologies for cataloging, comparing, and sharing developmental toxicity data. This work could help improve the reliability and regulatory acceptance of zebrafish embryos as a tool for evaluating developmental toxicity.

An interactive web resource, SEAZIT Data Integration and Visualization Enabling Resource (SEAZIT-DIVER), has been developed to allow the public to explore and visualize SEAZIT

data. This platform also provides methodological details, information on test chemicals, tools to evaluate data quality, and options to download data for additional exploration.

Development and validation of CYP26A1 inhibition assay for high-throughput screening

Tags: EPA, NCATS, NIEHS, developmental toxicity, metabolism, Tox21

Scientists at NCATS, EPA, and NIEHS are studying how chemicals interact with important biological systems to better predict potential risks, such as developmental toxicity. One key system involves all-trans retinoic acid, a molecule produced from vitamin A, which is crucial for regulating development and cell growth. Levels of all-trans retinoic acid are tightly controlled in the body, and the molecule is broken down by specialized enzymes called CYP26 (subtypes A1, B1, and C1) to maintain balance. This research focused on the detection of chemicals that inhibit CYP26A1, one of the enzymes involved in this process, using a new experimental method.

Researchers developed a high-throughput screening (HTS) assay using a system called P450-Glo (Sakamuru et al. 2024). This cell-free system quickly identifies chemicals that interfere with CYP26A1 activity. The assay performed well in terms of consistency, reliability, and ability to distinguish signals, with a signal-to-background ratio of 25.7 and a Z-factor of 0.7 (indicating strong performance). To validate the method, 39 chemicals were tested, including known CYP26A1 inhibitors, compounds related to retinoids, and control chemicals. The assay accurately confirmed known inhibitors and identified new ones, such as chlorothalonil, prochloraz, and SSR126768. This successful validation highlights the reliability of the new assay in the detection of chemicals that may disrupt all-trans retinoic acid balance, which is critical during development. This innovative tool could be pivotal for studying developmental toxicity and ensuring chemical exposure safety, particularly for substances that impact embryonic development.

NIEHS activities to develop new approaches for identifying potential immunotoxicants

Tags: NIEHS, immunotoxicity

To reduce and replace the use of animals in immunotoxicity assessments, the NIEHS Division of Translational Toxicology collaborated with NICEATM to develop a comprehensive human whole-blood new approach methodology (NAM) battery for in vitro assessment of immune function. The system uses human whole blood diluted in culture

media, to which an immune stimulant such as anti-CD3/CD28 antibodies or viral peptides may be added. The model immunotoxicants dexamethasone and benzo[a]pyrene were used to evaluate the utility of the screening assay. Following in vitro culture, immune cells were identified and counted by flow cytometric immunophenotyping and assessed for natural killer cell activity and T cell activation. Proinflammatory cytokine levels were measured in culture supernatants. In vitro treatment with dexamethasone resulted in concentration-dependent suppression of cytokine production, natural killer cell activity, and T cell activation. Treatment with benzo[a]pyrene caused suppression of cytokine production, but immunosuppression only occurred following metabolic activation by addition of S9 fraction enzymes. Advantages of this NAM battery include the ability to assess adaptive immunity and relate immunotoxicity to resistance to infectious diseases. These studies demonstrate that in vitro immunotoxicity assays can measure effects that reflect known outcomes from in vivo studies. This multiendpoint human whole-blood NAM battery may be useful for screening compounds for immunotoxicity hazard identification in a manner that reduces reliance on animal systems and increases translatability to humans.

High-throughput cytokine detection platform to identify potential neurotoxicants

Tags: EPA, NCATS, NIEHS, immunotoxicity, neurotoxicity, Tox21

Exposure to environmental chemicals, such as pesticides and heavy metals, may contribute to neurodegenerative disorders by inducing inflammation in nervous system tissues. EPA, NCATS, and NIEHS collaborated to develop in vitro models using human induced pluripotent stem cell-derived microglia to identify potential neurotoxicants (Yang et al. 2025). The assays measured cytokine secretion, which is a marker of inflammatory response. The cytokine response patterns in these cells closely resembled those under in vivo conditions, with significant increases in interleukin 6 and tumor necrosis factor-alpha levels. These initial results were used as the basis for development of a homogeneous, time-resolved fluorescence HTS assay platform in a 1,536-well plate format for screening of environmental chemicals. This assay was used to test nine environmental chemicals having evidence of cytokine-inducing potential in other in vitro assays. While all nine tested agents stimulated interleukin 6 and tumor necrosis factor-alpha production, three chemicals showed significant stimulation of both cytokines. This study establishes a reliable high-throughput platform for

detecting inflammatory effects of environmental toxicants in a microglial cell assay, data from which may provide valuable insights into their neuroinflammatory potential and possible contributions to development of neurodegenerative disorders.

Isolation and characterization of cell-free DNA from cerebral organoids

Tags: NIEHS, neurotoxicity

The early detection of neurological disorders is crucial for effective intervention and treatment, but current methods of studying brain tissue directly, such as biopsies, are risky and invasive. Scientists at NIEHS are investigating ways to measure biomarkers, molecules found in bodily fluids such as blood or cerebrospinal fluid, to monitor brain health. One promising biomarker is cell-free DNA, fragments of genetic material released by cells, which could provide insights into brain function and disease. However, understanding how cell-free DNA from brain tissue changes in response to damage or disease has been challenging because many tissues contribute to cell-free DNA levels in the body.

To address this challenge, NIEHS researchers utilized cerebral organoids, which are miniature, lab-grown models of the human brain. These organoids offer a controlled system to track cell-free DNA changes specifically related to brain tissue. The study demonstrated that cerebral organoids release enough cell-free DNA to be analyzed using advanced techniques including droplet digital PCR and whole-genome sequencing. Importantly, when researchers examined the genetic information in the cell-free DNA, they found that many of the fragments were associated with biological processes linked to neurodevelopment and neurological disorders.

This work highlights the potential of cerebral organoids to be powerful tools in the study of cell-free DNA biomarkers. These biomarkers could one day help identify early signs of neurodevelopmental and neurological conditions, paving the way for earlier diagnoses and better-targeted treatments. The findings provide a promising step forward in understanding how cell-free DNA reflects brain health and disease.

Design and application of an adept aerosol lung-on-a-chip and aerosol/vapor delivery system

Tags: DoW, inhalation toxicity, MPS

The U.S. Army Chemical Biological Center (CBC) has established cooperative research and development agreements with the tissue chip manufacturers Emulate, Inc., and TissUse GmbH to apply their products to investigations of aerosol toxicity.

Aerosolization of chemical agents is a health concern that presents unique technical challenges. When aerosols are generated in specific environments, the agent can be deposited on surfaces and then reaerosolized into the air due to environmental factors, 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. To investigate this concept, CBC scientists developed a novel system to simulate reaerosolization of chemical agents in soil with a high sand content. A vibration system with air inlet and outlet ports was developed to allow sampling of the reaerosolized soil-agent to determine a worst-case scenario concentration. Reaerosolized agent concentrations were identified and tested for toxicity in Emulate organ-chip models.

Toxicity of substances of concern will also be assessed in collaboration with TissUse using an open-top multiorgan chip system that can be exposed to an aerosolized agent or compound of interest. The system functions with an established TissUse 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. The InHALES system will be implemented at CBC in early 2026, and feasibility studies will begin in summer 2026 to better understand aerosol maturation and dissemination.

Additionally, CBC leveraged success with bespoke aerosol organ-chip delivery systems to establish a cooperative agreement with Emulate. The focus of the collaboration is to provide user input on Emulate's A1 open-top lung chip and to adapt the chip to CBC's existing aerosol generation infrastructure.

CBC recently began work that aims to characterize exposure routes in vitro. Human lung chips will be exposed to toxic compounds in liquid directly on the tissue or through aerosols generated by the VITROCELL® Cloud in vitro exposure system (VITROCELL GmbH).

Complex in vitro and ex vivo models to measure gastrointestinal endpoints

Tags: FDA, NIEHS, MPS

Conventional in vitro models of the gastrointestinal tract, such as Caco-2 monolayers, lack the cellular complexity, mechanical environment, and tissue architecture needed to accurately capture absorption, permeability, and barrier function in vivo. Studies by the FDA National Center for Toxicological Research (NCTR) explored how these systems could be improved to support drug development and safety testing.

- Ongoing studies are comparing animal and nonanimal models, including human intestinal tissue-based MPS, to evaluate the use of NAMs for assessing antiretroviral drug toxicity. This work will be presented at the 2026 MPS World Summit (Yanamadala et al. Abstract 797). Planned additional studies in collaboration with NIEHS will use multiorgan chip platforms to assess the extraintestinal impacts of the microbiome on the liver and heart.
- NCTR researchers have demonstrated the importance of continuous-flow systems for maintaining physiological relevance. Using resected human intestinal tissue, continuous-flow gut-on-a-chip models showed improved preservation of tissue function, greater sensitivity to dose-dependent responses, and distinct transcriptional, inflammatory, and drug-transport profiles compared to static systems. This project was presented at the 2025 Gordon Research Conference for Cellular and Molecular Mechanisms of Toxicity.
- To complement host-focused models, NCTR scientists developed a rotary culture-based NAM ex vivo system to study interactions between the microbiome and xenobiotics. This dynamic system enables controlled, mechanistic evaluation of microbial responses and has been directly compared to animal models to assess translational relevance. A presentation at the 2024 MPS World Summit (Khare et al. Abstract 481) described a bioreactor model that keeps microbiota and chemicals in motion, which mimics gastrointestinal conditions better than static systems. The study noted time- and dose-dependent changes in bacterial communities and metabolite production after exposure. The model may improve gastrointestinal toxicology risk assessment, support regulatory decision-making, and advance FDA goals to modernize toxicology testing.

Technical characterization of assays through investigation of sources of uncertainty

Tags: FDA, NIST

Technical characterization is a key component of the development of NAMs for widespread use. Technical characterization includes assessing sources of variability in a NAM, designing an assay to include relevant control measurements, evaluating the range of test substances for which the assay can be used, and developing a suitable statistical data analysis approach. A key aspect in demonstrating the scientific validity of a NAM is that the assay is sufficiently well-characterized from a technical perspective to ensure that it is robust, reliable, and reproducible. Researchers at NIST applied these principles to develop two assays: a nitric oxide nanobio assay and an oral mucosal tissue irritation assay.

- Engineered nanomaterials may induce oxidative stress in cells, potentially leading to cytotoxicity and DNA damage. This can be assessed using assays that measure cellular nitrites as a surrogate for nitric oxide production via macrophage activation in vitro. NIST collaborated with FDA to refine an assay used for this purpose to support its broader application (Fahmi et al. 2026). The assay has been recognized as a standard test method by the American Society for Testing and Materials.
- To support the broader application of an oral mucosal tissue irritation assay for the testing of biological effects from dental materials, NIST researchers utilized 3D human oral epithelium models to characterize irritation mechanisms and improve measurement robustness (Gutierrez et al. 2024). The team identified variability sources using statistical modeling and control charts to track assay performance over time. Robustness was tested under multiple conditions, highlighting the importance of pipetting procedures, exposure durations, and complementary measurements, such as transepithelial electrical resistance. Recommendations for protocol refinement and standardization have been made to ensure that this assay aligns with quality assessment procedures.

Error-corrected sequencing as an alternative to traditional rodent mutation assays

Tags: FDA, genotoxicity

Error-corrected sequencing technologies are emerging as highly sensitive alternatives to traditional transgenic rodent gene mutation assays because they can detect extremely rare genetic changes throughout the entire genome. These technologies can also reveal detailed patterns in these changes, helping scientists better understand how and why mutations occur.

In separate efforts, FDA NCTR and the NIEHS Division of Translational Toxicology have been exploring applications of these technologies in studies using human cell lines and in vivo rodent models. These projects address key methodological considerations for assay design.

- Molnupiravir, an antiviral prodrug used to treat COVID-19, can induce mutations in bacterial and mammalian cell cultures, raising concerns about patient safety. McKinzie et al. (2026) applied a novel error-corrected sequencing pipeline to peripheral blood cells collected retrospectively from molnupiravir-treated and untreated COVID-19 patients. No differences in mutation frequency, type, or mutational signatures were detected between groups, suggesting, within the limits of the study, that the authorized therapeutic dose carries no detectable mutational risk in treated patients.
- Commercially available TK6 and L5178Y cells have elevated background mutation frequencies compared to freshly derived single-cell clones, likely from accumulated replication errors during long-term culture. These elevated backgrounds reduce the relative signal upon mutagen exposure, potentially confounding error-corrected sequencing-based mutagenicity assessments. Miranda et al. (2025) used error-corrected next-generation sequencing to quantify the background mutation frequencies of freshly derived clonal TK6 cell populations and commercially available cell lines. The research team derived an alternative data analysis approach based on absolute mutation frequency changes within each cell population. This approach may provide more comparable results for commercial and clonal populations.

Addressing limitations of in vitro mutagenicity assays using liver cell lines

Tags: FDA, genotoxicity, hepatotoxicity, metabolism, MPS

Standard in vitro genotoxicity assays have limitations that can prevent broader use in replacing in vivo assays. Assays using cell lines that lack metabolic capability must be supplemented with exogenous rat liver S9 fractions, which do not accurately reflect human biotransformation. Additionally, conventional in vitro genotoxicity protocols have typically been of limited utility in predicting effects of longer exposures. NCTR researchers have been

applying advances in human-relevant genotoxicity testing that address these gaps through three complementary approaches: engineering metabolically competent TK6 cell lines to better recapitulate liver-like metabolism, using 2D and 3D HepaRG liver cell models to evaluate nitrosamine drug impurities, and applying extended-exposure HepaRG protocols to improve detection of low-level mutagens. Together, these approaches support a shift toward human-based systems for regulatory genotoxicity assessment.

- Li et al. (2025) developed TK6-4CYP, a human lymphoblastoid cell line coexpressing four liver CYP enzymes (CYP2A6, 2E1, 2C19, and 3A4) with metabolic capability, enabling bioactivation of indirect genotoxicants without reliance on a rodent S9 fraction. The cell line correctly identified known promutagens in standard genotoxicity assays. Promutagens such as N-nitrosodimethylamine and benzo[a]pyrene are not mutagenic in their original form but can be metabolized into mutagens. TK6-4CYP expands the existing panel of single-CYP TK6 lines and offers a human-relevant, metabolically competent platform for regulatory genotoxicity testing.
- Seo et al. (2025) assessed 10 nitrosamine drug substance-related impurities for genotoxicity in 2D and 3D HepaRG cultures. They used the CometChip assay, which can detect DNA damage at the level of individual cells, and flow cytometry-based micronucleus assays, which assess the presence of small extracellular nuclei containing chromosomal fragments. Five compounds that previously showed positive results in the enhanced Ames test induced DNA damage in both models, while five impurities with negative results in the Ames test showed no activity. The 3D spheroid format consistently outperformed 2D cultures in sensitivity, supporting its use as a more physiologically relevant system for drug impurity hazard evaluation.
- Building on prior work in which acute cytotoxicity limited mutation detection in HepaRG cells, Guo et al. (2025) used 7- and 14-day low-concentration N-nitrosodimethylamine exposures in both 2D and 3D formats to allow mutation accumulation. Extended treatment produced concentration-dependent increases in DNA damage, micronucleus formation, and mutation frequency, with 3D spheroids showing greater sensitivity. The results support longer-duration protocols in

metabolically competent human liver cells as a practical approach for the detection of mutagenicity at environmentally or pharmaceutically relevant concentrations.

Organoid models of KRAS-mutant cancer

Tags: NCI, carcinogenicity, genotoxicity

Anchorage-independent growth, or the ability of cancer cells to grow without being attached to a surface, is a hallmark of tumor development. While 3D spheroid cultures are valuable for studying this phenomenon, their use in research has been limited due to challenges in process scaling. NCI researchers have addressed this issue by developing new, cost-effective methods to grow tumor spheroids and organoids. One key focus of this research is the KRAS gene, mutations of which are linked to the development of several cancers, including lung adenocarcinoma. Scientists have successfully created scalable tumor spheroid cultures for studying KRAS-mutant epithelial cancers. These spheroids provide a more realistic model than traditional 2D cell cultures, allowing researchers to examine unique molecular and genetic features specific to 3D cancer growth.

NCI researchers also developed mouse organoid cultures using alveolar Type 2 cells, which are natural lung stem cells and likely the starting point of lung adenocarcinoma. The organoid cultures include both healthy and KRAS-mutant alveolar type 2 cells and have been used to study how the mutated KRAS gene drives cancer, including its effects on growth, signaling, gene activity, and stem cell behavior. Efforts are underway to establish similar organoids for human alveolar type 2 cells.

These advanced models help researchers better understand the role of the mutated KRAS gene in cancer development and its behavior in realistic 3D environments. This research helps support improved treatments, particularly targeted therapies for KRAS-related cancers, and enables tumor behavior to be further studied in a controlled yet realistic setting.

Methods for mammalian genetic analysis

Tags: NCI, carcinogenicity, genotoxicity

Cancer development research requires better methods to model the complex gene mutations and expression changes found in human tumors. NCI researchers have made significant progress in using advanced tools such as RNA interference and CRISPR/Cas9 genome editing to modify multiple genes at once, allowing more accurate simulations of cancer cell

behavior in the lab. Multiplexed RNA interference is an approach that enables scientists to silence up to seven genes simultaneously in human cancer cells. This approach helps uncover interactions between genes and identify redundant functions, providing insights into how genes work together to drive cancer. NCI researchers have also developed a multiplexed CRISPR/Cas9 system that can knock out three genes at the same time in human cancer cells. They are now adapting this CRISPR method for use in organoid cultures, which are 3D models that mimic tissue structure. This allows analysis of the effects of various genetic changes in organoids derived from both mice and humans, which better represent real-world tumor behavior. Additionally, the NCI team has created specialized CRISPR libraries for targeted genetic screens. These libraries are organized based on the functions of specific genes, making them highly flexible tools for studying cancer-related pathways. Finally, efforts are underway to develop methods to precisely edit genes in mouse organoids. This innovation allows researchers to rapidly create organoids with specific cancer-causing mutations, enabling detailed studies of tumor behavior and drug responses in lab-based models. These tools and techniques provide powerful methods to study the complex genetic environments of cancers and assess potential treatments, promoting the development of more effective cancer therapies.

High-throughput screening cancer panels of patient-derived organoid models

Tags: NCI, carcinogenicity, data sharing

The Patient-Derived Models Repository, hosted by NCI, provides resources to support cancer research and drug discovery. In addition to patient-derived xenografts, it offers NAMs such as patient-derived in vitro tumor cell cultures and patient-derived organoids. These specialized NAMs retain characteristics of the original tumors, making them valuable for understanding cancer biology and testing potential treatments.

The repository includes a diverse range of models developed from various types of cancer, reflecting both common and rare tumor types. This diversity helps researchers study different genetic and molecular aspects of cancer and investigate their influence on disease progression and treatment response. Additionally, the repository emphasizes quality control in the production and characterization of these models to ensure researchers using them to obtain high-quality, reproducible results. The repository also offers detailed information

about the models themselves. This includes their sources, tumor types, and other important characteristics, such as genomic data. Researchers can query the repository to find models specific to their study needs.

By using these well-characterized models, scientists can gain insights into cancer biology, identify new therapeutic targets, and evaluate the effectiveness of experimental treatments in settings that closely resemble human cancers. These tools ultimately facilitate advancements in cancer treatment and personalized medicine.

Data Resources

As momentum toward the adoption of alternatives to animal use in chemical safety testing increases, 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 data resource (ECOTOXicology Knowledgebase)

Tags: EPA, data sharing, ecotoxicity, metrics, PFAS

The EPA 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 from over 55,000 references in the scientific literature and over 1.2 million test records, covering more than 14,000 aquatic and terrestrial species and 13,000 chemicals.

There were eight updates to ECOTOX during 2024–2025. Recent additions include information on 6PPD-quinone, 6PPD-alternatives, arsenic, cyanotoxins, metolachlor, quizalofop, and per- and polyfluoroalkyl substances (PFAS). In addition to the data updates, the Explore Publications module launched in 2024 enables queries by citation information (author, title, ECOTOX ECOREF number, DOI) and displays toxicity data by reference. In 2024 and 2025, ECOTOX had an average of over 16,000 monthly page views and over 2,000 active monthly users. An ECOTOX training session was presented in April 2024 as part of a broader in-person EPA NAMs tools training workshop. Additional training resources for ECOTOX are available on the EPA NAMs Training webpage.

NICE data updates

Tags: NIEHS, carcinogenicity, eye irritation, PFAS, skin sensitization, Tox21

The NIH Integrated Chemical Environment (NICE) provides data and tools to help develop, assess, and interpret chemical safety tests. The March 2024 NICE v. 4.0.2 release improved support for chemical name searching in the NICE REST application programming interface (API). The September 2024 NICE v. 4.1 release added versioning information to datasets and added applicability domain information for chemical property predictions from the Open (Quantitative) Structure–activity/property Relationship App (OPERA). The July 2025 NICE v. 4.2 release updated curated high-throughput screening (cHTS) data to align with data in the EPA Toxicity Forecaster (ToxCast) invitrodb v. 4.2 release. Other updates and improvements to NICE datasets during 2024–2025 include:

- Links to the National Toxicology Program (NTP) Chemical Effects in Biological Systems database and the EPA CompTox Chemicals database in data downloads
- Data annotation aligned with Open Biological and Biomedical Ontology (OBO) Foundry ontologies
- Ability of users to download ClassyFire (Djoumbou Feunang et al. 2016) chemical taxonomies for over one million chemicals
- A new "PFAS" Chemical Quick List; a "Demo List of Chemicals" Chemical Quick List was also added to help new users explore NICE functionality
- An updated "RoC Classifications" Chemical Quick List that includes new data from the NTP's 15th Report on Carcinogens
- An update of the "Tox21" Chemical Quick List
- Continued harmonization of skin sensitization and eye irritation data

CAMERA beta testing and improvements

Tags: ICCVAM, NIEHS, data sharing, eye irritation, harmonization, skin sensitization

ICCVAM has identified a need to enhance access to information about NAMs that have been qualified and validated for regulatory application. The Collection of Alternative Methods for Regulatory Application (CAMERA) was developed in response to that need.
CAMERA is an interactive database and user interface that provides online access to validated alternative methods for U.S. regulatory use and other contexts of use. It is being

developed by NICEATM under guidance from an ICCVAM steering committee. CAMERA is a central hub and unified resource of validated alternative methods that enhances access to validation study reports, data, protocols, standard operating procedures, and information on regulatory guidance. It provides access to test method details, such as chemical applicability domain, related key events, and reference chemicals. Users can filter searches by alternative method type, defined approach (DA), test method endpoint, and regulatory guidance.
As of the end of 2025, CAMERA was in development, with some pages still having limited functionality. The database includes 27 NAMs that cover endpoints including skin irritation and corrosion, eye irritation, and skin sensitization. Updates planned for early 2026 will add tooltips to improve user guidance on the website, revise the chemicals and performance metrics tables, and add new definitions to the glossary.

Development of BioBricks, a bioinformatics repository

Tags: NIEHS, data sharing, 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 with Insilica in 2022 to provide support and testing of the BioBricks platform. The NICEATM team contributed to building numerous bricks (units that encapsulate datasets), including those derived from NICE datasets. NICEATM also supported the testing and expansion of the BioBricks interface through activities such as the development of a Python version. A complete description of BioBricks has been published in *Frontiers in Artificial Intelligence* (Gao et al. 2025), with NICEATM team members serving as coauthors. All databases integrated into BioBricks are fully and freely available; the code is open-source and available via a GitHub repository.

First-of-its-kind atlas offers a detailed look at rodent cells

Tags: NIEHS, data sharing

In 2025, NTP published a groundbreaking online resource called the Rodent Ultrastructure Atlas. This digital tool provides detailed electron microscope (ultrastructural) images of subcellular structures in rodent tissues, offering researchers an essential reference for identifying changes caused by diseases or chemical exposures. Understanding "normal"

cellular and tissue ultrastructure is critical for diagnosing abnormalities, and the Atlas serves as a baseline for comparison.

While light microscopy is commonly used for tissue evaluation, it cannot reveal structures within cells. In contrast, electron microscopy reveals subcellular structures, allowing researchers to pinpoint how a disease or chemical affects specific parts of cells. By providing reference photos, the Atlas can help scientists determine whether a compound has reached its cellular target or disrupted tissue and organ function.

This first-of-its-kind resource is freely accessible to researchers worldwide through the NTP website. Users can explore content by organ systems, such as endocrine, immune, or respiratory, and zoom in for detailed views of ultrastructural components. Each chapter combines overview images with detailed, high-resolution, ultrastructure images, making it easy for scientists to study tissues and identify cell components.

By providing researchers with a comprehensive library of high-quality images of healthy tissues, the Atlas reduces the need to generate new comparison samples from additional animals. Researchers can evaluate whether observed changes are meaningful by referencing existing images, helping minimize reliance on new in vivo studies. Although this Atlas currently focuses on normal ultrastructure, it will continuously expand to include examples of abnormal subcellular changes linked to toxicity and diseases such as cancer. Through collaboration with toxicologic pathologists globally, the NTP team will continue to expand and improve this resource, advancing research in toxicology and pathology.

DNT-DIVER: Integration and visualization of developmental neurotoxicity assay data

Tags: NIEHS, data sharing, developmental neurotoxicity

Increases in the incidence of neurodevelopmental disorders, such as attention deficit/hyperactivity disorder, dyslexia, and autism spectrum disorder, have prompted scientific interest in the potential contribution of environmental toxicants to the development of these disorders. To support these investigations, the NIEHS Division of Translational Toxicology launched the Developmental NeuroToxicity Data Integration and Visualization Enabling Resource (DNT-DIVER). DNT-DIVER allows users to analyze, compare, and visualize multiple developmental neurotoxicity (DNT) assays in an interactive web-based application. The application provides public access to and visualization of all data from

compounds screened by the division's Developmental Neurotoxicity Health Effects Innovation Program. The current version (v. 2.2) includes different tabs for experimental design summary, quality control, chemical-specific concentration–response curves, ranking of chemical toxicity per laboratory/assay, and comparison of results across assays. DNT-DIVER will also accept data from novel screening assays as they become available and are accepted by the international community of researchers investigating DNT.

Transfer and generation of new data from the developmental neurotoxicity in vitro battery

Tags: EPA, FDA, developmental neurotoxicity

In recent decades, the prevalence rates of neurodevelopmental disorders have increased. While improved diagnosis plays a role, environmental factors, such as chemical exposure during pregnancy, may also contribute. Despite this concern, data regarding DNT potential of most chemicals remain limited, and DNT testing is not typically required during chemical registration or reregistration. Current DNT testing methods rely on animal studies such as those described in Test Guideline (TG) 426 issued by the Organisation for Economic Co-operation and Development (OECD). Such studies are expensive, time-consuming, and ethically challenging, and they provide limited insight into how these substances affect human brain development.

To address these challenges, international researchers have developed in vitro NAMs to evaluate DNT more efficiently. This effort led to the creation of the DNT in vitro battery, a set of lab-based tests designed to assess key brain developmental processes without using live animals. OECD has published initial recommendations on how to use DNT in vitro battery data; however, for these methods to become officially recognized as OECD guidance, they must undergo testing in additional laboratories worldwide.

The FDA Human Foods Program has begun in-house validation of some DNT in vitro battery assays developed by organizations such as EPA, in order that they can be run within the Human Foods Program with the same reliable results. However, this transfer process faces challenges. For example, many required cell models are not commercially available. Additionally, there is no agreed list of "performance compounds" to reliably evaluate performance across laboratories. Successfully addressing these challenges will improve DNT testing worldwide, promoting consistency, reducing animal use, and advancing understanding

of how chemicals may affect brain development, a critical step in protecting public health, especially for children.

Perspectives on variability and reproducibility of in vivo toxicology studies

Tags: EPA, NIEHS, acute toxicity, developmental toxicity, eye irritation, skin sensitization

EPA and NICEATM researchers (Karmaus et al. 2026) reviewed the consistency of repeated traditional animal toxicity studies used in safety assessments. They examined published analyses covering various safety tests including eye and skin irritation, acute systemic toxicity, cancer studies, and developmental toxicity. The results showed that replicability, getting the same result when repeating a study, varied widely depending on the type of test and how the results are interpreted. For instance, a study could report a dose that caused toxicity, or it could be used in a categorical classification format with varying levels of concern. One recurring finding was that extreme results (finding that the chemical was either highly toxic or nontoxic) were more consistent than moderate effects. Evaluating replicability was difficult because studies are not frequently repeated for the same chemical, and the approach used in a subsequent study is not always the same as the approach used in the first. This variability comes from multiple sources, including differences in animal lines, experimental procedures, natural biological variation, and analysis methods. These findings are important because regulatory agencies are increasingly accepting new test methods that do not use animals, but these alternatives are often required to perform "as well as or better than" traditional animal test methods. Understanding that animal studies themselves have significant variability helps set realistic performance standards for new methods and suggests that some alternative approaches may be more reliable than the animal tests they are meant to replace.

Projects to extract and annotate legacy developmental toxicity study data

Tags: EPA, NIEHS, developmental toxicity, harmonization, metrics

Reviewing in vivo toxicity studies to assess the risk chemical exposures pose to human health is an important process. A foundational part of this process involves extracting and organizing information into a structured format. However, the scientific language used across studies may vary widely, requiring substantial manual work to standardize descriptions before the data can be analyzed effectively. To address this, EPA and NIEHS scientists

developed and applied a semiautomated workflow to map pre-existing controlled vocabularies (standardized systems of terminology) to ensure consistency in how toxicological effects are described.

In this study, the team reviewed over 40,000 toxicological data points extracted from prenatal developmental toxicity studies conducted by NTP and the European Chemicals Agency. They used a harmonized controlled vocabulary system that combined terms from three sources: Unified Medical Language System codes as implemented in the EPA Toxicity Reference Database; German Federal Institute for Risk Assessment DevTox harmonized terms; and endpoint vocabularies used by OECD. Leveraging a programmatic approach, standardized terms were successfully matched to 75% of the NTP data and 57% of the European Chemicals Agency data. Approximately half of the standardized matches required manual review to ensure accuracy. Data points that could not be matched automatically were often too general or required human judgment to interpret.

This semiautomated workflow saved over 350 hours of manual work and created useful resources, including reproducible code for implementing the mapping workflow and a comprehensive, accessible dataset of harmonized vocabulary for developmental and reproductive toxicity. This crosswalk effort can be readily adapted to other study types. The results demonstrate how automation can advance the organization of toxicological data by facilitating semantic interoperability, an essential prerequisite for integrating legacy datasets to support analyses and decision-making.

Open-access resource identifying molecules with androgenic activity

Tags: FDA, data sharing, endocrine disruptors, harmonization

The androgen receptor system is a critical component of the endocrine system that regulates male characteristics and reproductive health. Endocrine-disrupting chemicals can interfere with this receptor during critical developmental periods, potentially causing reproductive disorders, impaired fertility, and altered sexual development. FDA NCTR has developed the Molecules with Androgenic Activity Resource, the first comprehensive open-access database for assessing chemicals that may disrupt the androgen receptor (Dong et al. 2024).

The research team collected and carefully organized 125,519 data points on androgenic activity for 13,648 different chemicals from multiple public databases and scientific

literature. These data were produced by 923 different laboratory tests, including binding assays, cell-based tests, and animal studies.

The Molecules with Androgenic Activity Resource addresses a major challenge in chemical safety assessment; while extensive test data exist, the data are scattered across different sources, reported in various formats, and difficult to access and compare. This resource addresses this problem by providing a centralized, user-friendly web platform where researchers, regulators, and industry professionals can easily search for chemicals and find comprehensive information about their potential to disrupt androgen receptors. The database includes detailed information about each chemical's structure, test results, and the methods used to generate the data. By making this information freely available and easy to use, the Molecules with Androgenic Activity Resource enables better risk assessment of chemicals and supports the development of predictive models to identify potentially harmful substances before expensive animal testing is required.

Compilation and curation of an acute inhalation toxicity dataset

Tags: NIEHS, acute toxicity, inhalation toxicity, QSAR

Animal tests have traditionally been used to identify 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. These approaches have the potential to identify toxicants and elucidate toxicity mechanisms in a manner that is more relevant to human biology than animal models. However, developing and evaluating these NAMs requires robust, well-curated, and chemically diverse reference data.

Using data from open-access sources, NICEATM compiled a database of in vivo rat acute inhalation data for approximately 1,200 unique substances. 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 chemical set was found to represent a diversity of physicochemical properties and functional use categories represented in the EPA Chemical and Products Database. EPA hazard categories (e.g., nontoxic, toxic, highly toxic) were assigned based on LC50 values. While all hazard categories are represented in the database, about two-thirds of the chemicals were classified as Category IV (least

toxic/nontoxic). The final curated database contains 2,565 entries for 1,209 unique substances. Of these, 1,025 unique chemicals (2,109 entries) have chemical structures standardized for quantitative structure–activity relationship (QSAR) modeling.
A variability analysis was conducted on the 231 chemicals with two or more LC50 values by calculating mean absolute deviation, a measure of variability based on the absolute distance between each data value and the mean of the dataset. Principal component analysis was then used to evaluate any associations with inhalation LC50 variability, but no associations were found between LC50 values and either physicochemical properties or functional use categories. Conditional probabilities were also calculated for these chemicals to determine the probability that a chemical would be assigned a specific EPA hazard category given that it was previously assigned the same or another category on the basis of an LC50 from another study. This analysis showed Category IV categorization to be most consistent and Category III (mildly toxic) to be least consistent. The database can be downloaded from NICE, and a paper describing the database is in preparation.

Categorization of PFAS for tiered toxicological testing and hazard assessment

Tags: EPA, harmonization, PFAS, structure similarity

PFAS are manufactured chemicals known for their widespread use and environmental concerns, such as persistence, bioaccumulation, and toxicity. While some PFAS have been thoroughly studied, most lack detailed hazard profiles. To address this, EPA researchers developed a systematic categorization process to organize PFAS based on their chemical structures, enabling better prioritization for future research and risk assessment (Patlewicz et al. 2025).

Using the official PFAS definition outlined under the Toxic Substances Control Act, the study initially identified 13,054 PFAS from the EPA Distributed Structure-Searchable Toxicity database. For the 563 PFAS listed in the nonconfidential Toxic Substances Control Act inventory, plausible chemical degradation products were simulated, adding 2,484 degradants to the list. Altogether, the researchers compiled a comprehensive dataset of 15,538 PFAS.

Each PFAS was assigned to primary categories based on OECD structure-based classifications and further divided into secondary categories by chain length (shorter or

longer than seven carbons). These secondary groups were refined using chemical fingerprints, ensuring a balance between category size and structural similarity, resulting in 128 terminal structural categories. These categories were used to identify representative chemicals for data collection based on toxicity data gaps, environmental monitoring presence, and links to manufacturers/importers.

This innovative approach provides an organized framework for selecting PFAS for further toxicity testing. It has the potential to support hazard assessment, read-across methodologies, and the development of predictive tools such as QSAR models. These efforts will help address the lack of data for the majority of PFAS and improve understanding of their environmental and human health risks.

DILIrank database ranks liver injury risk of FDA-approved drugs

Tags: FDA, data sharing, hepatotoxicity

Drug-induced liver injury (DILI) is a serious safety concern that can lead to drug failure and patient harm. The FDA NCTR DILIrank database supports the development of NAMs to predict whether an investigational drug might cause DILI. The original database from 2009 contained information on approximately 1,000 drugs; DILIrank v. 2.0 (Olubamiwa et al. 2024) expands the database to 1,336 drugs approved through 2021, representing a 29% increase.

DILIrank categorizes drugs into four groups based on their liver injury risk: Most-DILI-Concern, Less-DILI-Concern, No-DILI-Concern, and Ambiguous-DILI-Concern. As part of the database update, researchers reviewed recent medical case reports and FDA drug labels, finding that some drugs previously considered safe now have associated documented liver injury cases. Interestingly, the analysis revealed that after the FDA issued specific liver safety guidance in 2009, newly approved drugs showed significantly improved safety profiles, with 39% classified as No-DILI-Concern compared to lower percentages in earlier periods. Cancer drugs (antineoplastic agents) continue to show the highest liver injury risk. The database also showed that drugs approved through expedited FDA programs in place between 1992 and 2008 had higher liver injury rates; however, many of these drugs were for treatment of life-threatening conditions, so higher liver injury rates were considered

acceptable. This comprehensive resource will help researchers develop better models to predict liver toxicity and support safer drug development in the future.

Generation of a drug-induced renal injury list to support development of NAMs for nephrotoxicity

Tags: FDA, data sharing

Drug-induced kidney injury affects more than 1.5 million people in the U.S. each year, but current methods for detecting kidney damage often fail to identify problems until significant kidney function has already been lost. To help improve drug safety testing, FDA NCTR researchers (Conor et al. 2024) created the Drug-Induced Renal Injury List, a comprehensive list of drugs with associated kidney damage risk levels. The research team compiled information from the scientific literature and FDA drug labels to create the list, which includes 317 orally administered drugs spanning all major drug categories. Of these drugs, 171 have potential to cause kidney injury, while 146 do not. This carefully curated list provides researchers with a valuable reference tool for developing NAMs that can potentially detect kidney toxicity earlier in the drug development process without relying solely on traditional animal models. Specifically, this list can be used to train and validate new predictive models, including computer-based approaches and advanced cell-based systems.

Mapping of Tox21/ToxCast assays onto characteristics of cancer

Tags: EPA, NIEHS, carcinogenicity, harmonization, Tox21

Key characteristics of carcinogens (KCCs) provide a foundation for organizing data on cancer mechanisms, with the potential to better characterize cancer hazard. To apply this framework to HTS assay data, a workgroup mapped assay data from EPA ToxCast invitrodb v. 4.2 to KCCs (Cuomo et al. 2026). The workgroup was made up of experts with diverse subject matter knowledge from NIEHS; EPA; California EPA; University of California, Berkeley; Texas A&M University; and the International Agency for Research on Cancer. Previous KCC assay mappings using the International Agency for Research on Cancer and NICE databases served as the starting point for this work. Reviewers developed a harmonized mapping approach to relate different assay technologies and endpoints to carcinogenicity. The workgroup's approach was to (1) clarify the contexts of the defined KCCs, considering directionality and biological coverage of the in vitro HTS assay space; (2)

develop a tiered approach to mapping assays to integrate key considerations about the direct relevance of assay platforms to carcinogenesis; and (3) map relevant assays to one or more KCC, each with a detailed documented rationale for the attribution. Of the 1,570 assay endpoints available in the database, 838 were identified as mappable to at least one KCC. For each mapped KCC, assays were assigned as either "Tier A", assays measuring a direct effect on the biology defined by a KCC or "Tier B", assays measuring an indirect or downstream effect on the biological process underlying a KCC. This comprehensive mapping, validated through thorough manual review and documentation by a panel of experts, enhances the utility of HTS data for integration into carcinogenicity evaluation and ultimately contributes to a deeper understanding of the complex mechanisms underlying chemical-induced cancer. This effort will be incorporated into NICE and the International Agency for Research on Cancer Monographs for use in weight-of-evidence analyses and other integrative approaches for the identification of cancer hazards.

Cancer Complexity Knowledge Portal

Tags: NCI, carcinogenicity, data sharing

The Cancer Complexity Knowledge Portal is a free, open-access platform funded by the NCI's Division of Cancer Biology and maintained by Sage Bionetworks through the Multi-Consortia Coordinating Center. NCI developed and is responsible for keeping the resource current. The portal serves as a central discovery hub for data, computational tools, publications, grants, and researcher profiles generated by several NCI-funded interdisciplinary research consortia. The portal is designed for a broad audience including cancer researchers, trainees, patient advocates, and funders. Visitors can search across all resource types simultaneously or browse by consortium, research theme, or cancer type. Practical uses include finding datasets related to a specific tumor type or experimental approach, identifying computational tools for data analysis, locating publications linked to a dataset of interest, discovering researchers working on related problems at other institutions, and accessing educational materials and tutorials.

Recent news releases from the portal illustrate the type of content being regularly added. The portal expanded its consortium coverage in 2024 and 2025 to formally include the Cellular Cancer Biology Imaging Research program, the Metastasis Research Network, the Cancer

Tissue Engineering Collaborative, and the Patient-Derived Models of Cancer program as contributors, alongside the original Cancer Systems Biology Consortium and Physical Sciences in Oncology Network. This expansion substantially broadened the scope of available datasets and tools. The portal's news feed publishes rolling updates on newly deposited items, including new datasets (for example, single-cell RNA sequencing data characterizing tumor microenvironments in metastatic breast cancer and melanoma drug-resistance studies) and new analytical tools (such as spatial transcriptomics software integrating tissue imaging with gene expression data).

Semiautomated extraction of literature data using machine learning methods

Tags: DOE, NIEHS, data sharing, machine learning

Accessing and organizing information from an increasing number of scientific studies poses a major challenge for researchers, especially in fields such as toxicology that rely on structured data from a variety of studies. Manual data extraction from scientific publications is time-consuming and labor-intensive. To address this issue, DOE and NIEHS researchers developed an automated workflow that uses large language models within the KNIME platform to extract structured data from scientific papers and general PDF files (Moreira-Filho et al. 2025). This workflow combines advanced document processing tools with large language models to extract variables from publications. The workflow has two modes: text mode, which extracts structured text and tables from sources such as peer-reviewed journal articles, and image mode, which processes more complex layouts such as nonstandardized reports or graphical content. The workflow achieved 81.14% accuracy in extracting data from scientific papers using text mode and up to 98.54% accuracy in handling general PDF files using image mode. The free and open-source KNIME data analytics platform, designed with user-friendly features, makes these cutting-edge tools accessible to researchers without specialized expertise. By automating data extraction, this approach saves time and effort while ensuring critical toxicological data are organized and ready for use in chemical assessments, study evaluations, and validation of NAMs. The success of this workflow demonstrates how advanced technologies such as large language models can revolutionize biomedical research by making data retrieval faster and more efficient. By

democratizing access to these tools, the platform empowers researchers to synthesize knowledge more effectively, driving progress in toxicology and other fields.

Translating nanoEHS data using the EPA NaKnowBase and the Resource Description Framework

Tags: EPA, data sharing, harmonization

Nano environmental health and safety (nanoEHS) is the study of environmental and health effects of nanomaterials. Multiple U.S. federal agencies have generated vast amounts of nanoEHS data, but sharing and organizing this information has been challenging for researchers, regulators, and partners due to differences in terminology and formats among datasets. To address this, EPA has developed two tools. The NaKnowBase is a curated, relational database that stores experimental results generated by the EPA Office of Research and Development from 2012 to 2019 on how nanomaterials interact with biological and environmental systems (Mortensen et al. 2024a; Boyes et al. 2022). The EPA OntoSearcher tool semiautomates the semantic mapping process. Additional training materials for these tools are available on the NaKnowBase tools webpage.

A federal nanoEHS consortium (Mortensen et al. 2024b), formed through collaboration with groups such as the National Nanotechnology Initiative's Nanotechnology Environmental Health Implications Working Group and the Database and Informatics Interest Group, aims to establish a "common language" for nanoEHS data. This aligns data with data findability, accessibility, interoperability, and reusability (FAIR) standards to improve their usability while also ensuring compatibility with European and global partners. EPA has incorporated semantic integration into the NaKnowBase, enabling data entries to be processed into standardized formats such as Research Description Frameworks (Mortensen et al. 2022) for better cross-dataset interoperability.

EPA also developed the OntoSearcher tool and corresponding training materials to assist federal partners with semantic mapping of agency-specific, siloed nanoEHS datasets (Mortensen et al. 2025). This reduces the manual effort required to connect nanoEHS data with ontologies (structured vocabularies), saving time and improving accuracy. These advancements support the development of computational tools designed to enhance data accessibility and integration. Through these efforts, EPA is promoting FAIR data standards

and creating tools that improve the organization, sharing, and accessibility of nanoEHS data. These developments set the stage for more efficient collaboration, better risk assessments, and expanded research opportunities in nanomaterial safety. U.S. federal partners have increased interoperability across publicly available but siloed datasets, making it possible to explore the utility of machine learning and super intelligence approaches to improve nanoEHS data content (Mortensen, Singh, et al. submitted).

Formatting ToxCast and NICE cHTS data into OECD reporting templates

Tags: EPA, NIEHS, harmonization

OECD has developed harmonized templates for standardized reporting of chemical information including potential human health and environmental effects. OECD Harmonised Template (OHT) 201: Intermediate Effects aims to capture mechanistic information from chemical test results generated by NAMs, such as in vitro testing or in silico methods. NICEATM developed a workflow for curating HTS assay data from EPA's invitrodb (v. 4.2) that included an annotation scheme to provide biological context to cHTS data and enable toxicological interpretation. The scheme incorporates annotations from the OBO Foundry, a harmonized and interoperable database consisting of multiple knowledge areas to encompass a broader range of biologic and toxicologic processes. These annotations are available through NICE. Assay description documents based on OECD Guidance Document 211, which outlines ways to harmonize nonguideline in vitro methods, have been made available for ToxCast assay endpoints through invitrodb. These documents describe the details of each assay technology, performance metrics, and data analysis procedures, which align with information requested for OHT 201 fields.

Ongoing annotation efforts by NICEATM, EPA, and the European Joint Research Centre include:

- Identification of HTS-relevant OHT 201 fields
- Alignment of OBO Foundry annotations and mapping information from NICE and invitrodb to OHT 201 fields
- Development of automation workflows to upload information to the International Uniform Chemical Information Database

Refinement of drug names in RxNorm to improve efficiency of drug identification

Tags: FDA, data sharing, harmonization

RxNorm is a widely used standardized drug naming system containing over 1.1 million drug names from 14 different sources. However, within this system, the same drug can be referred to by many different names (brand names, generic names, abbreviations), making it difficult for computer programs to accurately tally drug mentions and associated side effects. To address this problem, researchers at FDA NCTR developed an improved database of drug names to better identify adverse drug events from unstructured text sources such as medical literature and reports (Guo et al. 2025). Using RxNorm data, researchers systematically removed problematic entries including duplicates, extremely long names (over 200 characters), very short names that rarely appear in real-world documents, and names that would cause the same drug to be counted multiple times. The refined database contains approximately 102,000 drug names. When tested on 18,000 drug-related scientific abstracts from PubMed, the refined database identified drugs three times faster than the original RxNorm database while also improving accuracy by eliminating over 1,400 false or redundant drug name matches. The test found that most problematic drug names came from single sources rather than being validated across multiple databases, highlighting the importance of cross-validation. This refined drug name database provides a valuable tool for monitoring medication safety through automated analysis of medical literature, patient reports, and other unstructured healthcare data.

Computational Tools Development

Computationally generated predictions of toxicity endpoints can inform decisions about testing priorities and potentially 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).

Updates to CompTox Chemicals Dashboard

Tags: EPA

The CompTox Chemicals Dashboard is the primary web-based application for access to data and algorithms from the EPA Center for Computational Toxicology and Exposure. The Dashboard is a widely used resource for chemistry, toxicity, and exposure information for over a million chemicals. There were eight updates to the Dashboard released in 2024 and 2025, increasing the number of chemicals to 1,246,399 with all related data. These related data include mass spectrometry-ready/quantitative structure–activity relationship (QSAR)-ready structures, linked substances, and property predictions generated by the Open (Quantitative) Structure–activity/property Relationship App (OPERA) and EPA Toxicity Estimation Software Suite tools. The EPA Toxicity Forecaster (ToxCast) invitrodb v. 4.2 dataset was released in September 2024, and an updated version of the Chemical and Products Database was released via the Dashboard in June 2025 (Handa et al. 2025). The next Dashboard update is planned for August 2026.

A June 2025 virtual training on the Dashboard gave an overview of Dashboard functions along with its risk evaluation components, navigation, and a case study. Additional training resources for the Dashboard are available on the EPA NAMs Training webpage.

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.

There were four updates to SeqAPASS in 2024 and 2025, the most recent being the release of SeqAPASS v. 9.0 in September 2025. Improvements to SeqAPASS in these releases included:

- Protein structure generation and alignment capabilities to support detailed analyses of protein structural biology
- Incorporation of data from the World Register of Marine Species database to allow filtering of marine species
- Addition of a Focus/Filter toggle to customize results displays to either highlight optional selections from a full list of species or filter results to only display species that match the optional selection

- Data and executable updates

Training on new SeqAPASS capabilities was provided at an EPA virtual training session in May 2025. SeqAPASS v. 9.1 is scheduled for release in early 2026 and will include additional data and executable updates; v. 10.0 will be released later in 2026.

Updates to the Web-ICE application

Tags: EPA, acute toxicity, ecotoxicity

Ecological risk assessments aim to protect diverse ecological communities from chemical stressors through the evaluation of available species sensitivity data. However, toxicity data are typically available for only some standard test species, with limited or no data available for most species and taxa. Web-based Interspecies Correlation Estimation (Web-ICE), a web application developed by EPA, uses available data from appropriate surrogate species to predict acute toxicity for other species of interest lacking toxicity data. It can help address data gaps in species sensitivity and reduce reliance on uncertainty factors in ecological risk assessment.

Web-ICE v. 4.0 was released in May 2024. This release has over 4,700 models representing 478 aquatic animal species, over 100 models representing 69 algal species, and over 850 models for 156 terrestrial bird and mammal species. A new feature of Web-ICE v. 4.0 is the Bulk ICE module, which generates predictions for all available taxa for each user-entered surrogate, maximizing taxonomic diversity in the resulting model output. Slides from a December 2024 webinar describing features of Web-ICE v. 4.0 are available on the EPA website, and a video is available on YouTube.

NICE tools updates

Tags: NIEHS, exposure, IVIVE, structure similarity, Tox21

The NIH Integrated Chemical Environment (NICE), developed by NICEATM, provides data and tools to help develop, assess, and interpret chemical safety tests. All tool inputs now provide a “filter-as-you-type” function to help users find Chemical Quick Lists of interest. NICE tool help videos were updated in the September 2024 NICE v. 4.1 release and the February 2025 NICE v. 4.1.1 release. The updates also implemented improvements to many specific NICE tools.

- Major updates to **Search** result data visualizations in the March 2024 v. 4.0.2 release added immersive visualizations and functional use pagination; this release also updated filter chain options for Search results.
- The March 2024 update of the **In Vitro to In Vivo Extrapolation (IVIVE)** tool added new options for data overlays to contextualize model results. The July 2025 NICE v. 4.2 release also enabled users to provide their own physicochemical and absorption, distribution, metabolism, and excretion (ADME) data to **Physiologically Based Pharmacokinetics** (**PBPK**) and **IVIVE** tool queries.
- The July 2025 release revised the principal component analysis plots in the **Chemical Characterization** tool.
- Multiple improvements were made to the **Curve Surfer** tool, which allows users to view and interact with concentration–response curves from curated high-throughput screening (HTS) data. The tool now allows users to overlay the concentration that causes a half-maximal activity concentration (AC50) or the activity concentration at cutoff (ACC) values and includes technological interference flags and points-of-departure in results. Curve Surfer data are also now available via the NICE REST application programming interface (API).

CoMPAIT: Collaborative Modeling Project for Acute Inhalation Toxicity

Tags: NIEHS, acute toxicity, data sharing, inhalation toxicity, machine learning

NICEATM is working toward replacing traditional animal models with computational models to predict inhalation toxicity (LC50 values). The LC50 value represents the concentration of a substance that causes harm or death in half of a test population after inhalation and is an important measure used in chemical hazard assessment. NICEATM is developing a model to predict LC50 values that addresses an action item from a 2016 workshop focused on finding humane and efficient alternatives to animal tests for inhalation toxicity (Clippinger et al. 2018).

To support the project, NICEATM curated a dataset of LC50 values for roughly 760 chemicals to serve as the foundation for the development of predictive models. Unlike simpler endpoints, inhalation toxicity presents unique challenges due to complex data and regulatory requirements, making this initiative particularly innovative.

In mid-2024, collaboration with an international consortium of 35 participating groups began, with the goal of creating tailored computational tools specifically designed to predict inhalation toxicity. These models, combined into a consensus model and implemented in the free and open-source suite OPERA, will focus on meeting regulatory standards by classifying chemicals into hazard categories for systemic toxicity assessments. The collaborative process involves refining the methodologies and building consensus among researchers and regulators to ensure the tools are both scientifically robust and broadly applicable. The consensus model has been shared with project contributors. In 2026, developers will implement the consensus model in OPERA, making it publicly available. A paper on the modeling project will be submitted to a peer-reviewed journal.

This project represents a major step forward in toxicology, offering not only more humane testing strategies that eliminate reliance on animal studies but also efficient approaches to evaluating chemical safety. These computational models will help align with modern regulatory frameworks while advancing the science behind inhalation toxicity assessments.

STopTox: A computational tool for chemical hazard assessment

Tags: NIEHS, acute toxicity, eye irritation, inhalation toxicity, QSAR, skin sensitization

Reducing, refining, and replacing the use of animals (applying 3Rs principles) is a long-standing goal in chemical toxicology. To support this effort, NICEATM and collaborators developed STopTox (Systemic and Topical Chemical Toxicity), an in silico platform that uses advanced computer models to predict chemical toxicity without the need for animal studies (Borba et al. 2022). STopTox uses QSAR models, which analyze chemical structures to estimate toxicity. Designed as a user-friendly, accessible tool, STopTox provides a comprehensive alternative for identifying hazards.

During its initial development, STopTox was rigorously tested using large publicly available datasets, ensuring it met international standards, including principles for QSAR model development adopted by the Organisation for Economic Co-operation and Development (OECD). The models demonstrated high internal accuracy and strong external predictive capability. To further build confidence in STopTox as a substitute for animal testing, researchers conducted external validation tests using independent datasets. They assessed the ability of STopTox to predict toxicity across six major categories: acute oral, dermal, and

inhalation toxicity, as well as skin sensitization, skin irritation/corrosion, and eye irritation/corrosion. The validation results were highly encouraging, as STopTox performed reliably across all endpoints (Scheufen Tieghi et al. 2025). This supports the platform's potential as a dependable tool for regulatory toxicology decision-making. By offering accurate predictions and reducing the reliance on animal studies, STopTox represents an important step forward in advancing humane and efficient approaches to hazard identification in chemical testing. It highlights the promise of computational methods in revolutionizing toxicological assessments and contributing to animal replacement efforts.

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

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

There is a need for reliable and easy-to-use navigation tools capable of browsing, mining, and utilizing large and diverse repositories of chemical and biological data. To address this need, NIEHS in collaboration with Sciome, LLC, developed OrbiTox. OrbiTox uniquely offers an interactive and immersive 3D environment for the visualization of millions of chemical data entries, including both experimental and predicted biological activities toward various gene targets tested in vitro and/or in vivo. By organizing those data entries in concentric "orbits" (*e.g.,* chemicals, genes, pathways, species), OrbiTox facilitates translational discovery by inferring knowledge from connections across multiple data domains and sources (Ross et al. 2025). Through an intuitive graphical user interface, OrbiTox offers rapid and interactive navigation in the chemical space along with extensive gap-filling capabilities. Currently, OrbiTox employs over 150 predictive and cross-validated QSAR models, including individual models for Tox21 assays, bacterial mutagenicity, cardiotoxicity failure modes, and carcinogenicity classification and potency. These models use Saagar molecular fingerprints (Sedykh et al. 2021), which provide interpretable, chemistry-backed reasoning for each prediction with state-of-the-art atom contributions. As a result, users can hypothesize on the substructural origins and/or mechanistic steps highlighting why a given chemical exhibits a particular activity. Such identification of favorable versus unfavorable chemotypes for a given biological property can be of critical importance for prioritizing experimental testing. OrbiTox also provides rich, structurally

relevant analogs for read-across assessments, thanks to Saagar-based structural similarity and its vast database of bioactivity and metabolic profiles. Finally, OrbiTox provides users with opportunities to directly compare animal versus nonanimal data, which is important for building confidence in and establishing the relevance of new approach methodologies (NAMs), ultimately supporting the transition to human-based safety testing. The fall 2026 release will include an extended set of new QSAR models and advanced chemical filtering features to further refine chemical search results and predictions.

HPPT App: Web application to classify and subcategorize skin sensitizers using human data

Tags: NIEHS, skin sensitization

NICEATM and collaborators at the German Federal Institute for Risk Assessment developed an approach that incorporates data from human predictive patch tests (HPPTs) to consider skin sensitization potency in assigning chemicals to subcategories of United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) hazard classification (Herzler et al. 2024). This approach to potency classification is potentially more human-relevant than the current classification approach.

While the modified classification approach is useful, it can be challenging to understand and implement. To address this barrier, NICEATM developed the HPPT App, which provides an interactive platform for users to input HPPT data, visualize results, and understand classification outputs. The app is publicly available on the NIH website with no registration required.

Following the approach described by Herzler et al., the HPPT App uses data on both the number of sensitized human test subjects and the dose required to sensitize a single human test subject. If the data do not support a definitive classification, the HPPT App provides ambiguous classifications that indicate that there is either no evidence for the chemical being a strong sensitizer or that the data provided are insufficient to support any classification. The HPPT App includes a weight-of-evidence method to consolidate multiple HPPT results into a single GHS classification for each chemical. It flags questionable input data, and uploaded query data are not stored after the user ends their session. The app also provides a user guide and background information.

DASS App for skin sensitization prediction using defined approaches

Tags: NIEHS, defined approaches/IATA, skin sensitization

Regulatory agencies require that chemical products be evaluated for their potential to cause skin sensitization. Traditionally, such evaluations involved animal models, but more recently, nonanimal methods have been developed. Defined approaches for skin sensitization (DASS) use standardized interpretation procedures to combine data from multiple nonanimal tests, such as human cell-based experiments, molecular studies, and computational models, to determine if a chemical has potential to cause allergic dermatitis.

While regulatory agencies accept several DASS methods, applying these approaches manually can be complex, slow, and prone to errors. To address this, NICEATM researchers created the DASS App (To et al. 2024), a free, open-source web application that simplifies the use of three regulatory testing strategies: Two-out-of-Three, Integrated Testing Strategy, and Key Event 3/1 Sequential Testing Strategy. This web tool, first launched in 2023, allows users to upload their own data, identify inconsistencies, and generate predictions without needing advanced computational skills or special software installation. DASS App provides a data template and the option to select the assays from which the data are taken. Reference data can be imported from NICE. Outputs are provided in a user-friendly, downloadable format as tables and interactive graphics. By making the application freely accessible online, the developers ensure the tool is available for wide adoption and future improvements. This innovation supports faster, more reliable hazard assessments while advancing the goal of replacing the use of animals with humane and efficient alternatives.

Updates in 2024 and 2025 made the app more user-friendly, including simplified handling of borderline calls, as well as adding new test method inputs to cover similar updates to OECD Test Guideline (TG) 497, which contains the Two-out-of-Three and Integrated Testing Strategy DAs. DASS App now allows the user to evaluate performance using their own reference data or data from NICE chemical reference lists.

SARA-ICE model for identification of skin sensitizers

Tags: NIEHS, defined approaches/IATA, skin sensitization

Assessing the potential of chemicals to cause skin sensitization (allergic reaction) is essential for protecting public health. While nonanimal methods have been developed, single tests

alone are not sufficient to fully determine a chemical's sensitization risk or potency. DAs such as those described in OECD TG 497 (OECD 2025) combine multiple data sources for more reliable evaluations. However, existing DAs lack the ability to calculate a specific dose threshold, known as a point-of-departure (POD), for use in risk assessments.

The Skin Allergy Risk Assessment–Integrated Chemical Environment (SARA-ICE) DA for skin sensitization (Reinke et al. 2025) was developed by NICEATM and the consumer products company Unilever specifically to address this challenge. SARA-ICE employs statistical modeling to estimate a human-relevant metric called the ED01. The ED01 is the probability distribution for the dermal dosage at which there is a 1% chance of inducing sensitization, making it directly applicable to risk assessment. SARA-ICE integrates data from various in vitro tests as well as data from the local lymph node assay and HPPT, drawing upon historical data from 434 chemicals, and accounts for variability and uncertainty in input data. In addition to the ED01, SARA-ICE provides probabilities for GHS skin sensitization classifications. Specifically, the model calculates the probability that a chemical would be placed into a GHS subcategory: severe sensitizers (1A), moderate sensitizers (1B), or Not Classified (NC). In a case study focused on isothiazolinones, SARA-ICE demonstrated its ability to integrate diverse data inputs and calculate ED01 values for six isothiazolinone compounds that compared favorably with results based on historical datasets (Reinke et al. 2025).

In 2025, OECD revised TG 497 to include a more limited guideline-specific version of the SARA-ICE model, which provides a POD as the geometric mean of the ED01 and does not provide hazard or GHS classification predictions. It also assumes that any chemical evaluated is presumed to be a skin sensitizer due to model restrictions.

To broaden the accessibility of the SARA-ICE model, in 2025 NICEATM developed the SARA-ICE app. The app provides access to the two SARA-ICE models: the "OECD TG 497 DA", which aligns with TG 497 criteria, and the "Extended Model", which allows the user to make a hazard determination and predict GHS subcategorization and alternative "EDx" outputs (e.g. ED05, the dose at which 5% of a population would be sensitized). The Extended Model does not assume a chemical is a skin sensitizer and allows the user to adjust the GHS decision threshold depending on desired stringency. The OECD TG 497 DA is static because

it is based on the guideline; however, the Extended Model could be modified in the future to add in more test methods or outputs. Current outputs include a graphic representation of the uncertainty distribution and a tabular report of the probability distributions for various classifications. Future development may add methods to the Extended Model, in silico reactivity structural alerts, extrapolation of population-level effects, a user input feature, and a downloadable local version.

HuSSPred: A novel machine learning model and web portal for predicting the human skin sensitization effects of chemical agents

Tags: NIEHS, machine learning, skin sensitization, structure similarity

Skin sensitization, or an allergic reaction due to chemical exposure, is a critical focus in chemical safety assessments. Traditional animal tests often fail to accurately predict human responses, and ethical concerns further limit their use. To address this, NICEATM and University of North Carolina researchers developed HuSSPred, a reliable and ethical in silico tool designed using exclusively human data to predict human skin sensitization based on chemical structures. HuSSPred builds on HPPT data and aims to improve regulatory assessments while aligning with the 3Rs principles. The research team compiled an extensive HPPT database and grouped chemicals by their characteristics. They then developed and fine-tuned QSAR models using advanced optimization techniques. These models were rigorously tested using five-fold cross-validation and further validated on external data from the DASS App tool.

HuSSPred demonstrated strong predictive performance, with a correct classification rate ranging from 55%–88%, sensitivity (ability to correctly identify chemicals that would induce skin sensitization) between 48% and 89%, and specificity (ability to correctly identify chemicals that would not induce skin sensitization) between 37% and 92%. The tool's positive predictive value was particularly robust, ranging from 84%–97%, indicating strong reliability in identifying sensitizing chemicals. Validation results showed performance comparable to or better than existing tools, confirming its usefulness for regulatory assessments. HuSSPred is publicly accessible through a user-friendly web interface, providing an effective alternative to traditional methods. Its high accuracy, ethical approach,

and open access make it a valuable resource for predicting skin sensitization, supporting better regulatory decisions and reduced reliance on animal use.

A publication describing HuSSPred (Scheufen Tieghi et al. 2024) won the best paper of the year award from the Society of Toxicology In Vitro and Alternatives Specialty Section and was named an Editor's Choice publication in the journal *MDPI Toxics*.

MoVIZ: Interpretable chemical grouping using an automated KNIME workflow

Tags: NIEHS, eye irritation, machine learning, structure similarity

Analyzing large chemical datasets to identify patterns and categorize chemicals into meaningful groups is essential for advancing chemical safety and developing innovative testing methods. Chemical grouping involves organizing chemicals based on shared characteristics, such as physicochemical properties, biological activity, or intended uses. However, many existing tools for chemical grouping require advanced programming knowledge or expensive commercial software, making them inaccessible to many users.

To address these challenges, NICEATM developed MoVIZ, a user-friendly and automated pipeline of workflows using KNIME, a free and open-source data analytics platform (Moreira-Filho et al. 2024). MoVIZ tools include a chemical grouping workflow that guides users through complex tasks, such as calculating molecular descriptors, grouping chemicals based on structural and biological similarities, and applying machine learning techniques to identify patterns. It thus provides a comprehensive, no-code solution for chemical grouping and visualization. This workflow includes tools for calculating molecular properties, selecting important features, reducing data complexity, and applying machine learning methods (both supervised and unsupervised) to group chemicals effectively. Additionally, the workflow can generate interactive visualizations and natural language summaries to explain why specific groupings were made, making the results clear and interpretable. A case study using a dataset of over 2,200 chemicals tested for eye irritation successfully identified clusters of irritants and nonirritants and highlighted the molecular features driving these effects. To further demonstrate MoVIZ’s capabilities, developers applied the workflow to a dataset focused on eye irritation and corrosion, highlighting its effectiveness in categorizing chemicals and identifying key molecular properties for each group.

Designed for accessibility, MoVIZ workflows can be applied seamlessly on both the KNIME desktop application and the KNIME Server WebPortal, functioning as an interactive web application with guided steps. MoVIZ tools enable a broad range of researchers, regulators, and industry professionals to apply modern computational toxicology methods. This supports more efficient chemical prioritization, safer product design, and reduced reliance on animal models, aligning with global efforts to modernize safety assessment practices. Such innovative workflows empower users without coding expertise to perform advanced chemical analyses, democratizing access to powerful data analysis tools. MoVIZ represents a significant step forward in enabling efficient, transparent, and reproducible chemical grouping, supporting regulatory decision-making and chemical safety assessments.

DeTox: An in silico alternative to animal testing for the prediction of developmental toxicity potential

Tags: NIEHS, developmental toxicity, machine learning, QSAR

Understanding the safety of medications during pregnancy is critical, but many drugs are not adequately studied for their effects on the developing fetus. To help address this gap, NICEATM and University of North Carolina researchers developed advanced QSAR models to predict the likelihood of developmental toxicity from chemicals, including trimester-specific risks. These models aim to provide safer medication options for pregnant women while reducing the need for animal testing and aligning with 3Rs principles. The team collected and curated developmental toxicity data from FDA and Teratogen Information System databases, validating the information with thorough literature reviews. Using machine learning algorithms, they developed QSAR models that predict developmental toxicity with high accuracy: 76% accuracy for overall pregnancy risk and 80%, 95%, and 95% accuracy for risks specific to the first, second, and third trimesters, respectively. Sensitivity ranged from 53%–90%, while specificity varied between 46% and 100%, based on rigorous validation methods. These models have been made accessible through a public web platform called DeTox (Developmental Toxicity; Scheufen Tieghi et al. 2025). This tool supports regulatory evaluations of chemicals and drugs, helping the pharmaceutical and cosmetic industries design safer products for pregnant populations. Additionally, the curated dataset of developmental toxicants is available for public use.

Overall, DeTox represents a significant step forward in improving prenatal safety assessments, reducing reliance on animals, and advancing the development of safer treatments for expectant mothers and their babies.

Updates to ChemMaps

Tags: NIEHS, data sharing, Tox21

Access to visualization tools to navigate chemical space has become increasingly 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 ChemMaps.com to easily navigate chemical space. The first version of ChemMaps.com was limited to exploration of drugs and drug candidates. ChemMaps.com v. 2.0, 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 v. 2.0 incorporated 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 by both assay and target directly on the map and compare chemical spaces occupied by active and inactive chemicals. The latest update of ChemMaps.com (v. 2.1), released in October 2024, refined the chemical preparation pipeline to address previously unsupported chemistry. Chemical predictions were also updated to utilize the latest version of OPERA (v. 2.9).

FDA Expanded Decision Tree for toxic chemical screening in food

Tags: FDA, structure similarity

If not evaluated or used properly, some chemicals in food may have toxic properties that pose risks to human health. FDA helps to safeguard the food supply by evaluating the safe use of chemicals as food ingredients and food contact substances. The FDA also monitors the food supply for chemical contaminants and takes action to limit exposure when the level of a contaminant could cause a food to be unsafe. To do this, FDA uses scientific and regulatory

tools to assess the safety of chemicals in food while continually updating its methods to incorporate new approaches.

In July 2025, FDA released its Expanded Decision Tree chemical toxicity and risk screening tool that advances the agency's commitment to transparency and enhancement of the food chemical safety program. The tool provides a consistent, systematic, and science-based approach to support evaluation of the safety of chemicals in food based on their structure and estimated toxicity. The Expanded Decision Tree was evaluated through external peer review, marking a significant milestone in its development. The update also incorporated input from stakeholders such as EPA, the National Toxicology Program, the European Food Safety Authority, and experts from academia and industry.

FDA encourages interested parties to engage with the agency on the Expanded Decision Tree. Planned opportunities for public engagement include release of an informational video and listening sessions with technical, scientific, and trade groups to learn how the tool may impact their work.

Super intelligence tools to extract adverse event data from social media and drug labeling documents

Tags: FDA, data sharing, machine learning, QSAR

Researchers at the FDA National Center for Toxicological Research (NCTR) have developed advanced super intelligence (SI) tools to enhance adverse event detection from diverse data sources, such as social media posts and FDA drug labeling documents. These projects demonstrated high accuracy in extracting and classifying adverse event data, supporting faster drug safety surveillance and improving pharmacovigilance methods. Together, these tools show potential in streamlining the analysis of both informal real-world data and structured regulatory documents for better patient safety monitoring.

- FDA researchers developed an SI model to detect drug side effects mentioned in social media posts, addressing limitations of traditional reporting systems (Dong et al. 2024). The model performed well in identifying adverse events, achieving high accuracy scores in both testing phases. This approach shows promise for enhancing drug safety surveillance by quickly analyzing real-world patient insights from social media to complement traditional methods.

- FDA NCTR researchers introduced AskFDALabel, a large language model-based framework, to efficiently extract adverse event data from FDA drug labeling documents (Wu et al. 2025). Using a specialized workflow, it achieved high accuracy in identifying drug-induced liver injury (DILI), cardiotoxicity, and adverse events. The results suggest that AskFDALabel can improve the speed and precision of adverse event annotation for drug safety research.

SI tools to screen abstracts for NAMs relevance

Tags: NCI, machine learning

A major challenge in advancing NAMs is efficiently tracking their adoption across the rapidly expanding scientific literature and NIH grant portfolio. To support this need, in 2025 NCI began information-gathering efforts to better define and identify NAMs-relevant research. Building on this foundation, researchers are developing SI-powered tools to screen scientific abstracts and identify studies involving NAMs that can complement, reduce, or replace animal use in biomedical and chemical safety research. The NAM definitions used by the tools are based on publicly available NIH resources and can be shared upon request. Two complementary tools were developed within secure U.S. Department of Health and Human Services enterprise environments, one built on ChatGPT and one on Claude, both accessible exclusively to Health and Human Services staff. Each tool is guided by structured classifier instructions that include NAM definitions, decision criteria, and examples to ensure consistent and reproducible classifications. The tools evaluate titles and abstracts and assign one of three labels, YES, MAYBE, or NO, to indicate whether NAMs were involved in the study. The tools also provide plain-language justifications for the decision. Users can upload text or Excel files and interact with the tools conversationally to further explore classification decisions. The tools also remind users to follow agency-specific data use policies before uploading content. As NAM definitions and NIH policies evolve, the tools can be updated and reshared across the Health and Human Services community without requiring technical expertise from users.

This effort supports ICCVAM priorities by improving the ability to identify and track NAMs-related research, enabling more efficient evidence mapping and portfolio analysis.

Next steps include expanding testing across larger datasets, evaluating performance against expert-reviewed benchmarks, and exploring integration into broader workflows.

QSAR machine learning models to support lung toxicity prediction

Tags: DoW, inhalation toxicity, machine learning, QSAR

Inhalation exposure to toxic chemicals in the operational environment poses a significant risk to military personnel health and readiness. Many chemicals relevant to DoW activities lack acute inhalation exposure limits, necessitating the development of rapid and accurate predictive tools for chemical hazard identification. 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 U.S. Air Force (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 2,393 approved drugs collected in DrugBank and identified 138 potential novel NRF2 activators. PRT scientists also developed novel machine learning models to predict key events linked to acute inhalation toxicity: mitochondrial membrane potential depolarization and inhibition of peroxisome-proliferator-activated receptor gamma. Developed models are published in a 2025 Defense Technical Information Center report (Chushak et al.; not yet available online). These machine learning models offer an accurate, rapid, and cost-effective approach for prioritizing chemicals for further toxicological evaluation, addressing a critical gap in inhalation toxicity assessment for DoW applications. They 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.

Prediction of respiratory irritation and respiratory sensitization using machine learning QSAR models

Tags: DoW, inhalation toxicity, machine learning, structure similarity

Inhalation of toxic substances and contaminants can adversely affect the respiratory tract, leading to health problems such as irritation, inflammation, allergic reaction, asthma, lung damage, and even death. Experimental evaluation of respiratory toxicity for thousands of

existing chemicals is not feasible. USAF PRT generated structural alerts and developed machine learning classification models to predict respiratory irritation and sensitization hazards of chemicals using experimental data from publicly available literature and databases (Chushak et al. 2025). Thirteen structural alerts for respiratory irritants and 18 structural alerts for respiratory sensitizers were identified. Sets of models for irritation and sensitization were developed using different types of molecular descriptors and machine learning techniques. Five of the best performing models were combined into a consensus classification model for respiratory irritation. Four individual models were used to develop a consensus classification model for respiratory sensitization. The prediction accuracy of the respiratory irritation consensus model was 84% for the training set and 88% for the test set. The accuracy of the respiratory sensitization consensus model was 86% for both training and test datasets. A combination of generated structural alerts and machine learning models was used to screen occupational- and military-relevant chemicals. Of 687 screened occupational chemicals, 62 were identified as potential respiratory irritants and 121 as respiratory sensitizers. From a list of 525 military-relevant chemicals, 47 were predicted to be irritants and 36 to be sensitizers.

In vitro to in vivo extrapolation framework to predict in vivo gene expression from in vitro data

Tags: FDA, hepatotoxicity, machine learning

Results from cells grown in laboratory dishes do not always accurately reflect what happens in whole animals. To help address this limitation, FDA NCTR developed “artificial intelligence-aided in vitro to in vivo extrapolation” (AIVIVE), an innovative computer model that uses SI to predict how chemicals affect living animals based on laboratory cell studies, potentially reducing the need for animal models in drug safety evaluations (Chandra et al. 2025). AIVIVE accomplishes this by combining two SI technologies: a generative adversarial network that creates synthetic gene expression profiles mimicking animal data and specialized "local optimizers" that fine-tune biologically important genes often missed by standard SI approaches.

Testing the system on liver toxicity data from 140 different compounds, the researchers found that AIVIVE's synthetic profiles closely resembled real animal data, successfully

capturing important liver metabolism genes and toxicity pathways that are typically lost in cell culture studies. Notably, AIVIVE reproduced expression patterns of cytochrome P450 enzymes, critical liver proteins that break down drugs, even though these enzymes are poorly expressed in laboratory cell cultures. The synthetic data even slightly outperformed real data in predicting liver cell death (necrosis), suggesting AIVIVE could enhance drug safety predictions. This advancement supports the 3Rs principles by providing a scientifically robust alternative for toxicity assessment that could accelerate drug development while improving animal welfare.

Application of knowledge-based large language models for drug-induced liver injury and cardiotoxicity prediction

Tags: FDA, cardiotoxicity, hepatotoxicity, machine learning

For decades, scientists have relied on QSAR models to predict how toxic a chemical might be based solely on its molecular structure. However, this approach has limitations, especially for drugs where small structural changes can cause dramatically different toxic effects. Researchers at the FDA have developed a novel SI framework called quantitative knowledge–activity relationships (QKARs) that significantly improves the prediction of drug toxicity compared to traditional methods (Li et al. 2025). This method leverages advanced SI large language models such as ChatGPT to capture comprehensive knowledge about drugs, including their mechanisms of action, metabolic pathways, and known side effects, and converts this information into numerical representations that machine learning models can use for predictions.

The researchers tested QKARs on two critical drug safety concerns: DILI and drug-induced cardiotoxicity. Across multiple tests, the QKAR models consistently outperformed traditional QSAR models, demonstrating particular strength in distinguishing between structurally similar drugs with different toxicity profiles. For example, the drugs ibuprofen and ibufenac differ by only one methyl group, yet ibuprofen is safe while ibufenac caused severe liver damage and has been withdrawn from the market. This research supports FDA's ongoing efforts to reduce animal use in drug safety studies by providing more accurate computational alternatives that can assess toxicity earlier in drug development.

Machine learning and deep learning approaches for enhanced prediction of hERG blockage

Tags: FDA, cardiotoxicity, machine learning, QSAR

Cardiotoxicity is a major reason drugs are withdrawn from the market. The heart's electrical system depends on a specific ion channel called hERG. Interference with this channel by medications can lead to potentially fatal cardiac events. FDA NCTR researchers developed advanced computer models to predict whether new drug candidates might cause dangerous heart rhythm problems related to hERG blockage (Liu et al. 2024). The researchers trained six individual prediction models and three combined "ensemble" models using a large dataset of chemical compounds, employing both traditional machine learning and newer deep learning techniques. When tested on a subset of training data, the models performed somewhat better than previously developed models on the same dataset. However, when tested on external datasets that the models had never seen before, the new models significantly outperformed earlier models. These improved prediction tools can help pharmaceutical companies and regulatory agencies identify potential heart safety hazards earlier in the drug development process, potentially reducing animal use and preventing dangerous medications from reaching patients while reducing the time and cost of bringing safe drugs to market.

Derivation of an adverse outcome pathway linking vascular endothelial growth factor and cardiotoxicity

Tags: NIEHS, AOP, cardiotoxicity, endocrine disruptors, structure similarity, Tox21

Development of cardiovascular disease often involves factors such as the disruption of vascular endothelial growth factor (VEGF) signaling, an important process in blood vessel health. VEGF and its receptor play a role in the development of atherosclerosis, a major contributor to cardiovascular disease. Understanding how chemicals impact VEGF signaling can help assess their potential cardiotoxicity. NIEHS scientists used HTS data, which provide large-scale information about how chemicals interact with biological systems, to explore links between VEGF disruption and atherosclerosis (Ehrlich et al. 2024).
Researchers analyzed experimental data targeting the VEGF receptor for 4,165 chemicals from the ToxCast, Tox21, and PubChem databases. Using cheminformatics and clustering

approaches, they identified 109 structural fingerprints and 12 primary biological targets associated with VEGF receptor activity. These targets were linked to cardiovascular disease through an SI-assisted literature review, highlighting connections between VEGF signaling and other pathways, such as serotonin receptor, estrogen receptor alpha, and vasopressin receptor targets. This information was then used to construct an adverse outcome pathway, a framework that connects molecular-level events to broader toxic effects, such as atherosclerosis.

The study also identified several common endocrine-disrupting chemicals, such as bisphenols, triclosan, dichlorodiphenyltrichloroethane, and polychlorinated biphenyls, as potential contributors to VEGF-related disruption and cardiotoxicity. By combining computational methods, HTS data analysis, and literature insights, NIEHS researchers propose a data-driven approach to assessing environmental cardiotoxicity. This methodology enhances understanding of how chemicals may contribute to cardiovascular disease, supports regulatory toxicology efforts, and aligns with goals to reduce reliance on animal use.

Novel SI models to predict carcinogenicity

Tags: NIEHS, carcinogenicity, machine learning, Tox21

Carcinogenesis is a multistep process in which normal cells acquire properties that allow them to form benign tumors or malignant cancers. These properties have been associated with 10 well-established hallmarks of cancer. The concept of key characteristics of carcinogens has also 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 about the complex mechanisms involved in carcinogenicity. NIEHS scientists combined a novel QSAR modeling approach with modern machine learning to analyze the data of 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 simulating assay results for the Tox21/ToxCast inventory using structural and physicochemical properties and deep learning. Simulated in vitro assay results were enriched using data in the BioBricks platform, which compiles toxicity-relevant databases into a harmonized easily accessible format. This

enrichment allowed the inclusion of additional information, such as protein target binding or assay results, to the model. Finally, various machine learning approaches, including a multitask deep learning model, were applied to predict the likelihood of each chemical to induce 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.

Development of a multitask machine learning model for diverse toxicity data

Tags: DoW, machine learning, neurotoxicity

Machine learning models can support rapid chemical risk assessment, reducing the need for approaches using animals. In addition to requiring large, homogeneous datasets that are not available for most adverse outcome endpoints, single-task machine learning models train on one endpoint and lack transferability to similar endpoints. USAF PRT is collaborating with the Johns Hopkins University Applied Physics Laboratory to design a novel machine learning pipeline for molecular representation learning based on a multitask paradigm. A machine learning model was trained simultaneously on multiple tasks curated from moderate-sized EPA ToxCast datasets. To predict novel tasks from small datasets, the pretrained multitask model was combined with a task-specific predictor (random forest or a neural network). The resulting machine learning pipeline performed better than the single-task models for most tasks. Molecular representations from the multitask model combine information on multiple effects to provide a model of chemical space that captures generalizable information (Wichrowski et al. 2026). USAF PRT and the Applied Physics Laboratory are also investigating active learning approaches in which model development is integrated with laboratory testing to optimize the machine learning model-building process. A directed message-passing neural network and a specialized loss function were combined to select chemicals that would most improve model performance if tested using a specific in vitro assay. Assay results were used to update the model and select additional chemicals. The approach was tested on two endpoints: inhibition of solute carrier proteins 6A2 and 6A3 and inhibition of the monoamine oxidase A enzyme. Two learning cycles on the solute carrier protein endpoint produced modest but statistically significant increases in model performance. Monoamine oxidase A model improvements from a single learning cycle were

less substantial. The multitask pipeline combined with active learning offers a more generalized model of chemical space and a method to efficiently fill data gaps and strategically improve model performance. A manuscript describing the active learning approach is in preparation.

Predicting blood–brain barrier permeability using machine learning QSAR models

Tags: DoW, machine learning, neurotoxicity, structure similarity

In vitro studies are widely used to measure the neurotoxicity or neuroactivity of chemicals. However, to show similar activity in vivo, chemicals need to cross the blood–brain barrier, a membrane that controls transport of chemicals between the blood and brain. USAF PRT developed and validated a set of machine learning models to predict the blood–brain barrier permeability of chemicals using data from the B3DB database (Meng et al. 2021). After extensive data curation, seven individual classification models were built using various molecular descriptors, machine learning algorithms, and balancing techniques to address the dataset imbalance. Predictions from the individual models were combined into a consensus model, which demonstrated the most robust and stable performance, with a balanced accuracy of 0.81. Shapley Additive Explanations analysis (Lundberg and Lee 2017) identified the topological polar surface area as the most critical molecular feature for blood–brain barrier permeability. Additionally, 88 molecular fragments frequently found in blood–brain barrier-permeable compounds were identified, with nitrogen-containing rings being the most common. Regression model development was more challenging; the graph neural network model performed the best, with a coefficient of determination of 0.48. As a case study, the consensus blood–brain barrier classification model was applied to screen 6,031 compounds identified by in vitro assays as active against the dopamine D2 receptor. The model predicted that 27% of these compounds would not cross the blood–brain barrier in vivo, highlighting the importance of performing blood–brain barrier permeability screening early in the research process to eliminate false positives. A manuscript intended for Elsevier's *NAM Journal* is in development for submission in 2026.

PBPK modeling to predict chemical distribution in brain and adipose tissues

Tags: EPA, NIEHS, metabolism, neurotoxicity

PBPK modeling can be applied to high-throughput applications to facilitate decision-making in drug discovery and risk assessment. However, high-throughput pharmacokinetic modeling is limited to organs and organ systems represented by compartments included in the model. Many PBPK models lack brain and adipose compartments due to the complexity of chemical transport, partitioning, and release in these tissues. However, estimating brain and adipose tissue concentrations is important to predict neurotoxicity and effects of long-term exposure to persistent chemicals. To address these challenges, NICEATM scientists and collaborators developed a PBPK model using the EPA open-source httk R package (v. 2.2.2) that incorporates brain and adipose tissue compartments. The brain compartment accounts for chemical transfer across the blood–brain barrier. The concentration–time profiles generated by the revised model showed general concordance for both brain and adipose compartments in comparison to experimental data. For example, the maximum plasma concentration (Cmax) values predicted by the model for brain tissue were within 2-fold of experimental measurements for both water-soluble chemicals such as acetaminophen (predicted Cmax = 54.01 µM, observed Cmax = 74.03 µM) and hydrophobic chemicals such as benzo[a]pyrene (predicted Cmax = 11.21 µM, observed Cmax = 9.59 µM). This alignment between the httk-based brain–adipose model predictions and predictions from experimental data demonstrates the robustness of the model and its potential applicability for both drug development and chemical risk assessment. A paper describing this work is in preparation, and the model will be made publicly available.

Development of QSAR models to predict acute pesticide toxicity in fish

Tags: EPA, acute toxicity, ecotoxicity, QSAR

QSAR models may be used to assess the potential for acute chemical toxicity in fish of pesticides or pesticide degradation products for which there are little or no toxicity data available. However, data used to develop QSAR models 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 developed a random forest model to predict pesticide toxicity in fish. The model, Toxicity Model for Predicting Pesticide Lethality, was developed using data submitted to EPA for new pesticide registrations and performed better for this specific purpose than the three existing models to

which it was compared. This model is being incorporated into a graphical user interface tool with the goal of making it publicly available to web users via the CompTox Chemicals Dashboard.

Separately, the EPA Office of Chemical Safety and Pollution Prevention previously developed the Ecological Structure Activity Relationships predictive model. This model predicts toxicity of new or untested industrial chemicals by grouping structurally similar organic chemicals with available experimental effect levels that are correlated with physicochemical properties. It is continuously updated based on collected or submitted experimental studies from both public and confidential sources.

Integrating multidimensional in vitro data streams with machine learning for developmental neurotoxicity

Tags: EPA, defined approaches/IATA, developmental neurotoxicity, machine learning

A battery of in vitro assays has been developed to measure developmental neurotoxicity (DNT) potential for critical neurodevelopmental processes, including proliferation, differentiation, migration, apoptosis, neurite outgrowth, synaptogenesis, and neural network formation. Due to the complexity of these processes, these assays use both human and rodent cell models and various detection platforms, resulting in a highly complex set of endpoints. Currently, only a subset of chemicals has been tested across the full battery of assays, and only a few of these have in vivo DNT-relevant evidence for benchmarking. This limited dataset makes building and validating DNT predictive models difficult. EPA scientists leveraged the DNT in vitro battery data landscape from the EPA ToxCast program (invitrodb v. 4.3 dataset) to build a DNT predictive model using a Bayesian network approach. The ToxCast DNT in vitro battery is comprised of 18 distinct assays (66 endpoints) that map to seven neurodevelopmental processes, with 510 chemicals having data from at least one assay. The Bayesian network model was constructed based on expert knowledge of which nodes represented each DNT neurodevelopmental process, with an additional node capturing “DNT potential” based on reference chemicals. The results of the Bayesian network model were compared to the more rudimentary “1 hit” approach that has been previously used to classify the DNT of a chemical. Results suggested that the “1 hit” approach was more sensitive (more likely to detect true developmental neurotoxicants) than

the Bayesian network model but had less specificity (less likely to correctly identify chemicals that would not induce DNT). This work demonstrates how the implementation of Bayesian network modeling with the DNT in vitro battery data can support the classification of DNT reference chemicals compared to the "1 hit" rule. Bayesian network modeling offers advantages for regulatory assessment and provides transparent, interpretable results that quantify uncertainty and integrate expert judgment with limited data. This work will be presented as a poster (Truong et al., late-breaking session) at the 2026 Society of Toxicology meeting.

Predictive toxicology model of drug placental permeability using 3D fingerprints and machine learning

Tags: FDA, NCATS, developmental toxicity, machine learning

Placental transfer is an important factor that can inform regulatory decisions related to fetal safety and the inclusion of women with child-bearing potential in clinical trials. To address this concern, FDA NCTR developed an in silico predictive toxicology model based on 3D spectral data–activity relationships to determine the placental permeability potentials for drugs and new molecular entities with exposures during pregnancy. A fully referenced dataset of 148 chemicals with available fetal-to-maternal concentration ratios (F/M ratios) was used for modeling. To build a binary classification model, the continuous F/M ratios were assigned to two categories conditionally denoted as positive (drugs that readily cross the placental barrier) and negative (drugs with limited permeability). The negative class was defined as chemicals with F/M ratios ≤ 0.1, while an F/M ratio cutoff of ≥ 0.3 was used to define the positive class. Using these defined cutoffs, 44 chemicals in the initial dataset were classified as negative and 100 as positive. This dataset was algorithmically divided into modeling (67 compounds) and blind validation (77 compounds) subsets. Predictive model performance was evaluated using the validation set, resulting in an accuracy of 0.91. Simultaneously, classifications were also predicted for 1,530 drugs with unknown placental permeabilities. In vitro transwell assays developed by collaborators at the FDA Center for Biologics Evaluation and Research and NCATS are undergoing calibration and will be utilized to test the true predictive power of the model using a set of 10 positive and 10 negative chemicals with unknown placental permeability.

Tox24 crowdsourcing challenge to develop computational models to predict activity against transthyretin

Tags: ATSDR, NCATS, data sharing, endocrine disruptors, machine learning, QSAR

Endocrine-disrupting chemicals can harm the body by interfering with thyroid hormones, which are vital for growth and development. These chemicals often bind to transthyretin, a key protein that transports thyroid hormones. Many endocrine-disrupting chemicals remain unidentified, and assessing their risks is challenging due to current testing limitations and the complexity of interactions within the body. To address these challenges, the Tox24 Challenge was held to encourage the development of computational models to predict how chemicals interact with transthyretin (Eytcheson and Tetko 2025).

To help identify and prioritize potentially harmful chemicals, ATSDR researchers developed machine learning-based QSAR models as part of the Tox24 Challenge (Hou et al. 2026). Using a dataset of 1,512 chemicals, participating teams developed and evaluated diverse modeling approaches for predicting chemical binding to transthyretin and potential disruption of thyroid hormone transport. The challenge provided an objective framework for comparing model performance on held-out data and assessing the ability of computational strategies to prioritize potential thyroid-disrupting chemicals.

Researchers at NCATS and collaborators applied a deep learning consensus architecture (Zakharov et al. 2019), which integrates consensus modeling with deep learning to generate QSAR predictions (Cirino et al. 2025). Furthermore, using the 1,512-compound Tox24 dataset, researchers compared individual models from top-performing teams and developed consensus models by averaging their predictions. The consensus models generally exceeded the predictive performance and reliability of individual models, suggesting that different modeling strategies captured complementary information about chemical structure and transthyretin binding potential. This approach highlights the potential of consensus modeling to improve chemical risk assessments and supports efforts to refine data for better public health protection.

Model interpretation and substructure analyses identified molecular features associated with transthyretin binding, including hydrophobicity, branching, and electronic properties. These findings provide useful insight into structural characteristics that may contribute to thyroid

hormone transport disruption and can support future prioritization of chemicals for additional testing.

Together, these advancements align with global initiatives to modernize toxicology through computational and data-driven approaches. By integrating multiple modeling strategies, expanding the chemical dataset, and improving benchmarking practices, the identification and prioritization of harmful endocrine-disrupting chemicals can be strengthened. Importantly, these computational approaches can help reduce reliance on traditional animal models while providing scalable tools for chemical safety assessment.

Guided optimization of ToxPi model weights using a semiautomated approach

Tags: NIEHS, machine learning, PFAS

The Toxicological Prioritization Index (ToxPi) is an advanced tool designed to organize and visualize complex data for decision-making. Originally developed for comparing the relative toxicity of chemicals, ToxPi combines multidimensional data into visual profiles called "slices" and produces a score for ranking different entities. Beyond chemical toxicity profiling, ToxPi has been applied in other areas, such as in the analysis of geospatial data, including county-level COVID-19 risks and sites of historical exposure to per- and polyfluoroalkyl substances (PFAS). The ToxPi scoring system depends on user-defined weights for features (data attributes), but accurately assigning these weights can be a challenge, as users often lack the tools or statistical methods to determine them properly.

To address this, NIEHS researchers developed a semi-supervised method that predicts feature weights using ordinal regression (Fleming et al. 2024). This approach refines the predicted weights through a customized genetic algorithm to further optimize accuracy. A simulation study revealed that this method improves feature-weight prediction, even when only limited reference samples are available, making it effective in scenarios with sparse initial data. Researchers validated this method by building a ToxPi model using published data and comparing it to models built with expert-defined weights. The semi-supervised approach demonstrated strong performance, allowing users to generate accurate and meaningful rankings without requiring extensive prior knowledge or statistical assumptions.

This enhancement to ToxPi simplifies the process of weight assignment and improves the accuracy of profiling and ranking entities, making it a valuable tool for analyzing complex

datasets in toxicology, public health, and environmental science. It expands ToxPi's utility while providing a method to guide users with minimal initial data.

Continued development of httk

Tags: EPA, IVIVE, metabolism

To fully characterize the potential human health risk of a substance, data regarding the substance's toxicokinetics are often needed, including how a substance is absorbed, distributed, metabolized, and eliminated in the body. Traditional approaches for obtaining toxicokinetic data use animal models, 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 predict the fate of chemicals in humans, rats, mice, dogs, and rabbits. The latest version of httk, v. 2.7.4, was released in December 2025. This version provides quantitative structure–property relationship (QSPR) predictions of pharmacokinetic parameters for several thousand volatile organic chemicals. Other updates during 2024 and 2025 added models that describe absorption through the skin (Meade et al. 2026), partial oral absorption (Honda et al. 2025), and human pregnancy (Truong et al. 2025), as well as in vitro data and QSPR predictions of pharmacokinetic parameters for PFAS. More information is available on the EPA httk website.

OPERA updates

Tags: EPA, NIEHS, IVIVE, 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 to predict ADME endpoints that are important to PBPK modeling and IVIVE studies. All OPERA models were built using curated datasets 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 the development of scientifically valid, highly accurate 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 become available. In 2024 and 2025, OPERA models were expanded with newly curated datasets covering a number of physicochemical properties and ADME parameters, as well as new input/output features and bug fixes. OPERA predictions are available through the EPA CompTox Chemicals Dashboard and NICE. 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. It is also 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 and on the FDA's Precision platform.

Collaborative evaluation of in silico predictions for high-throughput toxicokinetics

Tags: EPA, NIEHS, metabolism

High-throughput toxicokinetic (HTTK) methods were developed by the pharmaceutical industry to estimate toxicokinetic statistics that can help predict chemical or drug metabolism. HTTK data are typically obtained from high-throughput in vitro assays that measure chemical-specific toxicokinetic parameters. These data are used as input for mathematical models that can, in turn, predict parameters for similar chemicals. EPA and NIEHS researchers evaluated the utility of seven QSPR models for estimating toxicokinetic parameters such as intrinsic hepatic clearance, fraction of chemical unbound in plasma, and toxicokinetic elimination half-life (Wambaugh et al. 2025). Performance of the QSPR models was evaluated using in vivo data, mainly from rats, obtained from the literature. Simulations of the in vivo data were made with a high-throughput physiologically based toxicokinetic model using the different QSPR model predictions as inputs. The study found that QSPR models may yield reasonable predictions of toxicokinetic parameters for novel compounds. The QSPR approach might also be sufficiently reliable in studies to prioritize further toxicological evaluation of chemicals when the error introduced by the predicted data

is less than a fixed factor, and the study included an analysis of error introduced by various approaches.

Rapid dosimetry modeling of drug disposition in long-duration spaceflight

Tags: DoW, metabolism

Spaceflight causes a wide range of physiological changes, some of which may affect the pharmacokinetics of xenobiotics, including medications. USAF PRT and the National Aeronautics and Space Administration conducted a study to investigate the impact of spaceflight-induced physiological changes on drug disposition. Astronaut demographics and spaceflight-specific parameters were integrated into a modified version of EPA's httk model to simulate drug concentrations in astronauts under spaceflight conditions. Additionally, cytochrome P450 polymorphism data were introduced into the model to account for metabolic mutations in the population. The httk model was used to assess how changing individual space-affected parameters (cytochrome P450 polymorphisms, metabolism modifiers, body weight, glomerular filtration rate, fraction of drug absorbed, cardiac output, and hematocrit) impacts the plasma concentrations of 99 oral and intravenous medications compared to terrestrial conditions. Results identified drugs that are impacted most by spaceflight conditions and highlighted the potential for both decreased and increased drug exposure in space that could require dose adjustments to maintain efficacy and safety. Furthermore, the model was used to estimate the impact of cytochrome P450 polymorphisms on drug concentrations and identify drugs with potential idiopathic changes during space travel. This computational approach offers a valuable tool for predicting individual astronaut responses to medication during space missions, paving the way for personalized medicine in spaceflight. A manuscript describing this study is in preparation.

NCBI Codeathon to build machine learning and SI solutions for biological research

Tags: NLM, machine learning

Machine learning and SI are transforming science and healthcare; thus, ensuring fairness and transparency in their use is increasingly important. To address this, the NLM's National Center for Biotechnology Information (NCBI) hosted a virtual codeathon, "Building Transparent ML/AI Solutions to Advance Biological Research", from February 26 to March 1, 2024. This event brought together developers, data scientists, and technology enthusiasts

to collaboratively build innovative solutions aimed at tackling challenges in healthcare, education, research, and more.

The codeathon emphasized social good, encouraging participants to design machine learning/AI models that prioritize fairness, transparency, and accountability. Teams of 5–10 people worked virtually to create meaningful solutions, particularly by leveraging publicly available scientific data and resources from the NCBI. Priority was given to projects that made use of NCBI tools and databases for machine learning applications.

Throughout the week, participants benefited from networking opportunities and expert-led sessions by professionals in academia, industry, and government. Unlike traditional competitions, the event promoted cooperation, with teams sharing ideas, skills, and knowledge to build impactful machine learning/SI learning solutions. By the end of the codeathon, teams presented their projects to one another, experts from the NIH, and broader public audiences.

This codeathon demonstrated how collaboration and transparency can drive the ethical use of machine learning/SI technologies in biological research. Participants not only developed innovative solutions but also fostered a sense of community and shared commitment to advancing science for the betterment of society.

QSAR-ready workflow for automated standardization of chemical structures

Tags: EPA, NIEHS, harmonization, QSAR

The increasing availability of public chemical data offers significant opportunities to create powerful QSAR models to predict chemical properties and biological effects based on molecular structures. However, inconsistencies in chemical structure information and experimental data quality can negatively affect model accuracy and reliability. To address these challenges, NIEHS and EPA researchers developed an automated workflow in the KNIME platform to standardize chemical structures and prepare "QSAR-ready" forms for molecular descriptor calculation, ensuring high-quality data for modeling efforts (Mansouri et al. 2024).

The automated workflow has three main steps: (1) reading and decoding chemical structures, (2) verifying consistency with chemical identifiers, and (3) applying a series of standardization operations. These operations include desalting, correcting valency and

tautomer forms, neutralizing molecules, standardizing nitro groups, stripping stereochemistry for 2D structures, and removing duplicates. This ensures the structures are uniform and compatible for QSAR modeling. Initially designed for collaborative QSAR modeling projects, the workflow was later adapted for broader use, such as preparing "MS-ready" structures for nontargeted analysis in mass spectrometry.

This innovative workflow enhances the accuracy and reliability of molecular descriptor calculation, a crucial step for producing dependable QSAR models. The workflows are freely available on KNIME and GitHub and as Docker containers, making them easily accessible to the scientific community. The workflow was also embedded in the OPERA suite of QSAR models to ensure consistency of predictions. By addressing data quality issues and promoting consistent methodologies, this resource empowers researchers across diverse fields to improve chemical modeling, facilitate collaborative projects, and advance chemical safety and analysis technologies.

In silico prediction of chronic oral reference doses for petroleum fuel components

Tags: DoW, QSAR

Characterizing human health risk is often challenged by a lack of published toxicity values. The acronym PIANO (paraffin, isoparaffin, aromatic, naphthene, and olefin) refers to a group of about 300 volatile compounds in petroleum fuels and the analytical method used to measure them. Of the compounds, 43 have published oral reference doses, with the remaining compounds typically assigned surrogate toxicity values based on similarly structured compounds. Researchers at the Defense Centers for Public Health and Navy and Marine Corps Force Health Protection Command developed QSAR models to predict oral reference doses for 294 PIANO compounds based on stepwise linear regression of 2D molecular descriptors and published toxicity values (Rockswold et al. 2026). The master dataset contained 1,123 compounds, specifically 294 PIANO compounds and 806 others. Five training groups, created by randomly selecting 80% of the non-PIANO compounds and 50% of the PIANO compounds that had oral reference doses, were used to develop five QSAR models. The geometric mean of QSAR results was used to predict oral reference doses for compounds lacking toxicological information. For compounds with known oral reference doses, 71% of the predicted oral reference doses were within an order of magnitude

of published oral reference doses. This is more precise than the acknowledged uncertainty inherent in published oral reference doses. This predictive approach has applicability beyond PIANO compounds and represents a NAM that may be used to reduce uncertainty in human health risk assessment and guide regulatory decisions.

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: EPA, FDA, NIEHS, and NCATS. 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, hepatotoxicity, Tox21

Gene expression profiling with alternative toxicological cell systems is a valuable tool for exploring 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 among environmental chemicals and specific molecular targets associated with biological responses, such as nuclear receptors, enzymes, and transporters. Over 300 reference chemicals have been identified and procured to build robust transcriptomic datasets in various cell systems, including the U-2-OS human osteosarcoma cell line and differentiated cultures of HepaRG and 3D microtissues of primary human hepatocytes, using the TempO-Seq™ technology for next-generation sequencing of cell lysates. Experiments covered a wide range of exposures to capture the initial stages of biological responses to environmental chemicals, using transcriptomic patterns to define similarity and help infer mechanisms for data-poor chemicals. This efficient strategy has the potential to greatly increase the mechanistic understanding of adaptive responses to environmental chemicals at the gene expression level and inform next steps in toxicology

research investigations. The research teams have completed data generation for all three cell systems, and manuscripts for each cell system are in preparation. Furthermore, these reference chemicals were shared with colleagues at the University of Leiden to add renal proximal tubule epithelial cells to the list of cell types. A manuscript by the researchers involved in this project has been prepared for submission in 2026.

Machine learning models to predict nonspecific thiol reactivity in HTS assays

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

High-throughput screening (HTS) programs such as Tox21 gather data on chemical bioactivity from 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, specific HTS assays have been developed to measure autofluorescence, and data from these assays have been used to train machine learning models to predict reactivity.

The fluorescence-based MSTI ((*E*)-2-(4-mercaptostyryl)-1,3,3-trimethyl-3*H*-indol-1-ium) HTS assay identifies thiol-reactive compounds. EPA and NCATS scientists used this assay to screen 7,872 unique chemicals in the Tox21 10K chemical library (Patlewicz et al. 2023). Active chemicals were compared to profiling outcomes using structural alerts encoding electrophilic information. A random forest classification model based on chemical fingerprints was then developed to predict assay outcomes. The classification model shows promise as a tool to screen untested chemicals for their potential electrophilic reactivity based solely on chemical structure. MSTI assay data were subsequently evaluated for their utility in predicting compound promiscuity and cytotoxicity by comparing the activity of the Tox21 10K compounds in the MSTI assay to their activities across the entire Tox21 assay panel (Kruger et al. 2024). The predictive performance showed a sensitivity of 55% and specificity of 97%; chemical features such as halides and carboxamides resulted in false negatives and false positives, respectively. While this high-throughput assay allows researchers to identify whether a compound will be cytotoxic and/or promiscuous in an efficient manner, this study also reveals potential challenges in its use.

Retrofitting existing Tox21 HTS assays with metabolic capability

Tags: EPA, NCATS, NIEHS, endocrine disruptors, metabolism, structure similarity, Tox21

HTS assays that have been used in the Tox21 testing program to date generally lack characteristics of metabolic activity occurring in living systems, which can potentially increase or decrease the toxicity evaluation of chemicals. As a result, HTS results may not accurately reflect in vivo activity. Scientists at EPA, NCATS, and NIEHS used 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 were 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 were further screened to identify the responsible metabolic enzymes, including the primary cytochrome P450 (CYP) drug-metabolizing enzymes CYP1A2, 3A4, 2C9, 2C19, and 2D6. The screening data were analyzed by grouping chemicals based on structural similarity, which enabled the identification of specific and nonspecific CYP inhibition patterns (Sakamuru et al. 2025). This information ultimately supports the development of predictive models for CYP inhibitory activities.

Retrofitted assays are also being used to explore how metabolism might affect interaction of chemicals with the androgen receptor pathway. Rat liver microsomes—small, membrane-bound vesicles formed from fragments of the endoplasmic reticulum—were incorporated into androgen pathway assays for screening the Millipore–Sigma Library of Pharmacologically Active Compounds (Lynch et al. 2025). Known androgenically active compounds, as well as at least one novel chemical, were identified using this newly optimized and physiologically relevant model. The 10K compound collection was screened during 2024 and 2025 to identify novel compounds requiring metabolism for androgen receptor antagonist activity; follow-up studies are ongoing in 2026.

Investigation of environmental determinants of pubertal timing in girls

Tags: NCATS, 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 NCATS and NIEHS examined the potential effects of endocrine-disrupting chemicals on important components of the biochemical pathway responsible for pubertal

timing (Yang et al. 2024). In these studies, 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. The 58 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.

Predictive toxicology of the retinoid signaling pathway

Tags: EPA, FDA, NCATS, NIEHS, 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. Earlier publications from the project:

- Identified candidate reference chemicals for use in in vitro assays relevant to the pathway (Baker et al. 2022)
- Described a computational model for fetal skeletal defects potentially linked to disruption of retinoic acid signaling (Pierro et al. 2022)
- Applied in vitro to in vivo extrapolation (IVIVE) to characterize potency of chemicals interacting with the pathway (Chang et al. 2022)
- Developed and validated a high-throughput assay measuring activity of the metabolic enzyme CYP26A1 (Sakamuru et al. 2024); this assay was then used to first screen a library of pharmacologically active chemicals and then the Tox21 10K compound

collection. Chemicals with inhibitory activity were selected for follow-up assays planned for 2026.

More recently, a high-throughput cytokine detection platform using human induced pluripotent stem cell-derived microglia was developed and validated to identify potential neurotoxicants (Yang et al. 2025) by testing nine environmental chemicals with evidence of cytokine-inducing potential in other in vitro assays.

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

Tags: FDA, NCATS, NIEHS, 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 underrepresented pathways in the current Tox21 assay portfolio may improve the predictivity of Tox21 data. Scientists at FDA, NCATS, and NIEHS are systematically identifying these underrepresented 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 CYP450 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). The impact of the newly generated assay data on improving model performance was evaluated, and predictions from the models were validated in relevant assays. The expanded Tox21 assay panel was found to significantly enhance the capacity of assay data for in vivo toxicity prediction, underscoring the importance of a diverse and comprehensive in vitro assay portfolio in advancing safety assessment (Xu et al. 2025).

Confidence

Establishing confidence in new testing methods requires engagement by both federal agencies and other 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 2024 and 2025 leveraged complex in vitro approaches, such as microphysiological systems (MPS), as well as defined approaches (DAs) and integrated approaches to testing and assessment (IATAs), in areas such as topical toxicity, ecotoxicity, inhalation toxicity, and identification of endocrine-active substances.

Validation of an in vitro human thyroid microtissue model for chemical screening

Tags: ICCVAM, EPA, NIEHS, endocrine disruptors, MPS

Current regulatory decision-making for potential thyroid-disrupting chemicals is based on in vivo apical endpoints, such as alterations in thyroid hormone levels. New approach methodologies (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 in 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 coordinated 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 was within the EPA Office of Research and Development, with three agrochemical industry and commercial laboratories participating in the transferability phase (Deisenroth et al. 2026). Goals of this effort included (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. Reference chemical

selection was based on previous work demonstrating disruption of thyroid hormone synthesis. These included one "true negative" and three "true positive" compounds. The study established standard operating procedures that were successfully transferred to multiple laboratories with no experience conducting the assay. Consistent results were generated among the testing laboratories using the same four blinded reference chemicals and among the three donor pairs. Independent human thyrocyte donors were matched between a testing laboratory and the lead laboratory to account for biological variability. In addition to the publication describing the study, a detailed report of the study is being prepared. A follow-up study to test the model with a larger chemical set and potentially improve replicability is currently being designed.

Validation of 96-well EASA to detect potential skin sensitizers

Tags: CPSC, DoW, FDA, NIEHS, NIST, AOP, skin sensitization, defined approaches/IATA

Skin sensitization, or an allergic skin reaction, is an important health concern in chemical testing. Assessment of skin sensitization hazard traditionally relies on animal models, such as the local lymph node assay (LLNA). Efforts to reduce animal use have led to the development of alternative methods based on the skin sensitization adverse outcome pathway (AOP).

The electrophilic allergen screening assay (EASA) is a promising in chemico method to identify substances that covalently bind to skin proteins, Key Event 1 in the AOP for skin sensitization. The assay was converted from a cuvette-based platform into a 96-well platform and characterized previously (Petersen et al. 2022). A multilaboratory validation study assessed intra- and interlaboratory reproducibility of the 96-well EASA. In addition to the CPSC/NIST lead laboratory, laboratories sponsored by FDA, DoW, and NIEHS participated in the validation study. Each participating laboratory confirmed transferability and assessed 20 reference chemicals. Results showed that the method is reproducible and reliable, supporting its potential inclusion in regulatory frameworks. A report of the validation study was published in August 2025, and a paper summarizing the study has been submitted for publication (Reinke et al. 2026).

Key Event 1 assays are incorporated in DAs for skin sensitization hazard and potency assessments described in Test Guideline (TG) 497 issued by the Organisation for Economic

Co-operation and Development (OECD; OECD 2025a). These DAs offer structured, fixed procedures for data interpretation, reducing reliance on expert judgment. By meeting performance standards for in chemico Key Event 1 assays, described by OECD in TG 442C (OECD 2025b), the EASA is a promising alternative to existing Key Event 1 assays. The EASA is currently under consideration by OECD for inclusion in TG 442C. Compared to the current TG 442C assays, the EASA offers higher throughput and requires less specialized equipment, making it more accessible and efficient. To support its inclusion in TG 442C, the OECD Skin Sensitization Expert Group has requested additional testing for predictive capacity and confirmation of molecular interaction between the probe and reference chemicals; this testing is in progress by NIST and the NIEHS-sponsored laboratory.

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

Tags: DoW, defined approaches/IATA, skin sensitization

Scientists in the U.S. Air Force (USAF) Force Health Protection program's Predictive Risk Team (PRT) performed a study using NAMs to characterize the risk of allergic contact dermatitis from occupational chemical exposures (Keebaugh et al. 2025). 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 the induction of sensitization. The kinetic direct peptide reactivity, KeratinoSens™, and human cell line activation test assays were used to predict LLNA effective concentration values using the regression models published in Natsch et al. (2022a) and Natsch et al. (2022b). In vitro assay results, in silico model predictions, and available human and animal data were used to predict sensitizer status using a weight-of-evidence approach. Preliminary candidate surface guidelines, derived from predicted effective concentration values for predicted sensitizers, can be used to evaluate the presence of these chemicals on work surfaces to better understand the risk of USAF personnel developing allergic contact dermatitis from occupational exposures.

Broadening applicability of DAs for identification of skin sensitizers

Tags: NIEHS, defined approaches/IATA, skin sensitization

Skin sensitization is an allergic reaction triggered by chemical exposure, which can cause significant health issues. To ensure safety, regulatory frameworks rely on methods to assess both hazard, the likelihood of a chemical to be a sensitizer, and potency, a prediction of the severity of sensitization that could be caused by the chemical. Traditionally, sensitization hazard and potency have been estimated using animal models, such as the LLNA. However, advances in nonanimal methods, such as in chemico, in vitro, and in silico tests, have enabled more ethical and human-relevant approaches through integrated strategies such as DAs, which combine data from multiple nonanimal tests to predict sensitization potential.

A May 2025 report summarized a NICEATM evaluation of test substances nominated by ICCVAM member agencies. In the study, 181 "difficult" test substances of regulatory interest were tested in individual in chemico and in vitro methods, and the results applied to three DAs for predicting skin sensitization hazard or potency. The in vitro methods evaluated were the direct peptide reactivity assay, the KeratinoSens™ assay, and the human cell line activation test. The three DAs used were the Key Event 3/1 Sequential Testing Strategy, the Two-out-of-Three strategy, and the Integrated Testing Strategy. Predictions were compared against reference data from LLNAs and human predictive patch tests (HPPTs) to assess concordance.

Results showed varying concordance with human and LLNA reference data. Individual methods had concordance rates ranging from 30%–93% and 52%–78% with LLNA data and human data, respectively. The DAs demonstrated better agreement among themselves (70%–92%) and showed higher concordance with human reference data (42%–47%) compared to the LLNA (24%–51%). Importantly, the DAs proved to be more accurate predictors of human skin sensitization hazard and potency than LLNA methods.

These findings confirm that DAs provide a more reliable, efficient, and ethical alternative to animal models for assessing skin sensitization. By integrating multiple nonanimal methods, DAs improve human health predictions, supporting regulatory decision-making and reducing reliance on animal-based testing.

Evaluation of the GARDskin sensitization test method using substances of regulatory interest

Tags: NIEHS, defined approaches/IATA, skin sensitization

NICEATM scientists have been working to replace the use of animal models in skin sensitization studies with reliable and ethical alternatives. A June 2025 NICEATM report summarized an evaluation of the performance of a nonanimal test method called the Genomic Allergen Rapid Detection (GARD™skin) assay, which tests whether chemicals can cause allergic skin reactions. The study specifically assessed the GARDskin assay's ability to predict both hazard, the likelihood of a chemical to be a sensitizer, and potency, a prediction of the severity of sensitization that could be caused by the chemical, utilizing DAs for skin sensitization. Results were compared against traditional animal test results, including LLNA and HPPT data.

The study examined 31 substances using the GARDskin assay, in conjunction with results from other methods, such as the direct peptide reactivity assay, the human cell line activation test, and the KeratinoSens assay. Predictions from these tests were compared against human and LLNA data to evaluate accuracy (an overall assessment of performance), sensitivity (ability to identify true positives), and specificity (ability to identify true negatives). The GARDskin assay performed well, ranking highest among the methods in terms of accuracy (63%), balanced accuracy (62%), and sensitivity (81%) when compared to LLNA data. It also had the lowest rate of false negatives (19%). Inclusion of GARDskin data improved the performance of DAs, which combine data from multiple nonanimal tests to predict sensitization potential. For potency, the DA containing GARDskin had higher accuracy than other DAs (53%) and was less likely to overpredict or erroneously assign a more restrictive hazard classification compared to other approaches.

While only 31 substances were tested, limiting broader conclusions, this evaluation shows promising results for the GARDskin assay in providing human-relevant, reliable predictions. Combining multiple tests into integrated approaches adds confidence in safety assessments, helping advance nonanimal methods for regulatory use. This study supports the shift toward modern, ethical testing alternatives for chemical safety evaluations.

Human relevance of in vivo and in vitro skin irritation tests for hazard classification of pesticides

Tags: EPA, skin sensitization

Skin irritation and corrosion testing for pesticides is traditionally conducted using the Draize rabbit test, which is required by many regulatory agencies. While the Draize test generally provides classifications designed to protect human health, it has limitations due to species differences between rabbits and humans, particularly for mild to moderate irritants. NAMs offer human-relevant alternatives, using reconstructed human tissues, biochemical skin models, and in vitro systems to better mimic human skin responses. However, these methods face challenges in regulatory adoption, mainly due to limited concordance with Draize test results and requirements to distinguish irritation potential across all hazard categories.

EPA researchers and collaborators evaluated how existing NAMs align with human skin anatomy, physiology, and the biological mechanisms underlying chemically induced irritation and corrosion (Raabe et al. 2024). The findings show that NAMs such as reconstructed human skin tissue models and synthetic barrier systems are biologically and mechanistically relevant to these endpoints and reflect human skin responses better than animal models. Notably, retrospective analyses revealed that the Draize test has low reproducibility (less than 50%) for mild and moderate irritants, with many replicate predictions varying across irritation categories. Based on these findings, the study recommends improved testing strategies for pesticide classification by using a combination of OECD-approved in chemico and in vitro methods that closely replicate human skin structure and function. Specifically, “top-down” approaches prioritize NAMs that identify corrosive substances, while “bottom-up” strategies improve sensitivity to accurately differentiate mild irritants or nonirritating substances from irritants and corrosives. Additionally, optimizing cytokine release protocols and analyzing reference test results could enhance discrimination within the mild irritation spectrum. This work underscores the potential of modern NAMs to replace animal models for skin irritation, advancing both regulatory science and human health protections by prioritizing more relevant and reproducible testing methods.

Evaluation and comparison of in vitro and ex vivo models for skin permeation testing

Tags: FDA, NCATS, NIEHS

Skin permeation is a primary consideration in the safety assessment of cosmetic ingredients, topical drugs, and human users handling veterinary medicinal products. While excised

human skin remains the standard model for in vitro permeation testing studies, unreliable supply and high cost motivate the search for alternative skin barrier models. FDA, NCATS, and NICEATM developed a standardized dermal absorption testing protocol to evaluate the suitability of alternative skin barrier models to predict skin absorption in humans (Salminen et al. 2023). Using this protocol, a reconstructed human epidermis model (EpiDerm-200, MatTek) and a synthetic barrier membrane model (Strat-M, Sigma–Aldrich) were assessed concurrently with standard excised human skin models. Ongoing follow-up studies to this project began in 2024. The number of reconstructed human skin models evaluated was expanded to seven (four reconstructed human epidermis and three full-thickness skin models; three batches per model), and the dermal chemical exposure time was increased from 6 to 24 hours. Caffeine, salicylic acid, mannitol, and testosterone were chosen as test chemicals for both models because of the abundance of historical in vitro and in vivo data and because they represent a wide range of physicochemical properties known to influence absorption. The endpoints assessed included permeability, transepidermal water loss, and histology. Tests were also run to characterize the lipid composition of the stratum corneum across skin models. All the in vitro models exhibited native human epidermis-like morphology, including a characteristic stratum corneum. The excised human skin models showed the lowest transepidermal water loss, with the Strat-M and reconstructed human epidermis models exhibiting a range of water loss equivalent to or higher than that of excised human skin. The full-thickness skin models exhibited the highest water loss and chemical permeation, making them the weaker skin barrier models. Overall, the results suggested that the reconstructed skin models have a weaker barrier than excised human skin, although the differences vary by model and test substance. Studies such as these to evaluate novel alternative skin barrier models may advance regulatory applicability of these models, potentially increasing the efficiency of regulatory review of new products. This project will be presented at the 2026 Annual Meeting of the Society of Toxicology (Modaresi et al.; Abstract 5193) and the American Society for Mass Spectrometry 2026 Conference on Mass Spectrometry and Allied Topics (Sun et al.; Abstract WP499). A peer-reviewed publication is in preparation for submission in 2026.

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

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

In 2025, NICEATM, PETA Science Consortium International e.V., EPA, and CropLife America member companies completed a collaborative effort to develop DAs for predicting United Nations Globally Harmonized System of Classification and Labelling (GHS) and EPA eye irritancy hazard classifications.

Using data from 29 agrochemical formulations tested in a common set of in vitro or ex vivo methods, four DAs were developed:

- The bovine corneal opacity and permeability assay (BCOP; OECD TG 437) with or without histopathology.
- The EpiOcular eye irritation test (OECD TG 492), followed by the BCOP with or without histopathology.
- The SkinEthic time-to-toxicity test for liquids (OECD TG 492B), followed by the BCOP with or without histopathology.
- The EyeIRR-IS assay (Cottrez et al. 2021), followed by the BCOP with or without histopathology.

Rather than evaluating direct concordance with historical in vivo rabbit eye test data, GHS and EPA predictions derived using the DAs were assessed using orthogonal validation and weight-of-evidence. Results showed that, for both classification systems, the predictions from each of the four DAs were as protective or more protective of human health than the in vivo test (Daniel et al. 2025). This study refines and builds upon two DAs described in a previous publication (van der Zalm et al. 2024). This work underscores the limitations of current animal-based reference data and highlights the need for a more human-relevant approach to eye irritation hazard classification. By improving integrated testing strategies, this research lays the groundwork for safer, more ethical, and scientifically meaningful alternatives to animal testing in regulatory toxicology.

Evaluation of fish cell line cytotoxicity assays for pesticides applied for nuisance fish control

Tags: DOI, acute toxicity, ecotoxicity

The application of pesticides to control invasive species remains a key component of integrated pest management. Historically, developing new chemical controls required extensive testing in live animals. In vitro cytotoxicity assays using mammalian or fish cell lines are increasingly utilized because they reduce the number of animals required and enable high-throughput screening (HTS) of candidate chemicals. However, it is still unclear how well in vitro cytotoxicity values represent in vivo toxicity values.

Scientists in the DOI U.S. Geological Survey have developed and evaluated fish cell line cytotoxicity assays for several indigenous and nonindigenous species to demonstrate proof-of-concept for pesticide screening targeting nuisance fish. Recent work evaluated antimycin A using the RTgillW1 cell line for rainbow trout (*Oncorhynchus mykiss*), following OECD TG 249 and using the Promega CellTiterGlo 2.0 assay. Results showed that in vitro–in vivo toxicity ratios were approximately one for most endpoints. In addition, a grass carp (*Ctenopharyngodon idella*) gill cell line assay demonstrated repeatability using the OECD positive control chemical 3,4-dichloroaniline. Together, these proof-of-concept assays and the strong agreement between in vitro and in vivo responses indicate that fish gill cell lines can reliably predict whole-animal toxicity. Continued development of these systems shows strong promise for pesticide candidate screening and related scientific applications.

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

Tags: DoW, acute toxicity, hepatotoxicity, neurotoxicity, structure similarity

USAF PRT is developing a NAM-based approach to support rapid risk assessment of novel and poorly characterized chemicals. A tiered approach is being evaluated, where lower tiers use in silico and high-throughput in vitro models to prioritize hazards, while higher tiers use organotypic in vitro models to perform quantitative risk assessments. To test the utility of this NAM-based approach on operationally relevant scenarios, researchers conducted a case study using several chemicals with known liver and central nervous system toxicity. The chemicals were assessed using the Edwards et al. 2022 in silico model (Tier 0) to evaluate mechanism of action and acute systemic toxicity. In vitro tests were then performed using assays appropriate for the mechanism of action identified by the in silico model (Tier 1A). Additional in vitro tests evaluated cell health (cell viability, cytokines, oxidative stress) in

glial and liver monoculture models (Tier 1B). Finally, Tier 2 human liver microtissue (3DInsight) and central nervous system (CNS-3D, 28bio) organoid models were used to evaluate tissue functional effects. Points-of-departure (PODs) were derived using benchmark dose modeling, converted to human-equivalent doses using toxicokinetic modeling, and compared to traditional testing data. Human-equivalent doses were generally more conservative than in vivo acute oral toxicity values, indicating that use of this metric to set exposure limits would be at least as health-protective as using metrics derived from traditional approaches. Neurotoxicity human-equivalent doses were generally similar to, or more conservative than, literature-derived traditional PODs. Liver microtissue testing results were also qualitatively representative of in vivo effects, and quantitative analysis of human-equivalent doses is ongoing. Findings from the project were presented at the 2025 and 2026 Society of Toxicology meetings and the 2025 Military Health Systems Research Symposium, and two publications are planned for 2026.

Advancing NAMs and organ-chip technologies for medical countermeasure development against chemical toxicity syndromes

Tags: DoW, acute toxicity, MPS, neurotoxicity

Supported by funding from the Defense Threat Reduction Agency's Joint Science and Technology Office, the U.S. Army Chemical Biological Center (CBC) is exploring the use of organ-chip technologies and other NAMs to advance medical countermeasures for chemical toxicity syndromes. The first project aims to create a detailed database of cellular markers from various organs exposed to chemical warfare agents or other toxic compounds. This will help identify new agents by analyzing overlapping mechanisms and symptoms, potentially leading to new drug targets for medical countermeasures development. The second study will identify the most predictive NAMs and develop a testing protocol or toolkit that will allow the rapid identification of potential neurotoxicants and promising medical countermeasures without extensive animal testing. NAMs that target key events in each of the endpoints of interest will be tested against known neurotoxicants, and in vitro qualitative and quantitative responses will be compared to concurrent in vivo studies to determine the most reliable and sensitive assays for inclusion in a testing protocol. Both efforts will result in the digital

curation of endpoint-specific biomarkers that can be used by researchers across DoW to identify additional targets for medical countermeasure development.

Validation of human reconstructed lung tissue in vitro models for acute lung injury from gas exposures

Tags: DoW, acute toxicity, AOP, inhalation toxicity, IVIVE

Comparing 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 PRT, in collaboration with the Air Force Research Laboratory's MPS team and the Naval Medical Research Unit Dayton, are evaluating the mechanistic relevance and the quantitative predictivity of in vitro lung models by comparing dose responses for key events to in vivo data following acute exposure to sulfur dioxide. Key events in the acute lung injury AOP, including oxidative stress, cytotoxicity, inflammation, and barrier integrity, are being measured in monoculture cell models (A549, HuArlo) and human reconstructed tissue models (MatTek EpiAirway™ and EpiAlveolar™; Epithelix MucilAir™) using gas exposure at the air–liquid interface. In vitro data collection is nearly complete. Concurrent studies in a rat model with corresponding sulfur dioxide exposures and correlated endpoints were recently completed at the Naval Medical Research Unit Dayton. Dose–response modeling is being used to identify points-of-departure from each endpoint, and in vitro to in vivo extrapolation (IVIVE) is being used to describe gas disposition in the lung (Tsujino et al. 2005; Nowak et al. 2025). Assays and measured endpoints that are most predictive of in vivo response will be incorporated into a recommended rapid chemical testing protocol. These NAMs are expected to facilitate scientifically sound chemical risk assessments on a timescale that would realistically support operational and acquisition decisions.

Alveolus-on-a-chip reveals distinctive soot-based toxicity biomarkers in extracellular vesicles

Tags: DoW, 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. An alveolus-on-a-chip platform was developed using primary alveolar epithelial and microvascular endothelial cells cultivated at the air–liquid

interface. The platform incorporates cyclical stretch and breathing mechanics to better emulate human physiology in a laboratory model. This model has been utilized to identify unique biomarkers of soot-based exposures, an emerging respiratory global concern to human health and performance. Initial results after 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. Extracellular vesicles isolated from particulate matter-exposed alveolus-on-a-chip platforms exhibited increased RNA content compared to the controls, suggesting that exposure alters the signaling cargo released by the lung. These exosomes were successfully labeled and traced for uptake by recipient cells. Multiple downstream models, including liver, skin, and immune cell systems, showed significant exosome uptake, supporting a mechanism for systemic communication beyond the lung following alveolar exposure. RNA from the recipient models after exosome exposure was isolated for global transcriptomic profiling. Investigators now aim to characterize pathways by which soot-based exposures drive whole-body effects through exosome-mediated transfer of molecular information to aid in biomarker identification for countermeasure development.

Evaluation of the use of NAM-based strategies for assessment of lung injury from $PM_{2.5}$ exposure

Tags: DoW, inhalation toxicity, MPS

This project aims to identify a suite of NAMs for predicting the qualitative and quantitative health risk from exposure to fine respirable particles ($PM_{2.5}$, or particulate matter less than 2.5 micrometers in diameter) using crystalline silica dioxide as a test material. Cytotoxicity, oxidative stress, tissue barrier disruption, and injury dose–response will be compared between concurrent in vitro and in vivo test models. Acute exposure and repeated exposure (daily for 1–2 weeks) experiments are currently nearing completion in HuArlo, BEAS-2B, Epithelix MucilAir, and MatTek EpiAlveolar models in studies performed by the Air Force Research Laboratory MPS Team. PODs will be derived for each endpoint. In vitro PODs will be converted to in vivo equivalent doses using multiple path particle dosimetry modeling, which will then be compared to in vivo exposures performed at the Naval Medical Research Unit Dayton. USAF PRT is performing multiple path particle dosimetry modeling, benchmark dose modeling, and risk assessment strategy. The product is a predictive risk

capability that will facilitate rapid, efficient, and scientifically grounded decisions for ensuring Force health protection in complex operational environments.

Validation of reconstructed human airway epithelial tissue models for use in dose–response assessment of reactive irritant gases

Tags: DoW, inhalation toxicity

Researchers at Battelle Memorial Institute are testing dose responses for multiple key events in lung injury using the EpiAirway reconstructed human airway in vitro model with the prototype lung irritants propylene oxide and epichlorohydrin. The goal of this project is to identify a suite of NAMs for testing lung toxicity risk from operational exposures to inhaled hazards. EpiAirway cells were exposed at the air–liquid interface to propylene oxide for four hours at concentrations of 0, 350, 750, or 1,250 ppm in a VITROCELL 24/48 exposure system. Initial range-finding studies were used to identify the three propylene oxide concentrations used in the definitive study. EpiAirway tissues exposed to propylene oxide demonstrated a concentration-dependent increase in cytotoxicity, barrier stress, cytokine production and oxidative stress in human bronchial reconstructed airway models. All endpoints were altered at 1250 ppm propylene oxide, with no effects at 350 ppm and only the most sensitive endpoints (e.g., adenylate kinase-indicated cytotoxicity, cilia loss) being disrupted at 750 ppm. A similar experiment using epichlorohydrin is currently ongoing. USAF PRT is comparing the dose–response results to in vivo data to evaluate the mechanistic relevance, predictivity, and repeatability of the in vitro model for testing reactive gases in support of health risk assessments for novel chemicals.

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

Tags: NIEHS, inhalation toxicity

To reduce animal use in the investigation of human-relevant inhalation toxicity, the NIEHS Division of Translational Toxicology is evaluating the use of in vitro lung models, including air–liquid interface airway tissues. Potential applications of these models include screening-level assessments to help predict the adverse airway effects of inhaled substances and prioritize them for further testing.

Initially, proof-of-concept studies were conducted to characterize and optimize the VITROCELL 48 2.0 Plus exposure system with human and rat tracheobronchial-derived air–liquid interface airway tissues. The chemical used was 2,3-pentanedione, a highly volatile component of artificial butter flavorings that has been associated with airway injury and fibrosis via occupational inhalation exposure. The studies were designed to explore the validity of extrapolations among rat in vivo, rat in vitro, and human in vitro airway effects in these models. They showed that exposure of the air–liquid interface tissues to 2,3-pentanedione vapor induces concentration-dependent changes in relevant airway toxicity endpoints. Changes in transepithelial electrical resistance, cytotoxicity, histopathology, and secreted biomarkers were comparable between species for most of the endpoints. Measurements of 2,3-pentanedione concentration were also performed on apical wash, basolateral culture media, and tissue lysate samples as indicators of dosimetry. One paper describing these studies has been submitted for publication and a second is in preparation. Further method development and optimization work are also in progress, including the use of alveolar-derived air–liquid interface models and incorporation of transcriptomics.

To help better assess the utility and application of in vitro lung (including air–liquid interface) models, NIEHS issued a Request for Information in April 2024 to gather information on the use of these models in inhalation toxicology research including knowledge gaps and technical challenges and limitations. Most responders indicated that they currently use in vitro lung models for inhalation/respiratory toxicity screening, although many also use them for chemical-specific mechanistic studies or IVIVE modeling. There is also interest in developing and validating the models for regulatory decision-making. The primary challenge or limitation identified in the use of these models was reproducibility (encompassing both data variability and method standardization), but other concerns included dosimetry (including test article generation and exposure), physiological/mechanistic relevance (model complexity, or lack thereof), model validation, and translational relevance/prediction (correlating in vitro model findings to data in vivo and in exposed humans).

To address the reproducibility concern, the NIEHS Division of Translational Toxicology has assembled a steering committee to oversee an interlaboratory reproducibility study that will involve multiple independent testing facilities. The steering committee is determining test

article generation and exposure systems and the source of air–liquid interface airway tissues used, appropriate chemistry and toxicological endpoints, standardizing the protocol according to the approach used by the Division of Translational Toxicology testing facility, formulating a plan for statistical analyses, and selecting three to five partnering facilities (federal or nonfederal) to conduct testing using 2,3-pentanedione.

Advancing immune-integrated MPS technology for pulmonary and systemic defense research

Tags: DoW, hepatotoxicity, immunotoxicity, inhalation toxicity, MPS

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 cell locations within airways and mucosal surfaces of the lungs. 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.

The U.S. Army CBC is incorporating immune components into commercially available MPS systems. CBC collaborated with the University of Colorado Anschutz Medical Campus to integrate a mast cell immune component into the Emulate system. CBC scientists observed significant increases in specific cytokine and chemokine production compared to chips without mast cells, which aligns with literature reporting. The findings from this study are captured in CBC Technical Report DEVCOM-CBC-TR-1941 “Incorporation of Immune Response into Organ-on-a-Chip Technology”.

This work has contributed to substantial efforts to incorporate other immune cells into the system to build a complete model of immune system infection to study prevention and pathogen defense. CBC recently received funding to establish a multiorgan chip system capable of recapitulating components of both the innate and adaptive immune system. Using the TissUse Chip3, they will combine liver, lung, and lymphoid follicle tissues along with peripheral blood mononuclear cells and other resident immune cell types. The long-term goal is to use this chip for viral infection challenge and vaccine candidate screening. Additionally, CBC has been working to bioprint human lung and skin tissues, complete with tissue-specific macrophages. This project aims to not only increase physiological accuracy for these tissue

types but will also provide a more robust model of immune response to microbial infections, which is largely lacking in most commercial organ-chip systems.

Liver-Chip drug development tool

Tags: FDA, hepatotoxicity, MPS

The FDA Center for Drug Evaluation and Research has marked a major milestone by accepting the first organ-on-a-chip technology into its Innovative Science and Technology Approaches for New Drugs (ISTAND) Program. This program promotes the use of innovative tools to improve how new drugs are developed. The accepted technology, called the Liver-Chip, is an MPS designed to predict drug-induced liver injury (DILI), a common reason drugs fail during early testing.

The Liver-Chip recreates the human liver environment by growing four types of liver cells in a small, engineered device. These cells mimic the natural physiology and mechanical forces they experience in the human body. This setup allows scientists to monitor in real time how drugs may cause liver damage compared to safe conditions. Importantly, the Liver-Chip evaluates whether a drug poses less, similar, or more risk of liver injury than other drugs in the same class. By improving preclinical testing, it can provide better data for drug investigational submissions and can help identify safer treatments earlier in development.

Initial Liver-Chip assessment has revealed the following:

- Conventional toxicity assays do not perform well in liver MPS.
- Alanine aminotransferase (ALT) activity is not stable in the Liver-Chip.
- Lactate dehydrogenase activity is prone to interference by medium components.
- ELISA is recommended for toxicity measurement in liver MPS.
- The chemical method is recommended for the urea assay in the Liver-Chip, offering adequate and improved specificity and sensitivity.

These results highlight the importance of reliable and reproducible assays for translational DILI biomarkers in liver MPS. Work is ongoing, demonstrating FDA's commitment to advancing new technologies, such as organ-on-a-chip systems, that have the potential to bring safer and more effective therapies to patients while reducing reliance on animal testing.

Liver-chip platforms for measuring drug injury biomarkers

Tags: FDA, harmonization, hepatotoxicity, MPS

Compared to conventional cell culture approaches, liver-on-a-chip models have the potential to better maintain cultures of primary hepatic cells, the current gold-standard model for drug metabolism and hepatotoxicity, and improve the prediction of DILI. Albumin, urea, ALT, aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) are proposed translational biomarkers for the prediction of DILI using liver MPS such as the Emulate® Liver-Chip. However, the performance of commonly used assays for these biomarkers in liver MPS may differ from clinical samples, posing a significant challenge in using NAMs data to predict clinical responses.

ALT and AST are both FDA-endorsed clinical biomarkers for DILI. While using the Liver-Chip, scientists in the FDA National Center for Toxicological Research (Shi et al. 2025) observed that when these biomarkers were measured using extensively validated reference procedures, the activity of ALT but not AST spontaneously decreased by approximately 50% every 24 h. Furthermore, ALT and AST activity remained below the detection limit (5 U/L) after acetaminophen treatment, when their protein concentrations were increased by approximately 20-fold as determined by ELISA. The protein concentrations of ALT and AST, but not their activity, showed good correlation with LDH activity, which was nearly eliminated after a freeze-thaw cycle. For viability of nonparenchymal hepatic cells, an unusually high background signal prevented the use of LDH activity assays; this was largely attributable to CultureBoost and fetal bovine serum in the perfusion medium, which are required to maintain key cell functions. However, LDH ELISA appeared to be a useful alternative. Of two widely used urea assays, the enzymatic approach was profoundly affected by medium supplements, wherein sample dilution increased the limit of detection, making it impossible to observe drug-induced urea inhibition. In contrast, the chemical assay offered adequate and improved specificity and sensitivity. For albumin, an ELISA had to be performed since routinely used dye-binding methods were not sensitive enough.

These findings provide a plausible explanation for many inconsistent reports in the literature and highlight the significance of establishing reliable and reproducible assays for

translational DILI biomarkers in liver MPS. Efforts are ongoing and will continue into 2026 and beyond to examine the performance of newly FDA-qualified and promising emerging clinical DILI biomarkers in liver NAMs models.

Survey of NAMs use to predict DILI risk and proposal of a reference drug list

Tags: FDA, data sharing, hepatotoxicity, MPS

DILI is a critical safety concern in drug development. The FDA is committed to reducing reliance on animal models in preclinical safety studies by promoting the use of NAMs. A review by scientists in the FDA NCTR evaluated the performance of NAMs, animal studies, and advanced MPS in predicting DILI risks (Shrimali et al. 2025). The study found that while in vitro NAMs showed advantages over traditional animal models in some contexts, there was significant variability in their predictive performance. Furthermore, the limited availability of data for MPS, a cutting-edge technology simulating human tissue-level responses, restricted the ability to make meaningful comparisons between these and other approaches. Overall, these findings highlight both the promise and challenges of integrating NAMs into DILI testing.

To systematically improve and benchmark NAMs for DILI prediction, the researchers proposed "DILIference", a curated reference drug list compiled from scientific literature. DILIference provides a standardized set of compounds with established liver toxicity profiles, serving as a critical resource for evaluating and refining DILI-predictive NAMs.

This work emphasizes the need for consistent benchmarks and robust datasets to further advance NAMs for regulatory use. By leveraging NAMs and tools like DILIference, researchers and regulatory bodies can reduce reliance on animal models while enhancing the reliability and human relevance of liver toxicity predictions in preclinical drug development.

Liver cell models for studies of cytotoxicity mechanisms of cannabidiol and analogs

Tags: FDA, hepatotoxicity, metabolism, stem cells

Researchers at FDA NCTR used complementary human liver cell systems to examine toxicity mechanisms of cannabidiol (CBD) and its analogs and metabolites. Together the studies illustrate the value of selecting the appropriate in vitro liver model for resolving metabolism-dependent versus metabolism-independent toxicity mechanisms.

- Chen et al. (2025) used mRNA sequencing in HepG2 cells to identify key cellular responses to CBD and its metabolites 7-hydroxy-CBD and 7-carboxy-CBD. Direct enzymatic assays confirmed strong inhibition of mitochondrial respiratory complexes IV and V by CBD and metabolite 7-hydroxy-CBD, with mitochondrial dysfunction further validated by glucose-galactose assay and loss of membrane potential by JC-1 staining. Autophagy, evidenced by autophagosome formation and enhanced autophagic flux, was the common cytotoxic mechanism shared across all three compounds, while mitochondrial complex inhibition was specific to CBD and 7-hydroxy-CBD.
- Campasino et al. (2024) exposed induced pluripotent stem cell (iPSC)-derived hepatocytes to CBD and hemp extract across a concentration range of 0.1 to 30 µM to assess hepatic viability and function. Overt cytotoxicity was observed only at 30 µM, but subcytotoxic concentrations of 0.1 to 3 µM produced significant changes in cytochrome P450 (CYP) enzyme activity, mitochondrial membrane potential, and lipid accumulation. Concentrations of 3 µM and greater additionally affected albumin and urea secretion, caspase 3/7 activity, and glutathione levels. The iPSC-derived hepatocyte model was thus able to detect functionally relevant perturbations below the cytotoxicity threshold, supporting its utility for early-stage safety evaluation of botanical compounds.
- Primary human hepatocytes and HepG2 cells were used by Li et al. (2025) alongside a panel of HepG2 lines individually overexpressing 14 human CYP enzymes to characterize the toxicity and metabolism of 7-hydroxy-CBD and 7-carboxy-CBD. Both metabolites induced cell cycle disturbances, apoptosis, and ER stress comparable to the parent compound. CYP2D6 was identified as the enzyme responsible for metabolizing 7-hydroxy-CBD, and its activity attenuated the associated cytotoxicity, apoptosis, and endoplasmic reticulum stress; 7-carboxy-CBD was not a substrate for any of the 14 CYPs tested. The study highlights how CYP-overexpressing cell lines can resolve metabolism-dependent differences in toxicological outcomes between structurally related metabolites.

TraCe MPS: Qualifying MPS as drug development tools

Tags: FDA, NCATS, developmental toxicity, hepatotoxicity, neurotoxicity, MPS

Many promising medications have failed to demonstrate safety and efficacy in human clinical trials despite encouraging results in preclinical studies. MPS, also known as tissue chips or organs-on-chips, offer a promising approach to generate more predictive preclinical data and improve the likelihood of clinical success. Built from human cells, MPS can replicate the structure and function of major organ systems, including the heart, kidneys, and lungs. Scientists are increasingly using tissue chips to evaluate drug effects on human tissues in a faster and more physiologically relevant manner than traditional preclinical methods, such as 2D cell cultures and animal models.

Since 2011, NCATS has supported the development and application of tissue chips for assessing drug safety and efficacy, modeling both rare and common diseases, informing the design of human clinical trials, and advancing precision medicine. In 2024, NCATS established four Translational Centers for Microphysiological Systems (TraCe MPS) to promote the broader adoption of tissue chip technologies, particularly in drug discovery and development (RFA-TR23-001). TraCe MPS is a partnership with the FDA ISTAND Program, with additional collaboration from the Critical Path Institute. The goal of the partnership is to qualify MPS, within defined contexts of use, as FDA-designated drug development tools. The overarching objective is to develop fit-for-purpose MPS platforms with clearly defined contexts of use that can support regulatory decision-making for new drugs and therapeutics.

The four Translational Centers are located at Texas A&M University in partnership with the University of Texas Medical Branch; the University of Pittsburgh; the University of Rochester in collaboration with Duke University; and the University of Washington in partnership with the Icahn School of Medicine at Mount Sinai. Current funded projects include models for kidney function (University of Washington and Mount Sinai), DILI and metabolic-associated steatotic liver disease (University of Pittsburgh), barrier tissues such as the blood–brain barrier (University of Rochester and Duke University), and placental–fetal development (Texas A&M University and the University of Texas Medical Branch). In addition to engagement with the FDA's ISTAND Program, NIH-funded awardees collaborate with biotechnology and pharmaceutical partners to refine contexts of use, broadening the pathway for adoption of MPS technologies.

Several translational centers have achieved notable progress within the ISTAND Program, with submissions publicly available on the FDA ISTAND website to support transparency and advancement in this rapidly evolving field. Accepted Letters of Intent for these models include:

- Two from the University of Pittsburgh: one focused on using their model to inform drug dosing in clinical trials for liver disease (DDT-IST-000034), and another on quantifying hepatotoxicity to support dosing decisions (DDT-IST-000044)
- One from the University of Washington and Mount Sinai team for assessing relative nephrotoxicity using a human kidney chip (DDT-IST-000045)
- One from the Texas A&M and University of Texas Medical Branch team for using a human chorio-decidual interface organ-on-chip to derisk positive rodent developmental and reproductive toxicity findings for novel investigational drugs (DDT-IST-000047)

Collectively, these efforts demonstrate the application of MPS technologies across multiple organ systems and regulatory contexts, including dose selection, toxicity assessment, and risk deconvolution. As of the end of 2025, the TraCe MPS is positioned to make a substantial contribution to advancing regulatory acceptance and adoption of MPS-based approaches.

Combinatorial NAMs approach to predict the placental transfer of drugs

Tags: FDA, defined approaches/IATA, developmental toxicity, machine learning

The ability to predict placental transfer of drugs administered during pregnancy is critically important to help manage the health of pregnant women and to include women of child-bearing potential in clinical trials. However, placental transfer assessments remain dependent on in vivo reproductive and developmental toxicology studies because there are no well-established NAMs-based approaches. Given the limitations of individual NAM platforms, a combinatorial approach integrating in silico and in vitro NAMs has greater potential for accurate predictions of placental transfer of drugs. FDA NCTR is evaluating the potential for an integrated approach using in silico, in vitro and ex vivo NAMs to predict placental transfer for a set of drugs. The in silico approach will combine rigorous model-building procedures utilizing 3D spatial molecular descriptors and advanced self-consistent algorithms free of overparameterization to generate accurate categorical models of placental

permeability. A combinatorial NAMs approach to predict placental transfer could inform weight-of-evidence-based decisions to enable earlier inclusion of women of child-bearing potential in clinical trials and expand access for pregnant women to experimental therapeutics, while reducing reliance on animal data.

Use of zebrafish embryos to assess protective effects of acetyl-l-carnitine

Tags: FDA, cardiotoxicity, developmental toxicity, small model organisms

An FDA NCTR study investigated the combined effects of verapamil (a drug used to treat high blood pressure, angina, and certain heart rhythm disorders) and sodium arsenite (a form of arsenic used in some pesticides, antiseptics, dye manufacturing, and chemical laboratories) on zebrafish (*Danio rerio*) embryos (Twaddle et al. 2025). Researchers aimed to understand how the interaction between these two chemicals affects development since verapamil can alter arsenic processing in the body. The results showed that zebrafish embryos exposed to sodium arsenite or verapamil alone survived without any major issues. However, when embryos were treated with both verapamil and moderate levels of sodium arsenite, survival rates decreased significantly to 23% and 17%, and the embryos showed developmental defects and heart damage. Interestingly, using a lower verapamil dose with arsenite resulted in no embryo deaths but caused developmental defects (cardiac and growth). Further analysis revealed that verapamil caused higher arsenic levels in the embryos by potentially blocking its removal. Verapamil also slightly reduced arsenic-induced cell death in the main body region (not yolk sac) but caused developmental and heart-related toxicity when paired with arsenic. However, the addition of acetyl-L-carnitine, a compound known to protect cells and mitochondria, completely prevented these toxic effects.

This study is the first to show that acetyl-L-carnitine can counteract the harmful effects of combined verapamil and arsenic exposure in zebrafish embryos, providing valuable insights into the mechanisms of these toxicities and potential therapeutic approaches. A second study showed N-acetylcysteine, another antioxidant and dietary supplement, also eliminated arsenic-induced cell death in zebrafish embryos (Gu et al. 2026). The zebrafish model has proven to be a useful tool for studying toxic elements, chemical interactions and their developmental impacts. Further studies are ongoing to determine the combined effects of arsenic and cadmium on development and the nervous system.

Pluripotent stem cells for target organ developmental toxicity testing

Tags: NIEHS, developmental toxicity, stem cells

Prenatal developmental toxicity research explores how environmental substances, drugs, and chemicals can negatively affect the growth of embryos and fetuses. Traditionally, these studies have relied on animal models, but ethical concerns and the need for human-relevant models have led researchers to explore new methods. One promising tool is pluripotent stem cells, which can differentiate into any type of human cell. Pluripotent stem cell models are increasingly used to simulate human development in the laboratory. Two-dimensional models are easier to use and can screen many chemicals quickly, but they lack the complexity of real human tissues. On the other hand, 3D models, such as organoids, create miniature versions of organs and tissues that mimic the conditions of a living body. While more realistic than 2D models, 3D models are challenging to produce and analyze, often requiring expensive tools and techniques.

A review by NIEHS scientists (Wu et al. 2024) highlights progress in the use of pluripotent stem cells to create models of brain and heart development, which are critical areas for understanding toxicity during pregnancy. Researchers are also combining pluripotent stem cell models with innovative technologies to improve testing methods and produce data that is more relevant to humans. These advancements help scientists predict potential developmental risks posed by chemicals, offering tools that are more ethical and accurate than traditional animal-based methods. Ultimately, these breakthroughs are paving the way for better decision-making in prenatal toxicity testing, helping regulators protect human health while advancing the science of developmental biology and toxicity assessment.

Application of IATAs to DNT evaluation of organophosphorus flame retardants

Tags: NIEHS, defined approaches/IATA, developmental neurotoxicity, endocrine disruptors, small model organisms

Organophosphorus flame retardants (OPFRs) are widely used chemicals that persist in the environment, yet limited information regarding their potential toxicity exists. Their structural similarity to organophosphate pesticides raises concerns about developmental neurotoxicity (DNT), particularly for human health. However, traditional in vivo testing methods are not suitable for evaluating the wide range of OPFRs that lack safety data.

To address this gap, NIEHS researchers applied an in vitro testing battery designed to assess key processes in neurodevelopment and function (Kreutz et al. 2024). Behavioral data from small model organisms were also included. This study used an IATA combining data from experimental assays, the NIH Integrated Chemical Environment (NICE) database, and existing literature. Human exposure data and physiologically based toxicokinetic models were used to compare in vitro toxicity thresholds with predicted human plasma concentrations.

Eight OPFRs, including aromatic OPFRs and halogenated flame retardants, were evaluated alongside two brominated flame retardants known to cause DNT. Results showed that several aromatic OPFRs (e.g., triphenyl phosphate and isodecyl diphenyl phosphate) demonstrated activity at concentrations comparable to the toxic brominated flame retardants, suggesting a need for further investigation. While the in vitro assays highlighted potential hazards, they provided limited insights into mechanisms of toxicity. By integrating additional information, endocrine disruption was identified as a potential mechanism of action for some OPFRs.

This study underscores the utility of an IATA for prioritizing chemicals of concern. It also found that human exposure to some OPFRs may result in plasma concentrations similar to those that trigger activity in vitro, raising concerns about potential human health risks. These findings provide a foundation for future investigations into OPFR safety and the mechanisms driving their neurotoxicity.

Integrating screening-level DNT information of chemicals in a NAMs battery

Tags: EPA, NIEHS, developmental neurotoxicity, small model organisms

Chemical exposure is believed to play a role in neurodevelopmental disorders, but most chemicals have not been tested for DNT potential. Traditional testing methods rely on animal models, but these tests are expensive, require many animals, and sometimes produce results that are not reliable for regulatory decisions. To overcome these limitations, the NIEHS Division of Translational Toxicology launched the DNT Health Effects Innovation program in 2019 to improve how chemical risks to the developing nervous system are evaluated.

The program applied a DNT screening battery to test chemicals quickly and efficiently using modern methods. This battery includes both 2D and 3D in vitro tests with human and rodent cells to assess key brain development processes, such as cell growth, migration, neurite

development, and neural network function. It also uses zebrafish embryos to study neurobehavioral effects. Over 300 chemicals nominated by stakeholders have been selected and distributed for testing using this battery.

A key accomplishment of the program is the creation of a unified data analysis system that integrates information from different tests. This system, which includes tools such as the Toxicological Prioritization Index (ToxPi), helps evaluate DNT hazards and prioritize chemicals for further study. The strategy demonstrates how the DNT screening battery can effectively identify chemicals for further evaluation, providing a systematic way to interpret results and guide decision-making. This innovative approach simplifies and modernizes DNT testing, ensuring timely, ethical, and scientifically reliable evaluations of chemicals for their effects on brain development. A paper describing the first testing phase of 95 chemicals has been accepted for publication in *Neurotoxicology* (Hall et al. 2026).

Application of human neural stem cell models to assess DNT potential of drugs of abuse

Tags: FDA, developmental neurotoxicity, stem cells

Two FDA NCTR studies used commercially available human fetal neural stem cells as a platform to assess the DNT potential of drugs often subject to abuse.

- Neural stem cells derived from fetal hippocampal tissue were exposed to CBD, its major metabolites 7-OH-CBD and 7-COOH-CBD, and delta-9 tetrahydrocannabinol across a range of clinically relevant concentrations for up to 7 days (Latham et al. 2025). All compounds caused dose- and duration-dependent cell death, and CBD and 7-OH-CBD additionally reduced the G1-phase cell population in cell cycle analysis. Combination exposures of cannabidiol with 7-OH-CBD produced additive cytotoxicity, providing direct evidence that the metabolite contributes to overall neurotoxicity in vivo. Gene expression studies also suggested that prenatal cannabinoid exposure may impair astrocyte maturation and endocannabinoid signaling in the developing brain.
- Human neural stem cells and cells differentiated from them were exposed to fentanyl at concentrations of 0.5 to 100 µM for 24 hours. Standard cytotoxicity assays detected no significant cytotoxicity or mitochondrial impairment at any concentration tested. However, chromatographic lipidomic analysis of differentiated neural cells revealed

dose-dependent effects indicating disruption of the ceramide/sphingolipid pathway at the highest dose. These findings demonstrate that lipidomic profiling can uncover early-stage neurochemical effects of opioid exposure that are invisible to conventional cell viability endpoints, highlighting the value of multiendpoint approaches when evaluating DNT (Wang et al. 2025).

Application of an in vitro human iPSC-based model for drug-induced DNT studies

Tags: FDA, developmental neurotoxicity, stem cells

Exposure to opioids during early pregnancy has been associated with a risk of neural tube defects in nonclinical animal models and human epidemiological studies. However, challenges stemming from study design limitations and conflicting outcomes in human and animal research have complicated risk assessment of the drug class. While animal studies are still required for embryo–fetal development assessments for therapeutics, the evolving regulatory landscape in drug development has recognized the value of NAMs to supplement current animal studies. Specifically, human iPSC-based models are increasingly being used in assessments of DNT potential.

FDA NCTR scientists used these models to recapitulate aspects of neural tube formation in culture to (1) determine which opioids pose greatest risk to the developing fetus, (2) identify the most susceptible neural cell types, and (3) investigate toxicological mechanisms of action without the need for animal studies. In 2024 and 2025, three human iPSC lines derived from healthy female donors were differentiated to neural progenitor cells using an optimized protocol for establishment of neural rosettes of embryoid bodies, three-dimensional aggregates formed by pluripotent stem cells. Opioids, including morphine, methadone, codeine, and buprenorphine, were tested in this protocol at concentrations ranging from 3x to 1000x the clinical maximum plasma concentration (Cmax). Opioid treatment had no clear effect on iPSC proliferation, differentiation, embryoid body development, or neural rosette formation at concentrations less than 30x Cmax. Methadone, however, inhibited neural rosette formation at concentrations exceeding 300x Cmax. Subsequent studies also identified potential adverse effects on neural differentiation and activity following high-dose methadone treatment. Follow-up gene expression analysis identified a potentially important pathway in methadone-induced DNT. This work was presented at the 2024 Annual Meeting

of the Society of Toxicology (Cai et al., Abstract 4461), with peer-reviewed manuscripts anticipated for publication by 2027.

Using nonhuman primate stem cell models to assess desflurane-induced neurotoxicity

Tags: FDA, developmental neurotoxicity, stem cells

Safety concerns about general anesthetics, such as desflurane, have arisen due to evidence of potential harmful effects on the developing brain after early-life exposure. A study by FDA NCTR and the FDA Center for Drug Evaluation and Research used neural stem cells from the hippocampus of monkey brains to investigate how desflurane affects brain development processes, including cell health, growth, and differentiation into specialized brain cells, namely neurons and glial cells (Wang et al. 2025). The goal was to establish a direct biological link between historical in vivo nonhuman primate data and cellular responses, providing evidence that could help reduce or replace the need for future nonhuman primate animal studies.

When exposed to desflurane at clinically relevant levels (5.7%), the neural stem cells showed no notable damage or changes in their ability to grow or survive after both short-term (3 hours) and longer-term (24 hours) exposures. However, once the neural stem cells were guided to differentiate into different types of neural cells, prolonged exposure to desflurane caused significant damage. Markers of cell injury, such as lactate dehydrogenase and increased inflammation-related proteins, were elevated after 24 hours. In addition, the number of neurons, as identified by a key marker called PSA-NCAM, was greatly reduced after prolonged exposure to desflurane. These results suggest that while desflurane does not harm neural stem cells directly, it can cause damage to newly formed neurons after long-term exposure. The study highlights PSA-NCAM as an important marker for understanding how anesthetics affect neuron health and provides insight into the mechanisms of anesthesia-induced neurotoxicity. These findings could help improve the safe use of desflurane and other anesthetics in children, reducing potential risks to brain development during early life.

Application of human brain organoids to investigate drug-related neuromodulatory effects and potential mechanisms of action of neuropsychiatric effects

Tags: FDA, neurotoxicity

Montelukast (Singulair®) is a G protein-coupled receptor antagonist targeting a specific receptor in the lung. Montelukast is prescribed to millions of patients every year, including very young children, to prevent and treat asthma, allergic rhinitis, and exercise-induced bronchoconstriction. However, montelukast has been associated with reports of serious neuropsychiatric adverse events. FDA NCTR scientists are using a central nervous system-3D organoid NAM to confirm the potential for off-target central nervous system receptor activation and downstream neuromodulation of human central nervous system cells. In 2024 and 2025, extensive secondary pharmacology studies were conducted; these studies identified several off-target candidate receptors expressed in the brain with significant binding and functional activity in non-neuronal cells overexpressing off-target candidate receptors. The human iPSC-derived brain organoids are being used to investigate the neuromodulatory effects of montelukast at endogenous receptors in the human brain and toxicological mechanisms of action. This study represents application of a human-based NAM to address a complex neuropsychiatric context of use for drug risk assessment. Planned future activities include further investigation of the neuromodulatory effects of montelukast at endogenous receptors in human brain 3D organoids and toxicological mechanisms of action.

Evaluation of NAMs for assessment of acute neurotoxicity

Tags: DoW, MPS, neurotoxicity

Assessment of health risks posed by chemical exposures is a fundamental component of warfighter health protection. Neurotoxicity is of particular concern for the military due to the potential for diminished performance and loss of able forces and an elevated risk of mission failure. USAF PRT is leading a cross-service collaboration with the Air Force Research Laboratory MPS Team, Army CBC, Naval Medical Research Unit Dayton and Sanford Burnham Prebys Medical Discovery Institute to evaluate the predictive capacity and reproducibility of several in vitro (2D, 3D, brain organoid, organ-on-a-chip) and ex vivo (rodent-derived brain slice) neurotoxicity models. These are being applied to qualitative (hazard identification) and quantitative (derivation of a POD) risk assessments of chlorpyrifos, chlorpyrifos-oxon, diisopropyl fluorophosphate, and carbaryl. In vitro data will be compared to concurrent in vivo rodent study data. Dose ranges for testing were initially

estimated using in silico toxicokinetic models and published literature. Data collection efforts are nearly complete and will be used to estimate PODs using benchmark dose modeling. PODs from in vitro and ex vivo models will then be translated to in vivo doses using IVIVE modeling, and derived external dose PODs will be compared to in vivo PODs. The end product will be a recommended nonanimal test strategy for acute neurotoxicity via acetylcholinesterase inhibition that can also serve as a foundation for other neurotoxicity pathways. Data and strategies developed in this project will improve our ability to protect the warfighter from neurotoxic injury due to operational exposures.

Application of NAMs to predict drug seizure risk

Tags: NIEHS, AOP, neurotoxicity

Animal studies have long been used to assess toxicity in drug development and environmental safety, particularly for central nervous system effects such as seizures. However, these animal studies often fail to accurately predict seizure risks in humans, leading to unexpected adverse events during clinical trials or leaving certain environmental compounds, like pesticides, untested due to limited resources.

To improve seizure risk assessment, NICEATM scientists and collaborators explored a novel approach integrating mechanism-based insights (Behl et al. 2025). The investigators identified 27 biological target families associated with seizure risk, combining drug discovery data with key events derived from AOPs. Over 100 in vitro assay endpoints were annotated to 26 of these target families, which included vital components such as neurotransmitter receptors, transporters, and calcium channels. Researchers reviewed a dataset of seizure-inducing and non-seizure-inducing chemicals, with 80% of these compounds tested in the in vitro assays. While the approach showed promise, certain target families lacked sufficient data, highlighting areas for further research.

The findings demonstrate that seizure risk has potential to be assessed using an AOP framework combined with in vitro testing. This approach improves early identification of seizure risk during drug development and environmental chemical evaluations. Expanding the assay panels to include additional targets will further enhance the predictivity of this methodology. By adopting this mechanistic and human-relevant strategy, researchers can better prioritize compounds for study, reduce reliance on less-predictive animal models, and

optimize resource use. Ultimately, this approach strengthens central nervous system safety evaluations, supports public health protection from neurotoxicants, and bridges critical gaps in scientific knowledge. This work was highlighted together with other NAMs in a *Drug Discovery Today* review of this topic (Roberts et al. 2026).

Application of a brain-on-a-chip model to understand mechanisms of Alzheimer's disease

Tags: FDA, MPS, neurotoxicity, stem cells

Alzheimer's disease is characterized by excess free and aggregated amyloid-beta peptides and by intracellular neurofibrillary tangles that contain hyperphosphorylated and misfolded tau protein. Moreover, research has reported neurovascular dysfunction in patients with mild cognitive impairment and Alzheimer's disease. These data suggest early neurovascular impairment may contribute to or exacerbate the underlying pathophysiology of Alzheimer's disease.

Researchers at FDA NCTR and collaborators at Emulate developed a brain-chip model of the neurovascular unit using patient-derived human iPSCs (Shen et al. 2026). Cells from an Alzheimer's disease patient and a cognitively normal individual were used to differentiate cell types that constitute the neurovascular unit, including brain microvascular endothelial cells, neurons, astrocytes, microglia, and pericytes. The model allowed researchers to provide proof-of-concept evidence that a brain-chip model was sensitive to neurovascular unit endpoints that are affected in Alzheimer's disease. The study found that Alzheimer's disease brain-chips showed increased paracellular permeability, lower tight-junction protein expression, and reduced activity of P-glycoprotein, a key blood–brain barrier efflux transporter, compared to cognitively normal brain-chips. Additionally, tau protein buildup was observed in the vascular channel, along with increased markers of inflammation. Notably, Alzheimer's disease brain-chips had lower levels of amyloid-beta 42 compared to the cognitively normal brain-chips, a finding that is not consistent with Alzheimer's disease pathology and supports the need to include multiple patient-derived human iPSC cell lines when studying heterogenous brain diseases like Alzheimer's disease. This brain-chip model provides a basis for researchers to investigate blood–brain barrier and neurovascular unit changes using NAMs. Importantly, the study emphasizes the use of a fully isogenic model and neurovascular unit-related endpoints, such as paracellular permeability and

P-glycoprotein functioning. While promising, the findings from the study highlight the need to include multiple human iPSC donor lines, as significant genetic variability is part of the model's limitation. Complete characterization and validation of these models will be necessary before they can be used with confidence in initial evaluations of safety and efficacy of novel therapies being developed for Alzheimer's disease.

Predicting oncology drug-induced cardiotoxicity with donor-specific iPSC-derived cardiomyocytes

Tags: FDA, cardiotoxicity, stem cells

Many cancer drugs, such as doxorubicin, can cause cardiotoxicity in some patients, reducing drug effectiveness as lifesaving treatments. To better understand differences in responses, FDA NCTR researchers used human iPSC-cardiomyocytes, human heart cells created from donor-specific stem cells, to study variability in how individual patients respond to doxorubicin-induced cardiotoxicity (Pang et al. 2024). The study found that iPSC-cardiomyocytes from different donors had varying responses to doxorubicin, resembling patterns seen in real-world clinical practice. By categorizing iPSC-cardiomyocytes into "resistant" and "sensitive" groups based on their reactions to doxorubicin doses, FDA researchers demonstrated that laboratory results could predict how likely a patient is to experience heart damage from treatment. Importantly, they identified a key gene, DND1, which was expressed differently in doxorubicin-resistant versus doxorubicin-sensitive cells, potentially playing a role in cardiotoxicity.
This research highlights the usefulness of donor-specific iPSC-cardiomyocytes for studying differences in drug-induced heart risk among individuals. The project will be continuing into 2026 and beyond to evaluate larger panels of iPSC-cardiomyocytes from diverse donors, aiming to identify new genetic and molecular markers for predicting cardiotoxicity from doxorubicin and other cancer drugs. By providing a more personalized method to understand drug risks, this approach could help reduce heart-related side effects, improve precision medicine in cancer care, and ensure safer treatment strategies for vulnerable patients.

Validating and using cardiac NAMs for toxicity screening and drug development

Tags: FDA, cardiotoxicity, MPS, stem cells

Advances in technology and a push to reduce reliance on animal models are driving the adoption of human-relevant models in drug development. At a May 2024 workshop held by the Health and Environmental Sciences Institute, FDA and other experts discussed how NAMs can improve the safety evaluation of drugs, especially in detecting potential heart-related risks (Pierson et al. 2025).

The workshop focused on innovative tools including human iPSC-cardiomyocytes, microfluidic systems such as BioFlux™, and engineered heart tissues. These systems recreate human heart functions more accurately than traditional animal models, allowing researchers to better predict how drugs might cause cardiac problems. Regulatory representatives emphasized the importance of creating uniform standards and validation protocols for integrating these methods into drug approval processes.

Case studies presented during the workshop highlighted how NAMs often outperform traditional methods in predicting drug safety and identifying issues early in development. These successes show the potential for NAMs to refine cardiovascular drug research, reduce failures during later clinical trials, and support the adoption of more ethical preclinical testing methods. However, the transition to the widespread use of NAMs will require collaboration between academic researchers, industry experts, and regulatory agencies. Together, these groups aim to validate the methods, improve drug testing efficiency, and help create a new era of drug research that is both scientifically advanced and less reliant on animal models.

Using engineered heart tissues to characterize sex differences in chemotherapy-induced cardiotoxicity

Tags: FDA, cardiotoxicity, population variability, stem cells

Cardiovascular toxicity from chemotherapy drugs affects men and women differently, but the underlying mechanisms remain poorly understood. Traditional preclinical cardiac safety studies rely heavily on animal models, often including only male participants, which fails to capture sex-specific differences observed in human patients. An FDA NCTR project addressed this critical gap by developing a human-based laboratory model to study sex-specific cardiac responses to cancer drugs. This model utilizes engineered 3D cardiac tissue constructs created from human iPSCs that can contract and function similarly to actual heart muscle. The engineered heart tissues offer a human-relevant alternative that can

maintain stable contractile function for over 100 days, enabling long-term toxicity assessment without animal use. This approach is anticipated to be faster and more cost-effective and provide human-specific data that may predict clinical outcomes better than animal models.

The ongoing project has three main objectives: (1) characterize baseline differences between engineered male and female heart tissues, (2) evaluate the role of sex hormones in cardiac function, and (3) test cardiotoxic chemotherapy drugs such as doxorubicin and paclitaxel. In 2024 and 2025, the research team successfully established protocols for generating stable engineered heart tissues in-house from human iPSCs and demonstrated their capability to detect drug-induced contractile dysfunction. In 2026 and beyond, the team will evaluate the effects of sex hormones and chemotherapy drugs on cardiac contractility using this platform. This innovative platform has significant potential for regulatory applications, enabling sex-specific risk assessment of pharmaceuticals while advancing the FDA's mission to expand nonanimal testing methodologies.

Use of human iPSC-cardiomyocyte data for safety and regulatory applications

Tags: FDA, cardiotoxicity, data sharing, harmonization, stem cells

Human iPSC-cardiomyocytes are increasingly being used in advanced NAMs to assess heart-related drug safety in regulatory toxicology. These stem cell-derived heart cells provide a promising alternative to traditional animal-based methods, offering human-specific insights in nonclinical drug evaluations. Scientists in the FDA Center for Drug Evaluation and Research reviewed the landscape of human iPSC-cardiomyocyte submissions to the FDA, highlighting trends and areas for improvement in their regulatory use (Simpson et al. 2025). The search method used was more comprehensive than those used in previous studies and emphasized the importance of standardized keywords in study titles for easier identification of NAMs submitted to the FDA. The study identified an increasing number of human iPSC-cardiomyocyte studies being submitted to the FDA. Most studies relied on the multielectrode array platform, which measures electrical activity in cardiomyocytes. While the review confirmed increasing adoption of human iPSC-cardiomyocyte methodologies, the researchers, who are also involved in nonclinical review of drug applications, noted several challenges. Specifically, study methodologies, context of use, and results of archived

submissions were often unclear, limiting their impact on FDA regulatory decisions. More detailed reporting of clinical relevance of human iPSC-cardiomyocyte findings in submitted studies and a better understanding of their predictive accuracy compared to traditional nonclinical methods could broaden the application of human iPSC-cardiomyocytes in regulatory contexts.

This review emphasizes the need for standardized reporting to build confidence in the reliability and relevance of human iPSC-cardiomyocyte data. Such improvements would help the FDA better integrate these innovative methods into decision-making processes. By systematically submitting robust and detailed human iPSC-cardiomyocyte data to the FDA and further demonstrating their predictivity, researchers and applicants can advance the regulatory acceptance of these human-based methodologies, paving the way for safer and more efficient drug evaluations.

Application of a 3D liquid cell culture model to study breast cancer metastasis

Tags: NCI, carcinogenicity, metabolism

Breast cancer is the most common cancer in women and second leading cause of cancer-related death. Breast cancer-specific mortality results from metastasis to other organs, which often develops many years after successful surgery and recovery from the initial primary diagnosis and treatment. NCI is investigating the molecular mechanisms that help cancer cells establish metastases, which are often unique when compared to the primary tumors. Through extensive multipronged characterizations, researchers have demonstrated that a specialized yet simple 3D liquid culture model more accurately replicates how tumor cells behave in the body than traditional 2D systems (Balamurugan et al. 2026; Sterneck et al. 2025). This model helped reveal how inflammatory signals from molecules, such as prostaglandins, stabilize adhesion proteins such as E-cadherin, enhancing cancer cell survival and metastatic potential (Balamurugan et al. 2023). Additionally, the 3D culture model allowed the researchers to discover a targetable molecular link between the E-cadherin cell–cell adhesion molecule and mitochondrial metabolism, which sustains tumor growth (Balamurugan et al. 2026). This innovative model is also being applied to better understand the biology of rare circulating tumor cell clusters, which are most efficient in seeding metastases, and the potential for liquid biopsy applications. This research provides new

insights into metastasis, creating opportunities to improve cancer diagnosis, personalized treatments, and patient outcomes.

Patient-derived tumor organoids for optimization of cancer therapies

Tags: NCI, carcinogenicity

Common epithelial cancers, including colon, breast, pancreatic, and biliary cancers, have patient-specific mutations that lead to the development of antigens that drive the T cell response to these cancers, called neoantigens. Therapies using T cells often succeed in treating these cancers, but when a patient does not respond to this approach, the treatment failure may be due to reasons related to the patient's specific tumor. NCI researchers are developing patient-derived tumor organoids to investigate the mechanisms of treatment failure and optimize subsequent treatment options (Gustafson et al. 2026). Because common epithelial cancers rarely grow in conventional 2D culture, 3D matrix-based organoids are generated from each treated patient to serve as autologous tumor surrogates. Studies using these models showed that organoid recognition by tumor-infiltrating lymphocytes correlates with higher clinical response rates. Ongoing work involves investigating discrepancies between organoid recognition and neoantigen presentation by normal antigen-presenting cells. In addition, whole-exome sequencing is being used to identify mutations, and the neoantigens related to these mutations are being cloned for further study.

Integrating transcriptomic and targeted NAMs into a tiered framework for chemical bioactivity screening

Tags: EPA, genotoxicity

With thousands of chemicals present in the environment, identifying which ones may pose risks to human health is a critical task. Scientists are using advanced laboratory methods like high-throughput transcriptomics to quickly determine how chemicals might harm biological systems. High-throughput transcriptomics looks at changes in gene activity (transcriptomics) when cells are exposed to chemicals, helping pinpoint which molecular targets or pathways might be affected.

EPA scientists developed an approach to link transcriptomic results to specific molecular targets, such as nuclear receptors, and combine those findings with more focused receptor-level tests to confirm their accuracy (Rogers et al. 2025). Using special gene-based

"signatures" developed from known reference chemicals, researchers screened 1,126 chemicals, identifying 201 that showed specific bioactivity linked to those targets. Of these, 57 chemicals were confirmed as selective nuclear receptor activators. When the researchers lacked data to confirm predictions, they tested 37 chemicals using five receptor-level assays, ultimately confirming that 18 (49%) of these had bioactivity as predicted. For prioritized chemicals, transcriptomics provided better or equally sensitive thresholds for potential effects compared to traditional pathway-based measures in 95.4% of cases. This innovative approach demonstrates how integrating high-throughput transcriptomics with targeted testing is highly effective at identifying chemicals with specific biological activity. The approach can help prioritize chemicals for further study, supporting risk assessment strategies that are faster, more efficient, and less reliant on traditional animal models. It offers a promising step forward in identifying potential chemical hazards more effectively.

Integration of NAMs for the assessment of data-poor chemicals

Tags: EPA, exposure

NAMs can be used to rapidly evaluate chemical safety without relying on traditional animal models. One key tool is the combination of in vitro high-throughput laboratory tests and in silico computer models to predict how chemicals might affect human health. These methods can help set a POD, or the level of chemical exposure that starts to cause harmful effects, and compare it to estimated exposure levels, resulting in a bioactivity-to-exposure ratio. This ratio helps prioritize chemicals for further testing based on potential risks. Additionally, these tools flag potential hazards, such as effects on hormones, brain development, or the immune system, for further investigation.

In a case study conducted by an international group of researchers, including researchers from the EPA, 200 chemicals with little traditional toxicity data were tested using NAMs (Paul Friedman et al. 2025). Scientists combined several advanced methods, including transcriptomics (the study of gene activity), phenotypic profiling (observing cellular traits), biochemical tests, and high-throughput toxicokinetic models, to estimate safe exposure levels for these chemicals. The results provided bioactivity-based PODs and helped identify potential hazards for further study.

This case study shows how NAMs can efficiently address gaps in chemical safety data, ensuring human health is protected while reducing the need for animal testing. By offering a reusable and flexible approach, this effort supports the use of cutting-edge science in managing chemical risks around the world. It demonstrates that combining NAM tools can provide reliable data for screening chemicals, improving our ability to assess risks quickly and ethically.

Toxicological profiling of environmental mixtures containing metals using in vitro NAMs

Tags: NCATS, NIEHS, ecotoxicity, genotoxicity, mixtures toxicity

Cleanup of industrial sites contaminated with hazardous waste is administered through the EPA Superfund program. At these sites, soil, air, and water may contain hazardous materials, including metals. Individuals residing and working in these areas can be exposed to mixtures of these substances, and such exposures can lead to many adverse health outcomes, including cancer. However, the risk presented by mixtures of hazardous materials compared to that of individual components has not been studied thoroughly. NCATS and NIEHS researchers investigated the toxicity of metal mixtures in five categories of in vitro assays (Ooka et al. 2025) measuring cytotoxicity, oxidative stress, genotoxicity, cytokine release, and blood vessel development (angiogenesis). Testing of these mixtures in primary cells and cell lines demonstrated that nickel/arsenic/cadmium and beryllium/arsenic/cadmium combinations exhibited higher cytotoxicity than their individual components, suggesting that the mixtures amplified the cytotoxic effect. Because oxidative stress is a common factor in most metal-related toxicities, the mixtures were further tested in cell-based assays to measure oxidative stress. Results showed that cadmium-induced oxidative stress was increased in some mixtures. The mixtures that induced oxidative stress also exhibited increased DNA damage, inhibited DNA synthesis, and activated the p53 transcription factor pathway. In addition, compared to their component compounds, the mixtures significantly increased interleukin-8 secretion, a marker of inflammatory response, and angiogenesis. These results demonstrate the utility of in vitro NAMs to explore mechanisms of mixture-induced toxicity.

Comparison of human versus macaque primary hepatocyte models for DNA damage

Tags: FDA, genotoxicity, metabolism

N-nitrosamines are chemical impurities increasingly detected in human drugs, and there is concern about their potential to cause mutations and cancer. To better understand their effects, FDA NCTR researchers (Seo et al. 2025) conducted a study comparing the DNA damage caused by eight nitrosamines in primary human hepatocytes and primary macaque hepatocytes. They measured DNA damage and analyzed cellular responses to validate potential genotoxic risks using tests including the comet assay. This is a sensitive technique used to detect DNA damage at the level of individual cells. It involves the electrophoresis of lysed cells in agarose gel, allowing damaged DNA to migrate and form a "comet" shape under fluorescence analysis.

The results revealed that all eight nitrosamines caused significant DNA damage in hepatocytes from both species. Interestingly, most nitrosamines resulted in higher DNA damage in macaque hepatocytes than in human hepatocytes, with variations linked to enzyme activity differences. Human hepatocytes showed greater variability in responses between donors, particularly in CYP enzymes, which influence how toxic chemicals are broken down in the body. Further tests, including western blot analysis, suggested that the presence of specific chemical groups, such as ethyl groups, may influence how nitrosamines promote DNA damage and cell death. Modeling results revealed more conservative threshold values (called benchmark concentrations) for DNA damage in human hepatocytes than in macaque hepatocytes, suggesting that human hepatocytes may have higher sensitivity to nitrosamines. The results align with findings from earlier studies using 3D HepaRG spheroid models, supporting the relevance of these results in human health risk assessments. Overall, this research highlights critical human-relevant findings of the genotoxic potential of nitrosamines and underscores the importance of investigating species differences to improve drug safety standards and toxicological evaluations.

Use of in vitro intestinal cell models to investigate effect of silver nanoparticles on intestinal pathogen barrier function

Tags: FDA

Silver nanoparticles (AgNPs), which are widely used for their antimicrobial properties in food packaging and dietary supplements, may pose risks to gut health. Previous studies showed that AgNPs affect gut microbial composition and epithelial function, and research by

FDA NCTR explored how AgNPs impact host-pathogen interactions at the intestinal barrier, particularly in the context of *Salmonella* serovar Heidelberg, a common cause of foodborne illness (Hanafy et al. 2026).

In laboratory tests, human intestinal epithelial cells were exposed to small (10 nm) AgNPs at safe, nontoxic levels. While AgNP exposure did not affect the ability of the bacteria to adhere to the cells initially, it significantly increased bacterial invasion and survival inside cells, particularly at the 10 µg/mL concentration. This indicates a weakened epithelial barrier, suggesting AgNP exposure can make cells more vulnerable to infection. FDA researchers observed changes in gene expression related to intestinal permeability, with affected genes controlling tight junctions, cell connections, and structural remodeling. Even though the cells activated repair mechanisms, these efforts were not strong enough to prevent *Salmonella* invasion and persistence. AgNP exposure also increased proinflammatory cytokines, worsening inflammation, while reducing key anti-inflammatory and repair factors.

These findings reveal that even low doses of AgNPs can disrupt the gut barrier, making it easier for pathogens including *Salmonella* to invade and persist. Given their increasing presence in consumable products, this study emphasizes the need to reassess the safety of AgNPs, especially regarding gastrointestinal health and infection risk. These findings also reveal that in vitro methods can successfully characterize toxicity of a nanomaterial.

Multiorgan-on-a-chip platforms for studying pathogen infection and antibiotic efficacy

Tags: DoW, MPS

The U.S. Army CBC recently completed a project aiming to determine if multiorgan chips can be effective models for the establishment of bacterial infection and for characterization of antibiotic efficacy and safety. This effort enabled CBC to be the first end-user of the Chip4 platform, a multiorgan chip that encompasses four different organ models containing two independent media circuits, representing blood and excretion. To evaluate this application, CBC used methicillin-resistant *Staphylococcus aureus* (MRSA) and its primary antibiotic, vancomycin. MRSA was selected as the target bacteria because vancomycin has been characterized in clinical data. The efficacy of vancomycin within the multiorgan chip platform after MRSA exposure was analyzed while the platform and potential compatible downstream analysis techniques were established. CBC scientists were able to establish

MRSA infections in the multiorgan chips and observe the efficacy of vancomycin. They were also able to characterize the safety of the antibiotic, specifically pertaining to cytotoxicity. The data generated from this project are included in the CBC technical report DEVCOM-CBC-TR-1996 "Incorporation of a Multi-Organ-on-a-Chip Platform to Assess Drug Candidate Characterization for Methicillin-Resistant *Staphylococcus aureus* (MRSA)". Follow-up work for this study is ongoing. The current goal is to establish a multiorgan chip viral infection model capable of characterizing biosafety level 3 and 4 viral pathogens of concern for medical countermeasure development and testing.

The CBC has also been working toward creating a more physiologically relevant organ-chip infection model through the incorporation of commensal microbial communities to human tissues. Skin and buccal chip models will be cocultured with microbial species representative of their specific microbiomes, and the chips will be subsequently exposed to relevant bacterial pathogens. By combining cutting-edge organ-chip technology with microbiome integration and sophisticated downstream analysis, this program seeks to advance the understanding of biological agent pathogen toxicity and improve predictive models for diagnostics development.

Advancing organ-on-a-chip technologies for biomarker discovery and translational toxicology research

Tags: DoW, MPS, metabolism

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. U.S. Army CBC scientists investigated how well bioinformatic data collected from organ chips match bioinformatic data collected from human samples, with the aim of using organ chips as an alternative approach for biomarker discovery in toxicity assessment and exposure identification. To do this, 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 the

data 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. A paper describing this work is currently under review with the *Journal of Proteome Research*. Follow-up work that aims to establish animal organ chips and examine their translational potential was recently funded. Currently, CBC is developing rat and nonhuman primate multiorgan chip systems and will compare the effects of acetaminophen on the incorporated tissues to results reported in historic and published literature.

Insights derived from testing a PFAS library in a battery of NAMs

Tags: EPA, NIEHS, PFAS, small model organisms

Per- and polyfluoroalkyl substances (PFAS) are a large group of human-made chemicals widely used in industry and consumer products. They are highly persistent in the environment, can bioaccumulate, and pose potential toxicity concerns. While a handful of PFAS have been studied for their hazard profiles, most remain poorly understood due to the number of chemicals involved. Evaluating thousands of PFAS using traditional methods would require substantial time, cost, and animal resources, prompting researchers to seek more efficient alternatives.

EPA and the NIEHS Division of Translational Toxicology launched a research project to address this challenge (Patlewicz et al. 2026). The study screened approximately 150 PFAS using a range of NAMs, including small model organisms such as zebrafish; in vitro assays that examine cellular and biochemical activities; and toxicokinetic assays that study absorption, distribution, metabolism, and excretion. The data were analyzed to identify chemical categories, prioritize PFAS for further study, and apply read-across approaches to predict toxicity for untested chemicals based on similarities within categories.

The findings from this effort helped categorize PFAS based on their structural and biological properties, facilitating streamlined prioritization for future research and regulatory actions. By leveraging alternative testing methods and computational tools, this work represents an efficient and ethical approach to tackling the extensive hazard evaluation needed for PFAS. It also provides a foundation for tiered-testing strategies, reducing reliance on traditional animal models while advancing understanding of PFAS toxicity and environmental safety.

Use of Tox21 screening data to profile PFAS bioactivities on nuclear receptors, cellular stress pathways, and cytochrome P450 enzymes

Tags: NCATS, NIEHS, metabolism, PFAS, Tox21

PFAS are synthetic chemicals widely used in commercial products. These chemicals are a global concern due to their persistence in the environment and extensive associations with adverse health outcomes. While legacy PFAS have been extensively studied, many nonlegacy PFAS lack sufficient toxicity information. NCATS and NIEHS researchers analyzed the bioactivity of PFAS using Tox21 screening data (Ooka et al. 2024). Over 75 assay endpoints including nuclear receptors, stress response, and metabolism were examined to understand the toxicity of nonlegacy PFAS and investigate potential new targets of PFAS. This analysis confirmed several known PFAS targets and pathways and identified several potential novel targets and pathways. The novel PFAS-affected targets included the metabolic enzyme CYP2C9, as well as ROR and sonic hedgehog, targets both related to tissue development. To confirm the effect of PFAS on these novel targets and pathways, follow-up studies were conducted using several cell- and enzyme-based assays. PFAS were found to inhibit cytochrome P450 metabolism enzymes, especially CYP2C9, which is important for metabolism of both drugs and endogenous compounds, such as fatty acids. This PFAS–CYP2C9 interaction was further investigated using molecular docking analysis, which suggested that PFAS directly bind to the active sites of CYP2C9. These findings are important contributions to understanding the mechanism of PFAS action and toxicity.

Predictive models to develop cytotoxicity flags for Tox21/ToxCast assays

Tags: NIEHS, machine learning, Tox21

Results from in vitro assays used in the Tox21 and EPA 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. To address this, concurrent cytotoxicity readouts from Tox21/ToxCast assays were mapped for hundreds of assay endpoints, allowing direct comparison of bioactivity potency against cytotoxicity. For chemicals tested in HTS assays that lack directly relevant cytotoxicity data, NICEATM scientists are developing machine learning algorithms to

predict chemical- and cell type-specific cytotoxicity concentrations across time trajectories. The models' predictions will be integrated with bioactivity data to strengthen confidence in assay outcome interpretation by identifying mechanistic vs. nonspecific/cytotoxicity-confounded bioactivities. The comparisons and predictions will be integrated into future versions of concentration–response visualizations for the curated HTS data in the NICE Curve Surfer tool. The direct comparisons will also be incorporated into the NICEATM project to harmonize curated HTS data to the OECD Harmonised Template 201, providing additional context for bioactivity calls to help facilitate the international adoption of standardized NAMs.

Leveraging biomarkers and translational medicine for preclinical safety: Lessons for advancing the validation of alternatives to animal testing

Tags: NCATS, NIEHS, MPS, machine learning

Biomarkers are measurable biological indicators, such as specific proteins or genes, that can provide insights into how the body reacts to chemical or drug exposure. Concepts from translational medicine, particularly the use of biomarkers, can help advance alternative testing methods to replace animal studies in preclinical safety assessments. By studying these indicators, researchers can improve the accuracy and human relevance of NAMs used in safety testing.

In a review article (Hartung et al. 2024), NIEHS and NCATS researchers outline how principles from translational medicine, such as "fit-for-purpose" validation (matching tools to specific tasks), evidence-based methods, and integrated testing strategies, are already being used to refine and validate NAMs. Biomarkers can help researchers better understand the mechanisms behind toxicity and relate observations in alternative models to outcomes from animal studies or clinical trials. This improves confidence in NAMs as replacements for animal testing.

Challenges, such as standardizing methods, demonstrating relevance, and achieving regulatory acceptance, still exist, but the field is advancing thanks to collaboration, technological innovations, and evolving regulations. The review also considers future trends, including combining data from multiple biological systems (multiomics), using organ-like MPS, and applying SI to analyze data. Successful case studies show how biomarkers are

already being applied in preclinical safety testing. By integrating biomarkers into NAMs, researchers can develop more predictive, ethical, and human-relevant safety assessment tools, ultimately reducing reliance on animal models and improving public health outcomes.

Evaluation of data levels during development and application of NAMs

Tags: FDA, NIEHS, data sharing, harmonization

Toxicological testing methods are used to assess the safety of chemicals, but the final results depend heavily on how the raw data is processed. This study focused on the importance of understanding and managing the steps involved in processing and interpreting data generated by NAMs. When toxicological tests are conducted, multiple layers of data are analyzed in sequence, starting with raw outputs from laboratory equipment and ending with a final classification of the test chemicals (e.g., whether they are toxic or not). Each step in the process involves specific data handling methods, and even small changes in these steps can alter the final outcome. For instance, the same raw data, when processed differently, could lead to different conclusions about a chemical's safety. This inconsistency can affect how reliable, robust, and acceptable NAMs are for regulatory use.

To address this, a review by FDA and NIEHS researchers emphasizes that detailed descriptions of data processing steps should be included in NAM reporting templates such as ToxTemp (Blum et al. 2025). This transparency helps ensure that results are reproducible, building trust in the method and supporting its acceptance by regulatory agencies. The review provides an overview of the key data levels involved in NAMs, explains how data is processed between these levels, and highlights the impact of these steps on the final results. By raising awareness of the importance of careful data management, this work aims to make NAMs more reliable, trustworthy, and widely accepted in toxicology, ultimately advancing the use of modern, animal-free testing methods.

From animal testing to in vitro systems: Advancing standardization in MPS

Tags: FDA, NIST, harmonization, MPS

Scientific communities and industry have long sought alternatives to traditional cell cultures and animal studies to improve human-relevant models for applications such as drug development, toxicology assessments, and preclinical evaluations. One promising innovation is microfluidic-based organ-on-a-chip technology, which mimics the structure and function

of organs and organ systems more accurately than conventional methods. Despite increasing interest from regulatory bodies and industry, implementation of this technology has been slowed by the absence of standardized guidelines.

To address this gap, a multidisciplinary team of stakeholders was formed, including representatives from FDA, NIST, the European Union, academia, and industry (Reyes et al. 2024). This collaboration aimed to establish a framework for developing guidelines and standards for the engineering and application of organ-on-a-chip models. By promoting consistency and reliability, these guidelines will help overcome technical and regulatory challenges, advancing the adoption of these systems.

The resulting framework encourages further conversations among stakeholders, including standards development organizations, to refine and finalize best practices for organ-on-a-chip use. This effort represents a critical step toward optimizing the potential of this innovative technology, ensuring it meets regulatory and industry needs while enhancing human-relevant testing.

Ultimately, this work lays the foundation for widely accepted standards that will improve the reliability, scalability, and impact of organ-on-a-chip models, supporting advancements in drug development, toxicology, and personalized medicine.

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: ICCVAM, EPA, FDA, NCATS, ecotoxicity, exposure, MPS

In 2015, ICCVAM initiated a series of Communities of Practice webinars to provide detailed presentations on a current topic relevant to alternative test method development. ICCVAM chair Natalia Vinas, Ph.D., presented a retrospective of ten years of Communities of Practice webinars at the 2025 meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM). She reviewed the topics covered in all of the webinars

and noted those that had set the stage for subsequent activities including collaborations and workshops.

- The January 29, 2024, Communities of Practice webinar was titled Implementing Computational Approaches for Regulatory Safety Assessments. It focused on how to establish confidence in computational approaches for regulatory applications. Michael Lowit, Ph.D., presented an overview of EPA Office of Pesticide Programs goals for applying new approach methodologies (NAMs), including computational models, to ecological risk assessments and described ongoing projects in support of those goals. Pras Pathmanathan, Ph.D., summarized recent FDA Center for Devices and Radiological Health guidance on reporting of computational modeling studies in medical device submissions. Jon Arnot, Ph.D., of Arnot Research and Consulting and the University of Toronto, described tools and approaches for bioactivity and exposure modeling and provided examples of how they can be used to support regulatory decision-making.
- The 2025 webinar was presented on July 21 and focused on Evolution of Complex In Vitro Models. Three scientists from government and academic laboratories and the private sector described how they are approaching validation of complex in vitro models such as microphysiological systems (MPS). Danilo Tagle, Ph.D., of NCATS presented an overview of their Tissue Chips for Drug Screening program. Ivan Rusyn, M.D., Ph.D., described activities of the Texas A&M University's Tissue Chip Testing Center, which is funded through a public–private consortium.
  Remi Villenave, Ph.D., from Roche, summarized pharmaceutical industry perspectives on NAMs use, including perceived barriers to NAMs usage.

ICCVAM Public Forums

Tags: ICCVAM

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.

The first ICCVAM Public Forum was held in 2014. At the 2025 SACATM meeting, ICCVAM member Anna Lowit, Ph.D. (EPA principal agency representative) described how both agency participation and audience engagement with the event has grown over time. She noted how public forum meetings provide a venue for agencies to announce and discuss projects and initiatives. They also facilitate engagement with specific stakeholder groups such as nongovernmental organizations and method developers.

The 2024 ICCVAM Public Forum was held in person on May 20 and 21 at NIH in Bethesda, MD, with an option for remote viewing. About 40 people attended in person, and nearly 300 viewed the webcast. The program featured 18 presentations from seven of the 17 ICCVAM member agencies 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 local and international activities. Public comments ranged from general suggestions about areas of focus for ICCVAM to specific discussions on the status of replacing animals for endpoints such as inhalation toxicity or developmental neurotoxicity (DNT). Specific comments recognized ICCVAM for publication of the document "Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies" and asked ICCVAM agencies to play an active role in the application of super intelligence (SI) in toxicology.

Due to uncertainty about travel and public communications, the 2025 ICCVAM Public Forum was held virtually on July 20 and 21 rather than in May as in the past. Between 150 and 180 people viewed the webinar over two days. Eight presentations provided updates on NICEATM and ICCVAM activities and activities of six ICCVAM member agencies. Five organizations submitted written comments, and six organizations presented public comments. Commenters recognized that federal agencies and stakeholders are undergoing a major transition toward NAMs that is driven by broader regulatory momentum and specifically noted new NIH policies and the 2025 FDA roadmap. However, they also noted uneven progress due to unclear guidance, inconsistent terminology, limited data sharing, and lack of standardized tracking of animal use. Organizations including advocacy groups and scientific consortia agreed on the need for better infrastructure, including training, transparent reporting, harmonized definitions, and validated methods, alongside increased international coordination. While some groups pushed for rapid replacement of animal testing where

alternatives exist, others advocated a balanced approach that continues improving animal models in parallel to ensure scientific rigor and public health protection during the transition.

ICCVAM advisory committee meetings

Tags: ICCVAM, FDA, NIH, data sharing, developmental neurotoxicity

SACATM is a federally chartered advisory group that advises NICEATM, ICCVAM, and the Director of NIEHS about ICCVAM activities. SACATM is directed by its charter to meet at least once each fiscal year.

The September 17–18, 2024, SACATM meeting was held at NIH in Bethesda, MD. About 40 people attended in person and over 100 people viewed each day of the webcast. Advisory committee members were briefed on federal government and international activities to support validation of new methods, including new ICCVAM guidance on validation, establishment of the Method Developers Forums, updates to Organisation for Economic Co-operation and Development (OECD) guidance on validation, and launch of the NIH Complement-ARIE program. Advisory committee members welcomed the publication of the ICCVAM guidance and encouraged continued progress on the other activities. The other focus topic of the meeting was DNT testing, and scientists from NIEHS, FDA, EPA, and the European Food Safety Authority presented on advances in the use of NAMs for this endpoint.

The September 11–12, 2025, SACATM meeting was hosted at NIEHS in Research Triangle Park, NC, and presented virtually. The meeting attracted over 300 viewers on the first day and nearly 200 on the second day. Many of the presentations at this meeting focused on recently announced initiatives by NIH and FDA to support implementation of NAMs in research and regulatory contexts. Advisory committee members welcomed these efforts and agreed that they would support efficiency in method validation. Data standards and harmonization were both noted as key to this effort. Updates were also provided on development of online tools such as the Collection of Alternative Methods for Regulatory Application, NIH Integrated Chemical Environment (NICE), and NICEATM-developed apps for predicting skin sensitization potential. A session on “ICCVAM Public Outreach and Education” presented retrospectives of the scope and impact of the ICCVAM Public Forum

meetings and ICCVAM Communities of Practice webinars, as well as a summary of NICEATM training and outreach activities during 2024 and 2025.

ICCVAM agency-sponsored workshops and webinars

Tags: EPA, FDA, NCATS, NCI, NIEHS, NIH, USDA, acute toxicity, AOP, carcinogenicity, ecotoxicity, exposure, eye irritation, IVIVE, machine learning, metabolism, MPS, PFAS, skin sensitization, small model organisms

ICCVAM agencies convened workshops and webinars from the beginning of 2024 and continuing into 2026, 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 25, 2024–December 10, 2025	EPA	Webinar series: Computational Toxicology Communities of Practice	These webinars covered computational approaches for assessing chemical safety and chemical risk assessment. Topics included NAMs, high-throughput screening (HTS), exposure science, and various EPA database tools.
February 21, 2024–December 11, 2024	EPA (PETA Science Consortium International [PSCI], Institute for In Vitro Sciences, California Department of Pesticide Regulation)	EPIC Webinar Series: Use of NAMs in Risk Assessment	This ongoing webinar series began in 2018 and will continue into 2026. Topics covered during 2024–2025 included skin sensitization, acute oral toxicity, eye irritation, and establishing confidence in nonanimal testing methods.

Meeting Date	Sponsoring Agency (and partner organization)	Meeting Title (with link to page if available)	Meeting Summary
March 6–7, 2024	NCI	Workshop: Digital Pathology Imaging-Artificial Intelligence in Cancer Research and Clinical Trials	This virtual workshop addressed data standardization, SI validation, image quality assurance, clinical trial integration, and regulatory compliance in digital pathology.
March 11–12, 2024	NCI	Exploratory Workshop on Math, Theory, and Cancer Biology	This workshop focused on mathematical theory development and cancer biology. Topics included manifold learning, topological data analysis, random matrix theory, high dimensional probability, and geometric measure theory applications in cancer research.
July 29–30, 2024	NIH	Workshop: 3-D In Vitro Tissue Systems for Research on Aging	This workshop focused on the development and utilization of 3D in vitro tissue systems and MPS to model aging processes in human cells and tissues as complements to animal models in aging research.
September 4–12, 2024	NIH, USDA (Johns Hopkins Center for Alternatives to Animal Testing (CAAT), Johns Hopkins University [JHU] Dept. of Molecular and Comparative Biology)	11th Annual 3Rs Symposium	This event covered topics including MPS, tissue sharing, breeding optimization, statistical design, euthanasia refinement, and alternative models.

Meeting Date	Sponsoring Agency (and partner organization)	Meeting Title (with link to page if available)	Meeting Summary
October 7–8, 2024	EPA, NIEHS	Workshop: Advancing Quantitative Analysis in Human Health Assessments through Probabilistic Methods	This workshop focused on advancing probabilistic methods in human health risk assessment. Topics included exposure modeling, toxicokinetics, benchmark dose modeling, and toxicity value determination.
October 23–24, 2024	NCATS (U.S. Health and Human Services Center for the Biomedical Advanced Research and Development Authority, University of California–San Diego, University of North Carolina–Chapel Hill)	Workshop: In Silico Drug Discovery	This workshop considered practical approaches and best practices to develop and integrate accurate, robust, and rigorous SI and machine learning methods/models into drug discovery pipelines.
October 28, 2024	FDA Human Foods Program (CAAT, International MPS Society, ILMERAC)	Webinar: MPS for Chemical Risk Assessment	In this webinar, international experts from regulatory agencies and industry discussed the use of MPS for safety testing in food and chemical risk assessment.
November 5–6, 2024	EPA	The State of Science on Development and Use of NAMs for Chemical Safety Testing	This hybrid meeting provided an update on implementation of the EPA NAMs work plan. Topics discussed included exposure NAMs, omics, and in vitro–in vivo extrapolation (IVIVE).

Meeting Date	Sponsoring Agency (and partner organization)	Meeting Title (with link to page if available)	Meeting Summary
November 22, 2024–December 12, 2025	NIEHS	Webinar series: Environmental Health Language Collaborative	These webinars supported the goals of the Environmental Health Language Collaborative and covered topics including common data elements, ontology development and curation, and application of large language models. This series will continue into 2026.
December 3, 2024	FDA Human Foods Program (Society of Toxicology)	Colloquia on Emerging Toxicological Science: Challenges in Food and Ingredient Safety	This webcast focused on emerging concepts in hazard identification and exposure assessment, particularly regarding food safety, environmental contamination, and human health risks from per- and polyfluoroalkyl substances (PFAS).
March 27–June 26, 2025	NCI	Webinar series: Beyond Mammals: Exploring Cancer Models	These three webinars featured researchers discussing *C. elegans* worms, *Drosophila* fruit flies, and zebrafish (*Danio rerio*), exploring their unique applications, differences from human cancer lines, and obstacles to adoption.
May 13–20, 2025	NCI	Virtual workshop series: Synthetic and Systems Approaches to Interrogate Spatiotemporal Processes in Cancer	These three workshops focused on transformation and cancer initiation, tumor-immune interactions, and drug response and resistance in spatially defined cancer processes.

Meeting Date	Sponsoring Agency (and partner organization)	Meeting Title (with link to page if available)	Meeting Summary
June 4–5, 2025	NIEHS	Workshop: Environmental Health Language Adverse Outcome Pathways Standards	The goals of this workshop were to bring together adverse outcome pathway (AOP) community efforts and increase alignment and collaboration within the biomedical knowledge base and ontology communities.
July 7, 2025	FDA, NIH	Workshop: Reducing Animal Testing	This hybrid event featured international regulatory representatives discussing novel methodologies, implementation strategies, successes, and challenges in minimizing animal testing requirements.
July 7–11, 2025	NCI	S3 Innovation Lab: Synthetic and Systems Approaches to Interrogate Spatiotemporal Processes in Cancer Initiation	This virtual event fostered interdisciplinary collaboration to develop pilot research proposals integrating computational modeling with synthetic biology experiments.
September 10–18, 2025	NIH, USDA (JHU/CAAT, JHU Dept. of Molecular and Comparative Biology)	12th Annual 3Rs Symposium	This symposium featured presentations focused on reducing, replacing, and refining animal use (3Rs) methods to improve laboratory animal welfare while maintaining or improving scientific results. Topics included liver-chip technologies, environmental health monitoring, refinement and enrichment strategies, and regulatory insights into the 3Rs.

Meeting Date	Sponsoring Agency (and partner organization)	Meeting Title (with link to page if available)	Meeting Summary
September 10, 2025	EPA, FDA (PSCI, Health and Environmental Sciences Institute, National Institute of Environmental Sciences [Japan], European Medicines Agency)	Webinar series: NAMs in Ecotoxicology	The September 2025 webinar considered integrated weight-of-evidence approaches for bioaccumulation assessment in environmental safety. This was the first of a series that will continue into 2026.
September 23–25, 2025	NCI	Workshop: Transformative Microsystems for Cancer Diagnosis, Treatment Monitoring, and Clinical Integration	This virtual event explored microfluidics, lab-on-a-chip platforms, and microscale technologies advancing personalized cancer care and clinical translation.
December 5, 2025	FDA (Triangle Centers of Excellence in Regulatory Science and Innovation)	Workshop: Pediatric Drug Development Safety and New Approach Methodologies (NAMs)	This virtual event covered federal programs, secondary targets, juvenile animal studies, in vitro tools, and model-informed techniques including quantitative systems pharmacology and SI applications.

ICCVAM Method Developers Forum

Tags: ICCVAM, carcinogenicity

As a follow-up to publication of the report on "Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies," ICCVAM and NICEATM envisioned a series of Method Developers Forums, each focused on a specific endpoint/toxicity. These forums would provide an opportunity for NAMs developers to present their methods and regulatory issues with relevant stakeholders.

The ICCVAM Method Developers Forum was held virtually in August 2024 and focused on NAMs for carcinogenicity testing. Ten method developers described their methods and proposed how they may be useful for regulatory and/or industry stakeholders in a webinar attended by over 200 viewers. Stakeholders representing potential government and industry users of the NAMs or the data they generate also participated in the webinar. The event was well-received with positive feedback from diverse stakeholders. Suggestions for improvement of future forums included:

- Increase engagement with industry, especially for preregulatory activities.
- Provide more time during the event for discussion between method developers and end users.
- Consider how to prioritize methods that are closer to being ready to apply to a regulatory purpose.
- Clarify evaluation criteria for proposals, which are needed to help both method developers to prepare their proposals and the steering committee come to a consensus on accepting proposals.

Planning for future iterations of the Method Developers Forum is currently underway.

Development of topic-specific searches for NAMs applications

Tags: NIEHS, USDA, carcinogenicity, developmental neurotoxicity, developmental toxicity, inhalation toxicity, IVIVE, MPS, QSAR, skin sensitization

In recent years, USDA's Animal Welfare Information Center (AWIC) has been developing PubMed search strategies, known as "hedges," for topics specific to alternatives. Hedges are complex search queries that can be used on their own or in combination with other terms to return the most current literature relevant to that query available in PubMed. The focus of AWIC's first hedges was refinement of animal use, and these hedges tended to be focused on animal species. Since 2023, NICEATM and AWIC have been collaborating to develop hedges on topics focused on replacement of animal use. In 2023 and 2024, hedges were developed for the topics MPS, NAMs for DNT, NAMs for acute inhalation toxicity, and IVIVE. These hedges were published in spring 2024 and are available on the National Toxicology Program website. All AWIC hedges are also available on their website. Additional search hedges on the topics of NAMs for developmental and reproductive

toxicology, NAMs for skin sensitization, quantitative structure–activity relationship models for chemical hazard, and reduction alternatives for the two-year rodent cancer bioassay were developed in 2025 and will be published on the National Toxicology Program and AWIC websites in 2026.

AWIC resources

Tags: USDA, ecotoxicity, metrics

The 1985 amendments to the Animal Welfare Act established AWIC, an information service at USDA's 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 or minimize pain and distress to animals (7 USC § 2143(e)).

In 2024 and 2025, AWIC continued to strengthen its support for the toxicology community by offering information on alternative methods in ecotoxicity testing and by maintaining resources that highlight mandatory and regulatory testing guidelines across federal agencies. The 3Rs in Ecotoxicity Testing webpage features curated searches for NAMs in the PubAg and PubMed databases, as well as a bibliography that was updated in September 2025 and provides access to nearly 800 abstracts on alternative testing approaches.

AWIC's website received substantial use during this two-year period, with 308,733 visitors generating 542,858 page views. These users accessed a variety of materials designed to facilitate the identification and application of animal use alternatives, especially within toxicology and related research disciplines.

In addition to online resources, AWIC engaged directly with the scientific and regulatory communities through presentations, posters, workshops, webinars, and conference exhibits. Across 40 outreach events in 2024 and 2025, AWIC interacted with approximately 3,191 animal researchers, veterinarians, Institutional Animal Care and Use Committee members, and others. These engagements emphasized best practices for conducting comprehensive literature searches, locating current research on alternative methods, and applying 3Rs principles within testing and experimentation, including toxicology contexts.

AWIC also continued to offer its workshops on meeting the requirements of the Animal Welfare Act. These sessions provided attendees with guidance on where to find literature and resources on the 3Rs, instruction on database literature search techniques (e.g., search syntax), and demonstrations of how to conduct literature searches to address requirements for consideration of NAMs. Four workshops were presented during 2024 and 2025, reaching a total of 927 participants. For those unable to attend live sessions, AWIC maintained access to online, on-demand training through its events webpage.

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 animals while continuing to protect human health and the environment. The Work Plan was 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. This program provides users courses and workshops with the appropriate level of oversight to enable them to use EPA NAMs tools and approaches in their own work.

In April 2024, EPA presented a comprehensive two-day NAMs Tools Training Workshop in person at their Research Triangle Park, NC, campus. It gave participants the opportunity to advance their knowledge about 10 different EPA NAMs tools through overview sessions, in-depth learning, interactions with tool and data owners, and tool-specific case studies. Materials from the workshop are available on the NAMs training program webpage.

EPA also presented several virtual training sessions during 2024 and 2025:

- June 2024: Chemical Transformation Simulator, a web-based tool for predicting transformation pathways and physicochemical properties of organic chemicals
- September 2024: Collaborative AOP Wiki
- May 2025: SeqAPASS online screening tool to extrapolate toxicity information across species

- June 2025: CompTox Chemicals Dashboard, the primary web-based application that provides access to data and algorithms from the EPA Center for Computational Toxicology and Exposure
- July 2025: httk R software package providing toxicokinetic models to estimate internal chemical concentrations from exposure and support in vitro to in vivo extrapolation (IVIVE)

Videos and other materials from these events are on the NAMs training program webpage. Other resources available on this page include links to online user guides and tool webpages.

DOI communications efforts to raise awareness of ecotoxicity NAMs

Tags: DOI, ecotoxicity, metrics

During 2024 and 2025, DOI engaged in communications activities to raise awareness of NAMs for ecotoxicity applications within the agency and among the general public.

- DOI generates scientific information needed to support the conservation and management of the nation's natural resources. This includes conducting ecotoxicological research and testing. In 2024, the U.S. Geological Survey, on behalf of DOI, launched a new website on "Reducing Animal Use in Ecotoxicity Testing and Biomonitoring." This website describes Geological Survey efforts to reduce animal use and employ in vitro and in silico replacement methods.
- To raise awareness of animal testing alternatives among agency scientists, the U.S. Geological Survey presented a July 2025 webinar on "Animal Alternatives for DOI Biomonitoring and Ecotoxicological Assessments." Presentations discussed:
    - Best practices for monitoring mercury contamination and assessing injury to wild bird populations
    - Cytotoxicity studies with fish gill cell lines: proof-of-concept for a screening approach to prioritize pesticide candidates
    - Cell culture models for screening environmentally safer tire additives
    - Development of water and sediment toxicity testing methods for a laboratory-reared mayfly

  The webinar was attended by 50 agency scientists.

Alternatives and NAMs webpage on the NIH OLAW website

Tags: NIH

The NIH Office of Laboratory Animal Welfare (OLAW) ensures the humane care and use of animals in U.S. Public Health Service-supported research, testing, and training. To support this mission, OLAW provides guidance and interpretation of the Public Health Service Policy on Humane Care and Use of Laboratory Animals, monitors compliance with the policy, and supports educational programs.

The 3Rs are foundational principles in responsible animal use. In particular, the consideration of NAMs, where scientifically appropriate, is important to ensure the judicious use of animals. The choice of model used in a study, animal or otherwise, is reviewed by subject matter experts during the process of grant application or contract proposal, and OLAW does not advise on the applicability or implementation of a particular alternative model in a study. While alternative approaches cannot completely replace the use of animals at this time, NIH is committed to transparently assessing where animal use can be reduced or eliminated by transitioning to alternatives. Areas where research using animals is currently necessary represent high-priority opportunities for investment in alternatives. OLAW is committed to providing helpful information to assist investigators who wish to explore human-based approaches and other methodologies.

The resources on OLAW's Alternatives and NAMs webpage were launched as an updated, consolidated Animal Welfare Topic page in September 2025. The updated webpage gives biomedical researchers and Institutional Animal Care and Use Committee members a place to learn about the 3Rs and NAMs, as well as resources for how to incorporate them into their work.

Resources available on OLAW's Alternatives and NAMs webpage include:

- Recent NIH announcements relevant to NAMs acceptance and use
- Frequently asked questions about NIH and NAMs
- Links to resources and tips for investigators, Institutional Animal Care and Use Committees, students, and the public
- Background information on NAMs and OLAW, and links to relevant laws, policies, and guidance

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 applications for acute oral or inhalation toxicity testing and for predicting whether chemicals could cause cardiotoxicity, developmental toxicity, or hepatotoxicity.

Screening-level estimation of chemical mixtures toxicity using in silico models: A jet fuel case study for environmental incidents

Tags: ATSDR, acute toxicity, machine learning, mixtures toxicity, QSAR, structure similarity

Environmental incidents, such as jet fuel spills reported in different regions worldwide, can expose communities to harmful chemical mixtures through contaminated drinking water. However, testing the toxicity of such complex mixtures with traditional methods can be challenging when data on component chemicals are unavailable. ATSDR researchers explored the use of in silico tools, which rely on computer models and predictions, to estimate health risks from oral exposure to jet fuel (JP-5) mixtures (Ji et al. 2026). Publicly available in silico tools, such as threshold of toxicological concern estimates, quantitative structure–activity relationship (QSAR) models, and read-across approaches, were used to evaluate the potential health hazards of JP-5 and its chemical components. Applicability domain assessments indicated broad QSAR model coverage, with OPERA and ToxTree fully inclusive and VEGA models showing mostly moderate-to-high confidence for most additives. The models were used to estimate points-of-departure (PODs), which were then extrapolated using dosage adjustments and uncertainty factors to obtain health guidance values. The study found that in silico-derived health guidance values for JP-5 mixtures generally aligned with existing safety guideline values and that threshold of toxicological concern estimates were consistently conservative, which means they erred on the side of caution. Additionally, the researchers gathered data from previous studies to estimate possible health effects. Overall, the research demonstrated that advanced computer models can effectively fill in toxicological data gaps and provide valuable screening-level insights into the safety of chemical mixtures when traditional data is unavailable. This approach is a

promising tool for addressing environmental health concerns and protecting public safety in situations involving chemical exposures.

Integrating NAM data and IVIVE for assessing inhalation toxicity risks

Tags: ATSDR, NIEHS, inhalation toxicity, IVIVE

Animal test data from evaluations of the toxicity of inhaled chemicals have limited applicability to humans due to anatomical differences between human and rodent respiratory tracts and complexities in the transport kinetics of inhaled chemicals through these organs. New approach methodologies (NAMs) have the potential to provide more human-relevant, mechanistically driven approaches to identify potential inhalation toxicity hazards. While in vitro assay data can provide valuable information about the effects of chemicals on biological systems, it is necessary to translate in vitro results to an in vivo context to determine safe exposure levels in real-life situations. In vitro to in vivo extrapolation (IVIVE) estimates the equivalent administered dose that would result in plasma concentrations equivalent to in vitro activity concentrations.

ATSDR and NICEATM scientists applied IVIVE to in vitro assay data from 20 volatile organic compounds with available pharmacokinetic and in vivo toxicity data covering multiple target organs via inhalation exposure. For each chemical, equivalent administered doses that would result in plasma or lung concentrations equivalent to the in vitro activity concentrations were estimated and compared to the in vivo PODs or ATSDR Minimal Risk Levels. While equivalent administered doses for most tested chemicals either underpredicted or were within 5-fold of in vivo PODs, substantial deviations from in vivo PODs warrant further investigation into the sources of uncertainty. However, most equivalent administered doses based on in vitro data were at least an order of magnitude higher than the corresponding Minimal Risk Levels, suggesting that an empirical or statistical modifying factor may be required to align equivalent administered dose-derived thresholds with public health-based guidance values. Physicochemical properties, particularly volatility, were found to significantly influence the reliability of in vitro assay results, and recognizing this and applying proper controls could improve results. There is also the possibility that incorporating experimental toxicokinetic data where available could improve the precision of

IVIVE predictions and strengthen alignment with in vivo PODs. A paper describing this study is in preparation for submission in 2026.

Application of CATMoS to predict pesticide toxicity

Tags: EPA, NIEHS, acute toxicity, ecotoxicity, QSAR

EPA currently relies on animal testing, specifically rat studies, to determine the LD50 (the dose of a substance that causes death in 50% of test animals) for pesticide safety assessments. This information is used for pesticide toxicity labeling and environmental risk assessment. To reduce animal use, a consortium of international researchers led by NICEATM developed a computer-based tool called the Collaborative Acute Toxicity Modeling Suite (CATMoS; Mansouri et al. 2021). CATMoS uses QSAR modeling to predict acute oral toxicity (LD50) values for chemicals.

EPA and NIEHS researchers compared CATMoS predictions to real-world animal data for 177 pesticides (Bishop et al. 2024). The tool was evaluated for its ability to classify chemicals into the EPA's acute toxicity categories and accurately predict LD50 values. Results showed that CATMoS was highly reliable at categorizing substances into EPA toxicity Categories III (moderate toxicity, 500–5000 mg/kg) and IV (low toxicity, over 5000 mg/kg), with an impressive 88% agreement between CATMoS predictions and animal test data for chemicals with LD50 values over 500 mg/kg. Additionally, for chemicals with LD50 values of 2000 mg/kg or higher, CATMoS predictions closely matched outcomes from high-dose animal tests.

These findings demonstrate that CATMoS is a reliable tool for predicting pesticide toxicity, particularly for less-toxic substances. By using CATMoS, regulators and industry can reduce the need for animal testing, making pesticide safety evaluations more ethical while still ensuring accurate risk assessments. This work represents a significant step forward in modernizing toxicity testing methods.

Integrating Bayesian networks and AOP networks for drug toxicity prediction

Tags: FDA, AOP, defined approaches/IATA, hepatotoxicity, machine learning

A study by the FDA National Center for Toxicological Research (NCTR) demonstrated how combining two analytical frameworks can improve predictions of drug toxicity while reducing reliance on animal models (Wang et al. 2025). The study compared and combined

adverse outcome pathways (AOPs), which map how chemical exposures lead to harmful health effects through chains of biological events, and Bayesian networks, which are statistical models that calculate probabilities of outcomes based on interconnected factors. Both approaches use similar diagram structures called directed acyclic graphs, but researchers had not previously verified whether they are mathematically compatible. Using drug-induced liver toxicity as a test case, the researchers confirmed that AOP networks align mathematically with Bayesian network properties. More importantly, they showed that applying specific mathematical concepts from Bayesian networks, particularly "Markov blankets" (the minimal set of factors needed to predict an outcome), can dramatically simplify toxicity prediction models. This simplification makes the models more efficient and practical for real-world use in drug development. The results support replacing traditional animal models with laboratory-based molecular assays that are more directly relevant to human biology. By demonstrating that these two frameworks work together effectively, the research provides a roadmap for developing better, faster, and more human-relevant methods to identify potentially toxic drugs earlier in the development process, ultimately improving drug safety while reducing animal use in testing.

Comparative study of molecular descriptors and SI-based embeddings for toxicity prediction

Tags: FDA, hepatotoxicity, machine learning, structure similarity

Researchers at FDA NCTR compared traditional chemical analysis methods with super intelligence (SI) language models in the prediction of whether certain drugs and chemicals are toxic. Scientists tested whether SI models such as GPT-3 could predict toxicity as well as or better than established methods that analyze molecular structure (Gray and Wu 2025). Both approaches were applied to three different toxicity databases containing information about thousands of chemicals. The traditional molecular descriptor methods, which mathematically describe chemical structures, generally performed best on complex multi-endpoint toxicity predictions, achieving accuracy scores of approximately 0.855. However, SI language models showed superior performance for specific toxicity types, particularly clinical toxicity and drug-induced liver injury (DILI), with GPT-3 reaching near-perfect accuracy (0.996) for one dataset. The SI models analyzed three types of text

inputs: chemical structure codes (SMILES strings), chemical names, and simple descriptions. Importantly, the researchers noted that the good performance of the SI models may partly reflect the models having encountered these well-known chemicals during training rather than true predictive ability. The study demonstrates that while traditional molecular analysis remains the gold standard for predicting the toxicity of new compounds, SI language models show promise as complementary tools, especially for screening known chemicals where existing toxicity information is available in scientific literature. Future work will combine both approaches to improve accuracy and reliability in drug safety evaluation.

Quantitative IVIVE for developmental toxicity

Tags: NIEHS, IVIVE, developmental toxicity, stem cells

Traditional research on the effects of chemicals on embryos and fetuses often relies on animal models, but use of human-relevant NAMs has the potential to improve safety assessments. An example is the Stemina devTOX quickPredict (devTOXqP) assay, which uses pluripotent stem cells to measure a chemical's potential to cause developmental toxicity. In a preliminary study using the known developmental toxicant valproic acid and its analogs (Chang et al. 2022), NIEHS, EPA, FDA, and Stemina demonstrated that the devTOXqP assay, when used with advanced pharmacokinetic models, could estimate safe exposure limits and rank chemicals based on toxicity risks.

To better understand the applicability domain and performance of the devTOXqP assay, NICEATM and Stemina evaluated the accuracy of the assay in predicting in vivo developmental toxicity in humans and rodents for a broad range of 198 chemicals: 117 developmental toxicants, 67 developmental nontoxicants, and 14 chemicals with unknown developmental toxicity in vivo. For chemicals with the highest level of in vivo evidence, the devTOXqP assay performed well in predicting in vivo outcomes. The study results suggest that the devTOXqP assay may be useful for the identification of substances likely to cause perturbations in early development, with a negative result in the test considered along with other evidence in decisions to prioritize chemicals for in vivo testing. As the devTOXqP assay informs on a specific biochemical aspect of fetal development, it may also be useful as part of research strategies to clarify AOPs for developmental neurotoxicity (DNT). A paper describing the study is in preparation for submission in 2026.

Comparison of PBPK modeling platforms for DNT IVIVE

Tags: NIEHS, developmental neurotoxicity, IVIVE

DNT occurs when exposure to a chemical causes an adverse effect in the developing brain or nervous system. NICEATM researchers and collaborators developed an approach that could be used to increase the speed and capacity of testing to predict whether a chemical might cause DNT without extensive animal testing (Kreutz et al. 2026). They used physiologically based pharmacokinetic (PBPK) models to translate doses that could potentially impair neurodevelopment from laboratory cell tests into estimates of human exposure levels that would be needed to disrupt brain development.

The team tested 91 chemicals that showed activity in cell-based tests for DNT, using three different PBPK modeling platforms. These PBPK models allowed for prediction of the amount of chemical that reaches the fetus and brain during critical periods of neurodevelopment. They focused on time points spanning pregnancy through six months after birth, when the brain is particularly vulnerable. The models predicted administered equivalent doses, the amount of chemical exposure that would be needed to produce concerning effects based on cell test results.

When compared against available animal study data for 31 chemicals, the predictions aligned well, with 78% falling within three-fold of observed effect levels. Data generated by the models were generally more protective compared with traditional animal model data. This work provides a faster, more cost-effective screening method for thousands of untested chemicals in commerce, helping regulators prioritize which substances warrant further investigation for potential DNT.

Understanding human variability through toxicokinetic modeling, IVIVE, and NAMs

Tags: EPA, NIEHS, IVIVE, population variability

Traditional safety assessments often rely on generalized assumptions and standard uncertainty factors that may not adequately protect vulnerable groups, such as children, elderly individuals, or those with underlying health conditions. A review by EPA and NICEATM scientists highlights major advances in how differences across populations can be accounted for through physiologically based toxicokinetic modeling, which allows scientists

to predict how chemicals move through and are processed by the human body (Kreutz et al. 2024).

Physiologically based toxicokinetic models incorporate detailed biological information about physiology and can be tailored to include age or population-related differences, such as differences in metabolism, genetic variability, and disease-related alterations in organ function. By integrating laboratory data from human cells and tissues with computational simulations, these models can translate in vitro findings into realistic human exposure scenarios. “Virtual populations” can also be modeled to simulate how diverse groups respond to chemical exposures, enabling more nuanced and inclusive risk assessments. Applications of physiologically based toxicokinetic models for variability include evaluating lifetime exposures, chemical mixtures, and sensitive life stages, such as pregnancy. Regulatory agencies such as the FDA and EPA are increasingly using these tools to refine safety limits and improve decision-making. Overall, the body of work captured in this review represents a significant step toward personalized and population-informed risk assessment, improving public health protection while reducing reliance on animal models.

Triazole fungicides risk characterization: Bridging human biomonitoring and computational toxicology

Tags: NIEHS, cardiotoxicity, exposure, endocrine disruptors, IVIVE

An NIEHS study combined biological measurements with IVIVE models in the NIH Integrated Chemical Environment to examine the real-world health impacts of triazole fungicides on coffee farmworkers and rural residents in Brazil (Marciano et al. 2024). Researchers detected multiple fungicides in urine samples from rural participants but not in samples from the urban control population, confirming that agricultural workers face significantly higher exposure rates. Importantly, male farmworkers showed reduced levels of key hormones including testosterone and androstenedione, which were associated with metabolic changes linked to increased risks of cardiovascular disease and diabetes. A notable aspect of the study was its integration of high-throughput laboratory data and computational toxicology tools into models to predict how these chemicals interact with human biological systems. These models successfully anticipated the hormonal disruptions and metabolic effects observed in the population, providing strong validation of nonanimal testing

approaches. The findings raise particular concern about epoxiconazole, which showed effects at exposure levels experienced in the field. From a policy and regulatory perspective, this work demonstrates that modern computational methods can effectively assess real-world risks, supporting more proactive and protective safety standards. It also highlights the need for improved monitoring and regulation of pesticide exposure in agricultural settings, particularly for vulnerable worker populations.

In silico hazard assessment of ototoxicants through machine learning and computational systems biology

Tags: ATSDR, endocrine disruptors, machine learning, QSAR, structure similarity

Hearing loss can occur from exposure to certain medications or environmental chemicals, raising concerns about the possibility of substances causing toxicity to the auditory system, or ototoxicity. ATSDR researchers and collaborators used advanced in silico models to screen and prioritize environmental chemicals for their potential to cause auditory system toxicity, focusing on identifying hazards rather than calculating actual health risks (Luan et al. 2026). The study analyzed 80 environmental chemicals, excluding four already known to cause hearing damage. Researchers used computational methods, including structural similarity assessments and machine learning modeling, to predict auditory system toxicity risks. A consensus model identified 18 of the remaining 76 chemicals as potential auditory system toxicants with high accuracy. These data provide valuable insights for prioritizing these chemicals for further study. To explore mechanisms of auditory system toxicity, the researchers compared a polychlorinated biphenyl compound with an ototoxic drug called mitotane. Both substances were predicted to disrupt thyroid-stimulating hormone receptor signaling, suggesting that thyroid-related pathways may play a role in hearing damage. Additional molecular targets included the aryl hydrocarbon receptor, transthyretin, and the pregnane X receptor, which are linked to metabolic and inflammatory processes. These findings suggest common underlying pathways that may connect chemical exposure to hearing loss. The research highlights the power of computational modeling to support virtual screening and prioritization of chemicals for ototoxicity assessments, offering a quicker and more efficient approach to identifying risks and protecting public health, particularly for vulnerable populations.

An in silico-to-in vivo approach to identify retinoid-X receptor activating tert-butylphenols used in food contact materials

Tags: NIEHS, endocrine disruptors, machine learning, small model organisms, structure similarity

Some chemicals used in food packaging and production may interfere with important genetic processes that regulate development and metabolism. A key concern centers on nuclear receptors, which control many biological processes, and retinoid-X receptors (RXRs). Disrupting RXRs can lead to harmful effects on multiple nuclear receptor pathways, impacting hormone function and metabolism.

To explore this risk, NICEATM researchers and collaborators used machine learning and computer simulations (in silico methods) to analyze over 57,000 chemicals and predict which would interact with RXR (Mengeling et al. 2025). Butylphenols emerged as the largest high-probability group, with 58 compounds identified, some of which are commonly used in food packaging. Further analysis of these chemicals using laboratory data from the EPA's Toxicity Forecaster (ToxCast) program suggested that modifications to their molecular structure (specifically at the C4 location) could enable RXR activation.

To validate these predictions, researchers tested six types of butylphenols in amphibians (*Xenopus laevis*) using a model that measures thyroid hormone signaling, a pathway controlled by RXR. Three of these chemicals, with larger substitutions at C4, increased thyroid hormone activity at extremely low concentrations. Molecular modeling confirmed that bulkier chemical structures improved the fit and stability within the RXR binding pocket.

This study demonstrates how machine learning combined with in vivo experiments can effectively identify chemicals that may disrupt critical genetic pathways. The findings highlight the significance of chemicals used in food packaging, such as butylphenols, and provide tools for assessing their safety, ensuring better protection of human health and the environment.

Unlocking the potential of clustering and classification approaches: Navigating supervised and unsupervised chemical similarity

Tags: NIEHS, structure similarity

Class-based methods, such as clustering and classification, are key to NAM development and application, aiding the understanding of hazard and risk concerns associated with groups of chemicals without additional laboratory work. Advances in computational chemistry, data generation and availability, and machine learning algorithms represent important opportunities for continued improvement of these techniques to optimize their utility for specific regulatory and research purposes. However, due to the intricacy of these methods, deep understanding and careful selection are imperative to align methods with their intended applications.

NIEHS organized a 2022 workshop to consider available clustering and classification approaches and how they might be appropriately applied in a regulatory context. A subsequent review (Mansouri et al. 2024) provides important guidance on how scientists and regulators can more accurately group chemicals based on their similarities using clustering and classification approaches to predict potential health risks without extensive use of data from animal models. NIEHS researchers distinguish between two key approaches: general endpoint-agnostic similarity, which compares overall structural features such as shape and functional groups, and endpoint-specific similarity, which focuses on linking structural characteristics to particular biological effects such as toxicity or receptor binding. A central finding is that using endpoint-agnostic similarity methods when endpoint-specific predictions are required can lead to incorrect conclusions, as visibly similar chemical structures may behave very differently biologically. To address this, the team developed an automated and user-friendly workflow tool that helps nonexperts apply the correct analytical approach. This framework is particularly valuable for "read-across" strategies, where data from well-studied chemicals are used to predict the safety of similar but untested substances. By improving the reliability of these predictions, the work supports regulatory decision-making at agencies such as EPA and the European Chemicals Agency. Overall, this research advances NAMs by making sophisticated computational toxicology tools more accessible, helping reduce reliance on animal models while improving the scientific basis of chemical safety assessments.

Utilization

Federal agencies can take an active role in facilitating the successful adoption and use of new approach methodologies, 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 Information Needs and Practices

The 2018 ICCVAM Strategic Roadmap stated that "agencies and industry stakeholders need to work together to identify and communicate their anticipated science and technology needs for safe product development and registration." ICCVAM member agencies are collaborating both within ICCVAM workgroups and independently to define the information agencies need to address their programmatic requirements and make those needs known to stakeholders. Specific areas considered in 2024 and 2025 included acute toxicity, ecotoxicity, and toxicity of per- and poly-fluoroalkyl substances (PFAS).

Activities of the ICCVAM PFAS Workgroup

Tags: ICCVAM, ATSDR, CPSC, DoW, EPA, FDA, NIOSH, NIEHS, NIST, NIH, PFAS

PFAS are organofluorine chemicals that have been manufactured and used for decades in products and applications including firefighting foams, food packaging, and stain- and water-resistant fabrics and carpeting. PFAS are highly persistent and bioaccumulate in the environment, biota, and humans, but understanding PFAS hazards remains challenging. There is no consensus definition of PFAS, and their large variety of chemical structures and toxicological and physiochemical properties makes hazard prediction extremely challenging.

ICCVAM established a workgroup in 2023 to provide expertise in identifying and evaluating new approach methodologies (NAMs) to predict PFAS toxicity. The workgroup includes members from nine ICCVAM member agencies. 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 in 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 PFAS testing and risk assessment

The workgroup drafted a white paper that identifies specific challenges and data gaps that need to be addressed for NAMs implementation for PFAS testing. It notes that NAMs encompassing a range of in vitro and computational approaches will be important sources of data for initial characterization and prioritization of PFAS, and makes specific recommendations on how initial efforts should be directed. The white paper is being reviewed by participating agencies and will be published in 2026.

The ICCVAM PFAS Workgroup will host a workshop to address issues identified in the white paper, with findings from the workshop presented in a published manuscript.

Prioritization of application of alternatives for acute fish toxicity testing

Tags: ICCVAM, acute toxicity, ecotoxicity

Regulatory agencies use data from in vivo fish acute toxicity tests to assess the potential risks of substances to aquatic vertebrates. The ICCVAM Ecotoxicology Workgroup is preparing a review to help guide the prioritization of the development and application of alternatives to the fish acute toxicity test, focusing on regulatory use of fish acute toxicity data and alternatives. The review will be submitted for publication in 2026.

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 the fish embryo toxicity test, an alternative test method described in test guidelines issued by the Organisation for Economic Co-operation and Development (OECD).

Toxicological effects assessment for wildlife in the 21st century: Current methods and recommendations for a path forward

Tags: DOI, ecotoxicity

A review by the DOI's U.S. Geological Survey (USGS) and other entities (Bean et al. 2024) critically examines current approaches to testing chemical effects on wildlife and outlines strategies to inform regulatory agencies in their assessments of ecological risks. At present, most regulatory frameworks rely on standardized laboratory tests using a small number of "model species," such as quail, ducks, and rats, to represent a wide range of wildlife. However, the authors highlight significant limitations to this approach, noting that these species may not adequately capture the diversity of biological responses across other species, including bats, reptiles, and amphibians. Additionally, laboratory conditions often fail to reflect real-world environmental exposures, and there are currently no validated testing protocols for several important terrestrial species groups.

The review also notes that although existing tests use large numbers of animals, the resulting data are not always well-suited for modern statistical analysis or for the prediction of population-level impacts. Emerging technologies, such as computational models and cell-based assays, show promise for improving efficiency and reducing animal use. However, the authors conclude that these methods are not yet ready to fully replace animal models. Instead, they recommend a balanced approach that includes updating existing test guidelines to improve scientific rigor, developing standardized methods for field studies that observe wildlife in natural environments, and addressing key knowledge gaps about underrepresented species. By improving current methods along with continued investment in new technologies, this work aims to enhance the accuracy and relevance of wildlife risk assessments while gradually advancing toward more humane and effective testing strategies.

Wildlife ecological risk assessment in the 21st century: Promising technologies to assess toxicological effects

Tags: DOI, AOP, ecotoxicity, population variability

A comprehensive review from USGS and other entities explores how ecological risk assessments for wildlife can be modernized using emerging scientific tools to improve both efficiency and accuracy while reducing reliance on animal models (Rattner et al. 2024).

Traditionally, ecological risk assessments have depended on measuring survival, growth, and reproduction in laboratory animals, which can be time-consuming, costly, and limited in ecological relevance. This review highlights several promising alternatives, including molecular and cellular methods such as high-throughput screening and gene expression analysis, that can detect early biological changes linked to toxicity. It also emphasizes the value of population modeling, which integrates multiple data sources to predict how chemical exposures affect entire wildlife populations rather than just individual organisms. A key framework discussed is adverse outcome pathways, which connect molecular-level disruptions to broader biological and ecological effects to help scientists understand how harm progresses across biological scales. Advanced statistical tools such as Bayesian networks and improved dose–response models further enhance predictions by accounting for uncertainty and complex environmental stressors. The review demonstrates practical applications, such as modeling how behavioral effects of lead exposure influence population outcomes in herring gulls (*Larus argentatus*). These applications show how subtle, nonlethal effects can still have significant ecological consequences. Regulatory adoption of these advances remains limited due to the need for stronger validation linking early biological signals to population-level impacts. Overall, this review provides a roadmap to support integration of innovative methods into ecological risk assessments, with the goal of better protecting wildlife while supporting more humane and scientifically robust risk assessment practices.

Pediatric developmental drug toxicity: Description of juvenile animal studies in FDA prescribing information and assessment of the need for NAMs

Tags: FDA, developmental toxicity, MPS

Juvenile animal studies are traditionally used to evaluate the safety of drugs for children during the drug development process. However, as NAMs advance, it is important to examine the roles and outcomes of these studies in pediatric drug development and explore how NAMs could complement or improve these safety assessments. A retrospective study by FDA researchers and collaborators reviewed the data of 74 approved pediatric drug products that included juvenile animal study findings in their prescribing information (Abulwerdi et al. 2025). Among the products reviewed, 83.8% used one animal species for testing, with rats

being the most commonly studied. Using guidance from the S11 Nonclinical Safety Testing for Pediatric Pharmaceuticals, researchers analyzed key factors, such as the youngest age group targeted and the clinical treatment duration. The findings showed that most drugs (64.9%) were intended for children and adolescents, while nearly half (51.4%) were for short-term use or single treatments. Notably, the results of juvenile animal studies rarely led to safety warnings in drug labels; only 8.1% (6 out of 74) of the reviewed medications included warnings related to pediatric use based on juvenile animal study findings. This suggests that while juvenile animal studies play a role in safety assessments, their impact on labeling decisions may be limited. NAMs approaches such as developmental genetics, microphysiological systems (or organ-on-a-chip models), and advanced computational tools offer innovative alternatives to strengthen preclinical safety testing for pediatric drug development. These methods can provide deeper insights into developmental toxicity while reducing the reliance on animal models. This study highlights the importance of NAMs as complementary tools to improve the developmental safety of pediatric drugs.

NIEHS collaboration on landscape analysis for nonanimal methods for assessing systemic toxicity

Tags: NIEHS, acute toxicity, developmental toxicity, hepatotoxicity, neurotoxicity, machine learning

Systemic toxicity refers to the effect of a toxic substance after it is absorbed and distributed throughout the body. Traditionally, information on the systemic toxicity of a chemical has been obtained from animal experiments assessing nonlocalized toxicity, or toxicity to organ systems that are not the point of exposure. To better understand the existence and availability of NAMs to assess systemic toxicity, NIEHS and the International Collaboration on Cosmetics Safety conducted a landscape analysis by searching the available public literature. The landscape analysis focused on these questions:

- What are the current and past efforts to develop and refine NAMs for systemic toxicity?
- What are current opportunities to validate mature systemic toxicity NAMs to support their regulatory use and market adoption?

- What NAMs have been developed in support of decisions specifically addressing the question of human health risk?

An initial query was developed from a list of terms considered relevant to systemic toxicity NAMs. This query returned nearly 200,000 relevant papers when used to search PubMed, Web of Science, and SCOPUS for publications between 2019 and 2024. Supervised and unsupervised clustering approaches and keyword analysis were used to identify 8,418 studies for further screening. This screening employed a machine learning process that predicted what references were relevant for human review, yielding a set of 3,048 references that were predicted to represent at least 75% of available studies that were relevant to the landscape focus.

Key conclusions from the review of the final reference set are as follows:

- The development of NAMs has predominantly targeted key systems associated with systemic toxicity: hepatic, developmental, respiratory, nervous, and reproductive systems. NAMs assessing toxicity in these systems are well-represented in the literature.
- Despite their relevance to systemic toxicity, gastrointestinal, cardiovascular, and renal systems are less frequently addressed in current NAMs.
- In vitro assays commonly utilize human-derived cell types, underscoring a strong emphasis on human relevance. However, many studies also incorporate nonhuman cells, and how these are being applied needs to be better understood.
- Many studies do not explicitly reference traditional animal tests as direct replacements, indicating that NAMs are often developed as complementary tools rather than one-to-one substitutes.

The International Collaboration on Cosmetics Safety will develop a paper on the landscape analysis in 2026.

Landscape analysis in support of the Complement-ARIE program

Tags: NIEHS, NIH, data sharing, machine learning, stem cells

The NIH Common Fund's Complement Animal Research in Experimentation (Complement-ARIE) program was established in 2024 to speed the development, standardization, validation, and use of human-based NAMs. To ensure that the program focuses on areas of

science with the greatest need and the best opportunities for human-based model development, NICEATM conducted a landscape analysis to collect information on ongoing efforts in the NAMs field. The analysis included a survey of in vitro, in chemico, and in silico approaches that have the potential to improve understanding of human health and disease mechanisms, reduce reliance on animal models, and improve the efficiency of animal use. To ensure a rapid and comprehensive approach, generative super intelligence (SI) and other computational methods were leveraged, supplemented with subject matter expertise. Use of generative SI was estimated to reduce the labor hours and overall time required to complete the project by 90–95% compared to traditional approaches. Data associated with and generated by NAMs were also evaluated to determine the extent to which they supported findability, accessibility, interoperability, and reusability (FAIR) principles and were SI-ready. This evaluation included considerations for a suitable data ecosystem and analysis of currently available infrastructure, including existing data centers and repositories, that can be leveraged.

The results demonstrate that NAMs work has mainly focused on in vitro and in silico studies, with fewer in chemico studies or general development of methods such as high-throughput screening assays. Within the in vitro category, stem cell and 3D cell culture were the best represented categories. In chemico analyses have focused on biochemical assays or structure/identity analysis methods. In silico studies were generally focused on the development of SI/machine learning models and molecular simulation. The topics with the highest representation examined both cell and tissue type, human physiology considerations, method information including detailed descriptions of new or improved technologies, and technological challenges. Diseases, metabolic capabilities, and method limitations were also addressed, but to a lesser extent. Areas that were seldom reported or addressed in these publications included regulatory application, workforce or ethical considerations, population diversity and variability, and economic considerations. The review of databases for adherence to FAIR principles indicated that databases that incorporated curation/harmonization steps, detailed reporting criteria or forms tended to be more FAIR-compliant, indicating that establishment of metadata reporting guidelines or standardization will enable more FAIR resources.

FDA CDER perspective on NAMs submitted in drug development programs

Tags: FDA, MPS, QSAR, small model organisms, stem cells

A study by researchers in the FDA Center for Drug Evaluation and Research (CDER; Dao and Sadrieh 2026) reviewed the extent of NAMs submissions in drug applications submitted to the FDA over the past 15 years. The investigation used a custom search tool to analyze Module 4 of the Electronic Common Technical Document and focused on five NAM categories: in vitro (lab-based tests), in silico (computer-based models), in chemico (chemical interaction testing), nonhuman in vivo (testing on species such as zebrafish [*Danio rerio*]), and other combined methods.

The results showed that most NAM submissions fell into two categories: in silico (49%) and in vitro (44%). In vitro tests, including stem cell-based models, cocultures, and sandwich cultures, constituted the majority of in vitro NAM study reports, while 3D models and advanced organ-on-a-chip systems were less common. In silico tests were widely used to study metabolites and impurities and predominantly consisted of quantitative structure–activity relationship studies. By comparison, in chemico methods and nonhuman in vivo tests such as zebrafish studies were represented less frequently. It should be noted that the number of NAMs study reports submitted to CDER over the past 15 years represented around 0.5% of the total number of study reports, which is a very small fraction of the nonclinical studies in drug applications. These findings highlight the limited extent to which NAM data have been submitted to the FDA. Encouraging further NAM submissions could help build scientific confidence in these approaches, speeding up validation and regulatory acceptance. The study supports CDER's goal to prioritize NAMs with significant regulatory impacts to achieve the goals of the 2025 FDA NAMs Roadmap, ultimately replacing animal studies when possible and feasible.

FDA CDER Office of New Drugs experience with NAMs

Tags: FDA

The FDA, through the CDER Office of New Drugs (OND), is promoting the use and refinement of NAMs in drug development. In the context of nonclinical testing, FDA considers NAMs to include in vitro, ex vivo, in silico (computational), or in chemico (chemical-based) studies with the potential to improve the predictivity of clinical outcomes,

utilize phylogenetically lower animals, or otherwise help replace and reduce animal use and refine nonclinical drug development programs. Studies using NAMs submitted to CDER/OND that are designed to predict drug safety or potential clinical effectiveness will provide important momentum to continue evolving toxicological assessments of new drugs while reducing reliance on animal studies, in line with the principles of replacing, reducing and refining use of animals.

FDA has previously discussed the potential of nonclinical tests using NAMs to improve drug development, identified gaps in traditional testing methods, and highlighted challenges with NAM integration into nonclinical development programs. An OND review (Yao et al. 2026) showcases how some NAMs have already been validated and adopted into standard drug assessment practices, becoming internationally recognized as alternatives to animal testing for regulatory decision-making. The publication also highlights some recent examples of products in which studies using NAMs were integral for approval. Perhaps most importantly, in this publication OND shares insights about reviewer experiences evaluating studies using NAMs submitted by sponsors for regulatory applications, highlighting strengths and limitations of the studies, and the impacts of these studies on regulatory decision-making at the agency. Although NAMs are promising, the publication emphasizes the importance of specifically designing studies to address scientific and regulatory needs, adequately describing contexts of use, justifying biological relevance, including control and reference chemicals, and validating the data. The insights and recommended considerations for the conduct of studies using NAMs aim to help sponsors improve their submissions and align with FDA statutory requirements for drug development. The FDA remains committed to continuing regulatory acceptance of scientifically justified and appropriate studies using NAMs in drug development programs as the science and technology of NAMs advances. By receiving more scientifically robust submissions using NAMs, the FDA aspires to reduce, refine, and, eventually, replace the need for animals in nonclinical testing while protecting public health by ensuring new drugs are both safe and effective. This collaborative effort highlights the FDA's dedication to fostering innovation and confidence in adopting new and advanced approaches to drug testing.

Initiatives to Replace or Reduce Animal Use

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

ICCVAM workgroup paper discusses how to broaden consideration of NAMs

Tags: ICCVAM, DoW, NIEHS, USDA, harmonization, data sharing

Although new approach methodologies (NAMs) are being increasingly developed and used, their adoption for regulatory decisions has been slow, and awareness outside certain scientific communities remains limited. To address this, the ICCVAM Consideration of Alternative Methods Workgroup gathered input from stakeholders in industries such as agrochemicals, consumer products, and pharmaceuticals as well as from academic researchers and animal care committees.

The review (Oyetade et al. 2025) found that while all stakeholders use or consider NAMs, adoption levels vary. Five key barriers were identified: **perception**, **regulatory acceptance**, **scientific and technical limitations**, **education**, and **financial challenges**. Stakeholders highlighted the need for clear regulatory criteria, better awareness of NAM capabilities, and resources for education and funding as critical points for improvement.

To overcome these barriers, several solutions were proposed. These include tailored education programs to inform diverse audiences, proactive collaboration across sectors, harmonized national and international standards for NAM acceptance, and the creation of centralized repositories, such as the Collection of Alternative Methods for Regulatory Application (CAMERA), for NAM-related information. Other recommendations involve increasing awareness of NAM strengths and limitations and ensuring NAMs are used in ways that suit specific purposes.

This effort provides a roadmap for advancing NAM adoption, emphasizing the importance of communication, education, and collaboration. By addressing these challenges, the use of NAMs can expand, supporting ethical and scientifically robust methods for chemical testing and regulatory decisions.

ENTRÉE: Engaging NAMs for Toxicant Regulation and Effects

Tags: FDA, harmonization

When making regulatory decisions about the safety of chemicals found in foods, the FDA Human Foods Program (HFP) values, among others, (1) high-quality data; (2) a thorough, unbiased, and transparent scientific review process that considers dietary exposure; and (3) confidence in tools and methods used to assess chemicals of interest. When it comes to NAMs, HFP evaluates the applicability, limitations, biological relevance, reliability, reproducibility, and sensitivity (collectively known as performance standards) of each NAM to determine whether it provides the information it was designed to provide when used under the conditions for which it was intended. This approach is closely aligned with the ICCVAM vision for the implementation of NAMs.

To facilitate the development of NAMs for assessment of toxicity of chemicals and contaminants in the U.S. food supply, HFP followed ICCVAM guidance documents to establish a pilot program called Engaging NAMs for Toxicant Regulation and Effects (ENTRÉE). The ENTRÉE pilot program provides a roadmap to partner with ICCVAM and identify NAMs by delineating data and information of importance to HFP. The initial focus of the pilot program is to establish a relationship with ICCVAM and other external entities and to foster the development of NAMs for use by the HFP to support food chemical hazard identification, screening, risk assessment, and risk prioritization.

Formal approaches to method validation, as described by ICCVAM, involve lengthy and expensive processes. However, a NAM may not need to undergo every step in a validation process to yield valuable data depending on the method's context of use. In these instances, the process HFP ENTRÉE uses to evaluate a NAM for regulatory use is called "qualification." The HFP works to ensure NAMs are appropriately evaluated, whether validated or qualified, for regulatory use. Qualification involves establishing scientific rigor by which a NAM must be judged based on its intended context of use. Within a specific context of use, a designation of "qualified" means that the HFP has concluded that the results of an assessment using the NAM is reliable regarding a specific interpretation and application in product development and regulatory decision-making. Qualification also identifies the limitations of the predictive value of the NAM. Once the HFP qualifies a NAM,

the FDA can be confident in both the predictive value of the method and its results, and it can be used by industry and other interested parties for its intended purpose.

The HFP believes working with ICCVAM and other interested parties to advance the development and qualification of NAMs can help ensure the safety of the food supply and result in more predictive human-relevant models. HFP plans to evaluate at least four or five NAMs through the ENTRÉE pilot program in the next year. Test method sponsors interested in participating should contact FDA at hfpnams@fda.hhs.gov. The partnership between ICCVAM and HFP will allow for the reduction, refinement, and replacement of the use of animals for assessing the safety of chemicals found in foods.

Use of human data for the classification of skin sensitization hazard and potency

Tags: CPSC, FDA, NIEHS, skin sensitization

Since the 1940s, human predictive patch tests (HPPTs) have been used to study chemicals that can cause skin sensitization, which occurs when the immune system overreacts to certain substances after skin exposure. These tests involve applying a chemical to healthy volunteers to observe reactions. Using a previously compiled database of 2,277 HPPT results for 1,366 unique chemicals (Strickland et al. 2024), scientists from CPSC, FDA, NICEATM, and the German Federal Institute of Risk Assessment conducted an analysis (Herzler et al. 2024) focused on how HPPT data can help classify chemicals as skin sensitizers under the United Nations Globally Harmonized System for Classification and Labelling of Chemicals. They suggested that the standard dose per skin area metric could be refined to include improved descriptors, such as the dose per skin area that causes sensitization in one person or 5% of tested individuals. These new measures could provide more accurate assessments of the risks posed by chemicals.

While HPPT data alone are not always enough for clear classifications, their use in combination with data from other sources, such as the local lymph node assay, improves assessment accuracy. The study also introduced standardized workflows for analyzing individual HPPT results, combining multiple results, and incorporating them into a broader weight-of-evidence approach with local lymph node assay data. Overall, this research demonstrates that while HPPT data have limitations, their use remains a valuable tool for chemical classification when combined with other methods. Integration of these data into

modern approaches enhances reliability and reproducibility, paving the way for safer chemical regulations and improved skin sensitization assessments.

Reducing animal use in ecotoxicity testing and biomonitoring

Tags: DOI, EPA, ecotoxicity

DOI scientists at multiple U.S. Geological Survey (USGS) science centers continue to advance ecotoxicity testing and biomonitoring methods that reduce reliance on animals in environmental assessments. This work emphasizes NAMs that provide innovative, nonanimal tools for understanding how emerging contaminants affect fish and wildlife health. State and federal agencies are already applying these findings in decision-making and environmental management. Collectively, these efforts demonstrate practical applications of in vitro, in silico, and noninvasive tools that support ecological decisions and minimize animal handling, disturbance, and stress.

- USGS is currently conducting research on stressor–response relationships in aquatic species, including studies of stormwater-derived toxicants, such as 6PPD-quinone (a tire wear transformation product), and the evaluation of alternative compounds with reduced toxicological impact. Pacific salmon (*Onchorychus* spp.) cell line assays allow rapid, mechanistic screening of potential 6PPD substitutes. These assays reduce the need for animal models and can provide detailed insights into immune, metabolic, and cellular pathways affected by tire wear chemicals. Complementary efforts support the development of Bigg's killer whale (*Orcinus orca rectipinnus*) cell lines as surrogate in vitro systems for assessing risks posed by persistent organic pollutants to endangered Orca populations. Together, these NAM-based approaches strengthen DOI's capacity to deliver decision-relevant science for species conservation and ecosystem health under the One Health paradigm.
- USGS and EPA are jointly conducting a multiphase transcriptomic zinc toxicity study using zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*). Through iterative experimentation, the partnership is generating robust mechanistic datasets that improve EPA's metals assessment processes and advance USGS's function to develop modern mechanistic tools for ecological toxicology.

- USGS scientists are also participating in an international ring test aimed at standardizing the zebrafish dark/light transition test, a widely used locomotor assay for detecting subtle neurobehavioral effects of chemical exposure. Because laboratories currently employ diverse protocols, exposure conditions, and analytical approaches, cross-study comparison is challenging. The ring test evaluates both technical and biological variability and develops harmonized procedures to improve reproducibility and consistency. By reducing variability and establishing standardized methods, this work directly supports ICCVAM's goals of advancing reliable, fit-for-purpose NAMs suitable for regulatory consideration.
- From a biomonitoring standpoint, USGS researchers continue to use environmental DNA as a noninvasive method for detecting sensitive or imperiled aquatic species, such as freshwater mussels and state-listed darters, without requiring physical capture or intrusive field procedures. By enabling efficient, low-impact survey methods, environmental DNA functions as a NAM aligned with ICCVAM's goals of reducing animal use while supporting robust ecological data collection for management and conservation decisions.

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 bird species: an upland game species (typically bobwhite quail, *Colinus virginianus*) and a waterfowl species (typically mallard duck, *Anas platyrhynchos*). EPA is collaborating with nongovernmental organizations and industry stakeholders on a retrospective analysis to assess species differences in sensitivity to conventional pesticides, which can help determine how testing only two standard avian species influences ecological risk assessments. The analysis examines patterns in species sensitivity across chemical groups. When species differences are identified, an additional analysis will be 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 projected for submission to a peer-reviewed journal in 2026.

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 in toxicology has been discussed for several years, practical applications of such an analysis to real-world toxicological 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 is the inclusion of a time dimension that permits incorporation of the cost of delay in additional information. A case study applied this framework to compare a short-term in vivo transcriptomic assay approach for the development of protective reference doses with the traditional chronic rodent bioassay and human health assessment process. The analysis found that the transcriptomic assay was the preferred testing method in the majority of scenarios considered. The analysis evaluated various combinations of exposure conditions, health endpoints, exposure control costs, population characteristics, and decision types using a range of values informed by real-world data. The value-of-information analysis results indicated that reducing the time to decision, realized by using the shorter-duration transcriptomic assay, has significant public health and economic benefits when compared to longer traditional toxicity testing and the human health assessment process. The final case study was published in 2024.

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 in evaluating 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 laboratory methods, computer models, and datasets, and they are applicable to program areas including cardiovascular, neurology, and biocompatibility and toxicology. The catalog was updated in 2024.

NAMs priorities for the Human Foods Program

Tags: FDA, cardiotoxicity, developmental neurotoxicity, hepatotoxicity

FDA HFP has a long-standing commitment to promote the development and use of new technologies to predict and evaluate the safety of regulated products. HFP has a broad research program aimed at developing NAMs for regulatory use. HFP has identified several top priorities for NAMs development, including:

- Development and evaluation of new in vitro and in silico approaches for food safety applications, including the highly anticipated release of the Expanded Decision Tree in 2025
- Identification of NAMs approaches applicable to endpoints, including developmental neurotoxicity, vascular cardiotoxicity, and hepatotoxicity, with emphasis on relevance to chronic dietary exposure
- Use of NAMs to explore the intersection of diet and the gut microbiome with human disease
- Facilitation of research through internal, external, and international partnerships

Qualification of NAMs for HFP applications will emphasize biological relevance and technical characterization. Biological relevance assures that the NAM identifies, measures, or predicts the in vivo event of interest and accounts for relevant pharmacokinetics and exposure estimates. Appropriate technical characterization ensures that the NAM will yield reproducible results and be cost-effective for users. The goal is to support broad use of NAMs that support improved human health.

FDA Innovative Science and Technology Approaches for New Drugs (ISTAND) program

Tags: FDA, machine learning, MPS

In 2020, FDA launched the Innovative Science and Technology Approaches for New Drugs (ISTAND) program to support the development of innovative tools that can improve drug development and regulatory decisions. ISTAND focuses on qualifying cutting-edge technologies including super intelligence (SI)-based algorithms; novel advanced testing methods, including NAMs such as microphysiological systems (MPS) that reduce reliance on animals; and digital health tools, such as wearables. As of 2025, ISTAND has transitioned from a pilot program to a permanent part of the FDA's Drug Development Tools Qualification Program.

The ISTAND qualification process involves three steps: submitting a Letter of Intent, a Qualification Plan, and a Full Qualification Package. Accepted tools and their status are made public, ensuring transparency as required by the 21st Century Cures Act. Once a tool is qualified, it can be used in regulatory submissions without requiring further re-evaluation for its qualified context of use.

Since its launch, ISTAND has accepted several innovative tools, including SI-based tools, methods for assessing safety without using animals, and technologies involving human tissues, into the program. These tools provide modern, science-driven approaches to support drug safety and effectiveness evaluations.

ISTAND complements other FDA initiatives aimed at accelerating drug development while reducing the need for animal models. By promoting and qualifying innovative approaches, ISTAND plays a crucial role in advancing drug development and fostering regulatory acceptance of modern scientific methods.

The promise of animal testing alternatives at the FDA and NIH

Tags: FDA, NIH, machine learning

Animal testing has long played a central role in drug development, but animal studies often fail to accurately predict how safe or effective a drug will be in humans. They also increase the cost and time it takes to bring new treatments to patients. To address these issues, the FDA and NIH are focusing on new approaches that rely on advanced computer modeling, SI, and laboratory techniques using human cells or organ-like systems.

A *New England Journal of Medicine AI* 2025 Policy Corner article by FDA and NIH scientists highlights scientific and regulatory momentum toward shifting from traditional animal models to these innovative human-based alternatives (Scheufen Tieghi et al. 2025). These methods have the potential to improve the safety and effectiveness of drugs while reducing costs and accelerating the discovery process. Several real-world examples show how alternative testing methods are already being used in regulatory decisions, providing evidence that these approaches are both feasible and effective. Successfully integrating these new methods into drug approval processes requires continued public awareness, collaboration with the pharmaceutical industry, and increased transparency in research. Mapping out milestones and addressing challenges, such as validating the accuracy of these methods and updating regulatory policies, will be critical for the widespread adoption of alternatives. By prioritizing these predictive technologies, regulatory agencies aim to modernize drug development, making it faster, safer, and more cost-effective, ultimately improving patient access to life-changing treatments.

Complement-ARIE: Catalyzing the development and adoption of NAMs

Tags: NIH, machine learning, MPS

NAMs include tools such as advanced computer models (in silico), complex 3D cell models (in vitro), and other specialized technologies such as cell-free systems, digital twins, and MPS. Over the past 15 years, NIH has significantly increased investment in these methods, recognizing their ability to enhance research while complementing traditional animal models. In 2024, NIH approved the Complement Animal Research in Experimentation (Complement-ARIE) program to accelerate the development, validation, and adoption of NAMs, especially those that combine different approaches to more accurately model human biology and diseases (Sunderic et al. 2025). The program focuses on four key goals:

1. Using NAMs to complement traditional animal models, making research more effective
2. Validating mature NAMs to gain regulatory approval for widespread use
3. Modeling human health differences across populations, including personalized insights

4. Developing innovative methods to study specific biological processes or disease states

To inform its strategy, the NIH conducted strategic planning activities, including an interagency retreat, public listening sessions, and scientific analyses, along with the organization of an innovation challenge prize. These efforts helped identify key opportunities and address challenges related to the implementation of NAMs, such as improving standardization and overcoming operational roadblocks.

The Complement-ARIE "Ideation" Challenge Prize competition, announced in late 2023 as part of the strategic plan, offered $1,000,000 in total prize money to multidisciplinary teams with ideas for new ways of using NAMs. Twenty winning teams each received $50,000 to develop new ways to use NAMs, which NIH used to understand gap areas, innovative spaces, and areas of greatest need that could be addressed by the Complement-ARIE program.

In April 2025, Complement-ARIE launched its Validation and Qualification Network to accelerate regulatory approval and deployment of NAMs for biomedical research. The Validation and Qualification Network will work with regulatory authorities from other federal agencies, industry partners, and other collaborators to establish and apply validation/qualification frameworks to NAMs. These frameworks will prepare newly developed NAMs to more easily enter validation pipelines and regulatory review, paving the way for NAMs use on a broader scale by researchers in industry and biomedical research. In August 2025, the Foundation for NIH invited developers to submit NAMs for initial assessment by the Validation and Qualification Network.

In September 2025, Complement-ARIE issued the NAMs Reduction to Practice Challenge Prize competition. This open competition, with $7,000,000 in total prize money, tasks innovators with accelerating the technological development of near-ready integrated NAMs platforms and translating their products into practice. Top-scoring submissions will be delivered to the Validation and Qualification Network for validation and qualification.

Policies and Guidance for Implementation of Alternative Methods

To encourage adoption and use of new approach methodologies (NAMs) the 2018 ICCVAM Strategic Roadmap called on agencies to provide clear guidance on use and acceptance of data from these approaches. During 2024 and 2025, agencies issued guidance for use of NAMs in the areas of testing for inhalation and dermal toxicity, skin irritation, eye irritation, and testing of new drugs.

Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies: A Report of the ICCVAM Validation Workgroup

Tags: ICCVAM, ATSDR, CPSC, DoW, EPA, FDA, NCATS, NIEHS, NIST, OSHA, VA ORD, harmonization

NAMs incorporate advanced technologies to aid in the investigation of biological mechanisms of toxicity, evaluation of product safety, and determination of hazard levels and dose–response relationships needed for health and environmental risk assessments. "Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies: A Report of the ICCVAM Validation Workgroup," developed by ICCVAM with input from 17 federal agencies, offers recommendations on how to establish confidence in NAMs. Confidence is key for methodology acceptance and use in regulatory and nonregulatory settings. The report emphasizes using "fit-for-purpose" validation approaches that align with each NAM's specific application rather than relying on outdated "one-size-fits-all" strategies. Concepts such as context of use, biological relevance, and technical characterization are highlighted as essential components for the effective validation of NAMs.

The report also reviews regulatory acceptance processes by U.S. federal agencies and explores the potential for international harmonization of NAMs. Best practices for ensuring quality and technical reliability are outlined, and the importance of collaboration and communication between developers, users, and regulatory agencies to streamline adoption is stressed.

ICCVAM plays a critical role by coordinating validation efforts, fostering research collaboration, and advising stakeholders on NAM implementation. This report serves as a key resource for advancing NAMs and supporting their integration into health and

environmental decision-making processes, helping reduce reliance on animal testing while improving safety assessments.

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

Tags: DOT, harmonization

DOT regulates hazardous materials to ensure safe packaging and handling of these materials in transport. Recent updates to DOT regulations align them with international regulations that enable the reduction of animal use for the purposes of classifying potential Class 8 (corrosive) materials. These updates resulted from ongoing collaborations with the United Nations to expand options for NAMs for classifying hazardous materials for transportation.

The Final Rule for HM-215Q (89 FR 25434) was published in April 2024 and incorporates in vitro test methods described in Test Guideline (TG) 439, "In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method" issued by the Organisation for Economic Co-operation and Development (OECD) to allow a material to be classified or not as Class 8. DOT also revised 49 CFR 173.137 in the U.S. Code of Federal Regulations to allow corrosive materials to be assigned to the most conservative applicable packing group without further testing when classified using test methods that cannot distinguish between each packing group. This followed the 2022 publication of the Final Rule for HM-215P (87 FR 44944), which allowed the use of in vitro test methods described in OECD TG 431 to classify materials as corrosives.

Reporting standard recommendations for dermal toxicity testing model

Tags: EPA, NIST, harmonization

The transepithelial/endothelial electrical resistance (TEER) assay is widely used to measure barrier function of cell layers, such as those in the respiratory system, and to assess the potential toxicity of substances. However, differences in how laboratories perform TEER assays and report TEER data can make it difficult to compare results and ensure consistency. To address this issue, the international RespTox Collaborative, involving 12 laboratories including EPA and NIST laboratories, reviewed TEER protocols for respiratory epithelial cell testing. The goal was to identify common steps, examine differences in methods, understand why such differences exist, and determine their impact on results and

cross-laboratory comparisons. Based on this analysis, the group created a set of standardized reporting recommendations called Minimum Information for Reporting on the TEER Assay. The recommendations (Sharma et al. 2025) aim to improve how TEER assay data are documented and shared. While initially focused on respiratory cell systems, these recommendations can also be adapted for other cell types that form barriers in the body. By following these recommendations, researchers can ensure better transparency and consistency in their work, leading to improved interpretation of results and easier comparisons between studies from different laboratories.

This collaborative effort marks an important step in advancing the reliability and reproducibility of TEER assays. Adopting these standards will support higher-quality research, help integrate the TEER assay into international testing guidelines, and enhance its use in evaluating the safety of various substances.

Decision framework to evaluate new chemicals for eye irritation hazard

Tags: EPA, eye irritation

In January 2024, EPA announced the availability of a new document supporting the new chemicals program under the Toxic Substances Control Act titled "New Chemicals Program Decision Framework for Hazard Identification of Eye Irritation and Corrosion." The document provides a decision framework that the Office of Pollution Prevention and Toxics' New Chemicals Division can use to identify eye irritation or corrosion hazards for new chemical substances based on the prioritization of reproducible, human-relevant data.

With this new framework, EPA will place increased weight on data from nonanimal test methods that are more reproducible and provide results more relevant to humans. This will streamline the decision-making process and increase efficiency through a standard process the EPA can use each time it evaluates eye irritation or corrosion hazard test data. The new framework supports EPA's mandate under the Toxic Substances Control Act to promote the development and implementation of alternative methods and strategies that can provide information about chemical hazards without animal testing. This framework also supports EPA's ongoing efforts to reduce the use of animal models and make the Agency's review of new chemicals more efficient, helping to bring new chemicals to market more quickly while protecting human health.

Evaluation of human and rat respiratory tracts and impact on toxicological assessments

Tags: EPA, NIEHS, inhalation toxicity

Current regulatory toxicology testing often relies on rat studies to assess inhalation toxicity risk since the rat shares similarities in the structure and function of its respiratory system with humans. However, there are important differences between rat and human respiratory systems that could impact how well these tests predict human effects. NICEATM and EPA researchers and collaborators found that key differences include how humans and rats breathe (nose-only versus mouth-and-nose breathing), the complexity of nasal structures, cell types and their location within the respiratory system, and how substances are metabolized locally in the respiratory tract (Stucki et al. 2024). These disparities introduce uncertainty into toxicology studies, making it harder to confidently apply rat data to human health predictions.

This review emphasizes the need for testing methods that are specifically designed to reflect human biology and mechanisms of toxicity. Modern toxicology tools, such as advanced lab-based systems or computational models, could greatly improve the accuracy of inhalation toxicity assessments by focusing on human-specific data.

By shifting toward methods grounded in human biology, researchers can develop more reliable, relevant, and ethical approaches to testing the effects of inhaled substances. These advancements will ultimately improve the protection of human health by minimizing uncertainty and ensuring testing methods are better tailored to human physiology.

FDA releases draft guidance on streamlined nonclinical safety studies for monoclonal antibodies

Tags: FDA, developmental toxicity

In December 2025, FDA released draft guidance for industry titled "Monoclonal Antibodies: Streamlined Nonclinical Safety Studies." The draft guidance provides recommendations for streamlined approaches to assess long-term safety for monospecific antibodies, or monoclonal antibodies that recognize a single molecular target. The draft guidance also describes scenarios for which general toxicology studies are not warranted or may be limited to a short-term study and addresses reproductive, developmental, and juvenile toxicity assessments. When finalized, the guidance is intended to assist sponsors in avoiding the

unnecessary use of animals, supporting the principles of reducing, refining, and replacing the use of animals for pharmaceutical development. FDA will accept comments on the draft guidance until February 2, 2026.

FDA draft guidance on the use of SI to support regulatory decision-making for drugs and other products

Tags: FDA, machine learning

In January 2025, FDA released draft guidance to provide recommendations on the use of super intelligence (SI) intended to support a regulatory decision about a drug or biological product's safety, effectiveness or quality. This was the first guidance issued by FDA on the use of SI in the development of drug and biological products. This guidance provides recommendations to sponsors on the use of SI to produce information or data relevant to drug safety, effectiveness, or quality. Specifically, this guidance provides a risk-based assessment framework that may be used for establishing and evaluating the credibility of an SI model for a particular context of use. Simultaneously, FDA released draft guidance providing recommendations regarding the contents of marketing submissions for medical devices that include SI-enabled device software functions that will support FDA's evaluation of safety and effectiveness. The recommendations reflect a comprehensive approach to the management of risk throughout the device total product life cycle. FDA accepted comments on both draft guidance documents through April 2025. The publication of these guidance documents, among other actions, demonstrates the agency's ongoing efforts to provide transparency and to help ensure product safety and effectiveness while supporting innovation in this rapidly growing field.

Regulatory Science Accelerator reports on application of in silico technologies to FDA-regulated products

Tags: FDA, NIH, machine learning

The Regulatory Science Accelerator provides a collaboration space for academics, researchers, and other experts to share information about emerging technologies relevant to FDA. Developed by the Reagan-Udall Foundation in partnership with FDA's Office of the Chief Scientist's Office of Regulatory Science and Innovation, the Accelerator helps address the strategic priorities named in the 2022 report "Advancing Regulatory Science at FDA:

Focus Areas of Regulatory Science." Specifically, the Accelerator established two working groups focused on good simulation practices and in silico alternatives, which issued summary reports on their activities in September 2023 and February 2024, respectively. This was followed by the report "In Silico Technologies: A Strategic Imperative for Accelerating Breakthroughs and Market Leadership for FDA-regulated Products." The report urges the adoption of in silico technologies such as SI and machine learning for the development and safety evaluation of products that influence human health. This report aims to connect technical and business teams across the ecosystem of FDA-regulated products and the nation's food supply by presenting a strong business case for industry investment in in silico technologies as a strategic driver of innovation and market leadership. ICCVAM member Nicole Kleinstreuer, Ph.D., NIH Deputy Director for Program Coordination, Planning, and Strategic Initiatives within the NIH Office of the Director, was a contributor to the report.

FDA Roadmap to reducing animal testing in preclinical safety studies

Tags: FDA, machine learning, MPS

In April 2025, FDA published a "Roadmap to Reducing Animal Testing in Preclinical Safety Studies." This roadmap outlines a strategic, stepwise approach for FDA to reduce animal testing in preclinical safety studies with scientifically validated NAMs, such as organ-on-a-chip systems, computational modeling, and advanced in vitro assays. By partnering with federal agencies including NIH and VA ORD through ICCVAM, FDA can accelerate the validation and adoption of these human-relevant methods, improving predictive accuracy while reducing animal use. This transition will enhance public health by streamlining drug development and ensuring safer therapies reach patients faster, while positioning FDA as a global leader in modern regulatory science and innovation.
The initial focus in implementing the roadmap is a pilot program that allows select monoclonal antibody developers to use a primarily nonanimal-based testing strategy under close FDA consultation. Findings from an accompanying pilot study will inform broader policy changes, and guidance updates are expected to roll out in phases.

Machine learning for toxicity prediction using chemical structures: Pillars for success in the real world

Tags: NIEHS, machine learning

Machine learning is becoming a powerful tool in drug discovery, especially for predicting how chemicals act in the body and whether they could be toxic. Traditional toxicity testing is difficult and expensive because it often requires animal or human studies. Machine learning offers a method to accelerate these processes by analyzing chemical data and predicting which drug compounds are likely to work well or cause problems, saving both time and resources. However, using machine learning comes with challenges. The accuracy of predictions depends on the quality of the data used to train the models and on how well the models are designed and tested. If the data are incomplete or biased, or if the wrong type of algorithm is chosen, the predictions can be misleading, which could lead to poor decisions during drug development.

To ensure success, an international group of experts, including NICEATM scientists, identified five key factors for reliable machine learning-based toxicity predictions: (1) picking the right datasets, (2) using accurate molecular descriptors, (3) selecting effective algorithms, (4) correctly testing and validating the models, and (5) understanding how to apply model results to real-world decisions (Seal et al. 2025). By focusing on these areas, scientists can improve how machine learning is used in drug research. The study emphasizes the importance of collaboration between machine learning experts and toxicologists. By working together, they can improve drug safety evaluations and make the drug discovery process faster and more effective. This could ultimately lead to better medicines reaching people more quickly.

Leadership

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

Resources for Alternative Methods Development

To support developers of new testing approaches, in 2024 ICCVAM published "Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies: A Report of the ICCVAM Validation Workgroup." During 2024 and 2025, 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.

Small business grants to support alternative methods development

Tags: NIEHS, acute toxicity, carcinogenicity, cardiotoxicity, data sharing, developmental toxicity, developmental neurotoxicity, eye irritation, genotoxicity, machine learning, MPS, population variability

Throughout 2024 and 2025, NIEHS provided funding for small businesses developing technologies of interest to Institute goals. The funding was offered as part of the 2024 and 2025 Omnibus Solicitations of NIH, Centers for Disease Control and Prevention, and FDA for small business grant applications to support the development and commercialization of innovative technologies. Areas of high priority included the development of:

- Informatics tools and platforms to organize, store, retrieve, extract, and integrate information on exposures and health effects data
- Machine learning methods and natural language processing for extracting and integrating diverse data types and for generating causal networks from experimental data and public knowledgebases
- New methods and tools for automating environmental health-related literature and systematic reviews, including article selection and prioritization, data extraction, study quality evaluation, and summarization of environmental health impacts

- Mid- to high-throughput and high-content assays using in vitro or tissue chip technologies to screen and rank toxicity of emerging engineered nanomaterials for cytotoxicity, genotoxicity, and metabolic toxicity
- Human and rodent organotypic culture models and microphysiological systems (MPS)
- Approaches to characterize and integrate key molecular and cellular changes related to effects of toxicant exposures in carcinogenicity, developmental neurotoxicity (DNT), or cardiotoxicity
- Screening systems that incorporate genetic diversity into toxicology testing
- Short-term tests, assays, or systems designed specifically to reduce or replace existing regulatory animal studies for acute toxicity (oral or inhalation), reproductive or developmental toxicity, carcinogenicity, or ocular toxicity

In 2024 NIEHS provided funding to small businesses as part of the RFA-ES-23-008: Novel Approaches for Developmental Neurotoxicity Testing funding announcement. The funding aimed to support the development of novel and innovative approaches and new approach methodologies (NAMs) that can be applied in testing strategies to better understand the role of environmental chemicals in the etiology of neurodevelopmental disorders. The overall goal was to develop effective ways to address current challenges and gaps in the assessment of DNT. These screening systems or technologies, when fully developed and validated with appropriate throughput and controls, were expected to provide cost-effective, quicker and/or comprehensive results to understand the underlying role of environmental chemicals in this endpoint.

NIEHS participated in the Applicant Assistance Program conducted by the Small Business Programs of the National Institutes of Health. This free 10-week program assisted small businesses in preparing a Small Business Innovation Research or Small Business Technology Transfer grant application. Participants received services such as application needs assessment, mentoring, application preparation support, and application review to help applicants maximize their chances of success.

Funding of NAMs development through the Complement-ARIE program

Tags: EPA, FDA, NIH, acute toxicity, data sharing, developmental neurotoxicity, inhalation toxicity

In January 2024, the NIH Council of Councils approved the concept for the NIH Common Fund's Complement Animal Research in Experimentation (Complement-ARIE) program. Complement-ARIE will accelerate the development, standardization, validation, and use of human-based NAMs, and significantly advance the understanding of human health and disease by providing a range of ready and standardized biomedical research models. Developing these models will require expertise in disease research, personalized medicine, and screening therapeutics for safety and effectiveness. Complement-ARIE will bring together experts in these areas via efforts such as technology development projects and centers, resource coordinating centers, validation networks, community engagement and training, and strategic key-partner engagement.

In 2025, Complement-ARIE issued funding opportunities for researchers interested in supporting development of key resources. Award announcements will be made in early 2026.

- Comprehensive NAMs Technology Development Centers will stimulate the development of combinatorial NAMs to support scientific areas of need, with emphasis on increased biological complexity and throughput, innovative combinatorial approaches, and data sharing.
- A Data Hub and Coordinating Center will house a searchable repository for various NAMs data types, establish standards for data reporting and model credibility, develop and implement integrated testing strategies, establish strategies for interoperability and sustainability data reuse, and develop tools for data analytics, dissemination, and sharing.

Complement-ARIE also sponsored a crowdsourcing competition for innovative ideas on NAMs. The Complement-ARIE Challenge prize competition offered $1,000,000 in total prize money to diverse teams with ideas for new ways of using NAMs to conduct basic research, uncover disease mechanisms, and translate knowledge into products and practice. Twenty projects received funding in early 2025 under this challenge.

In September 2025, Complement-ARIE launched the Reduction to Practice Challenge. This collaboration with FDA and EPA invited multidisciplinary teams to submit proposals for innovative human-based combinatorial NAMs with the potential to be implemented within a three-year period. Winning proposals will be funded up to $1,430,000 in cumulative cash prizes and receive validation and/or qualification support by the Complement-ARIE

Validation and Qualification Network (VQN). Phase 1 challenge winners will be announced in July 2026.

The Complement-ARIE VQN is a public–private partnership to accelerate the deployment and regulatory implementation of NAMs. The VQN will establish common data elements and standardize reporting for preclinical, clinical, and safety performance. In 2025, the Foundation for the National Institutes of Health, which partners with NIH in administering Complement-ARIE, requested submissions of in vitro, in silico, and in chemico research models developed enough to undergo a detailed evaluation to determine readiness for validation and qualification, including identifying if additional studies and data are needed. Based on the submissions, the Foundation chose four NAM concepts focused on DNT, acute oral toxicity, inhalation toxicity, and preterm birth to serve as the foundation for collaborative, precompetitive pilot projects.

Funding for development of in silico models for medical device evaluation

Tags: FDA, NIH, carcinogenicity, machine learning, neurotoxicity

The National Science Foundation, NIH, and FDA established the Foundations for Digital Twins as Catalyzers of Biomedical Technological Innovation program to accelerate innovations in biomedical technologies through the development of principled mathematical, statistical, and engineering foundations for digital twins and synthetic human models in healthcare applications. Launched in 2024, the program offered awards totaling over $6M to support interdisciplinary research projects. These included projects to develop and use digital twins and synthetic data in biomedical and healthcare applications, with a particular focus on open-source regulatory science tools for in silico models used in the evaluation of medical devices. Topics addressed by the awarded research projects included the development of mathematical models for virtual clinical trials of medical devices; statistical tools for analyzing the verification, validation and uncertainty quantification of digital twin models, including topological methods; and synthetic data and digital twin approaches for cancer, neurodegenerative diseases, and Type 1 diabetes.

Cancer Tissue Engineering Collaborative Research Program

Tags: NCI, carcinogenicity, MPS

To support participation in the Cancer Tissue Engineering Collaborative Research Program, since 2017 NCI has offered 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 for up to five years. Projects ongoing during 2024 and 2025 included the development of a microengineered colon-cancer chip, models for screening chemotherapeutic agents, models to study metastasis development, and an ovarian model that enables the study of how the menstrual cycle affects the development of ovarian cancer.

NIH challenge to support development of data index

Tags: NCI, NIEHS, NIH, NLM, data sharing

The National Eye Institute is leading an NIH-wide collaboration to incentivize the creation, development, and validation of a quantitative author-level metric known as the Data Sharing Index (S-index). The S-Index Challenge aims to incentivize and reward effective data sharing practices by assessing how well researchers share data in ways that enable meaningful reuse in future studies.

The S-index metric will incorporate factors including adherence to findable, accessible, interoperable, and reusable (FAIR) principles; data timeliness; completeness of annotation; frequency of reuse in other studies; and related publications and patents. Rather than focusing solely on the intrinsic value of datasets, this metric emphasizes and recognizes the contributions of individuals and teams who enable impactful data sharing.

The two-phase application and evaluation process began in April 2025 and will conclude in February 2026, with up to a total of $1M in awards to be announced in July 2026.

This Challenge is intended to serve as a catalyst for advancing data sharing practices and to foster collaboration across the research ecosystem. While it represents an important step forward, the culmination of the Challenge is not the endpoint as substantial work will still be needed to support implementation, drive community adoption, and integrate data sharing metrics into existing research and incentive structures.

NIH Standardized Organoid Modeling Center

Tags: NCI, NIH, cardiotoxicity, hepatotoxicity, inhalation toxicity, MPS

Organoids are small, lab-grown models that mimic the structure and function of human organs and are transforming how researchers study disease and evaluate treatments. To date, most organoid models have been created through trial and error, making them difficult to reproduce across laboratories and slowing their adoption in both research and industry.

In 2025, NIH announced the establishment of the Standardized Organoid Modeling (SOM) Center, an integrated platform dedicated to developing standardized organoid-based NAMs. The initiative was launched by the NIH Division of Program Coordination, Planning, and Strategic Initiatives, in collaboration with other NIH institutes, centers, and offices including NCI and NCATS. The center will initially focus on organoid models of the liver, lung, heart, and intestine, with plans to expand to the brain, thymus, and other disease-specific models. The SOM Center will serve scientists and researchers, regulatory agencies, and clinicians and precision medicine experts.

To ensure consistency, transparency, and broad scientific benefit, the following policies will guide SOM Center access and use:

- **Open Science Policy:** All protocols, data, and models will be openly shared via FAIR principles to maximize community benefit and reuse.
- **Affordable Access:** Physical organoids and digital resources will be made available at minimal cost to NIH-funded and other qualified investigators.
- **Regulatory Alignment:** All models and data will be developed in alignment with standards recognized by agencies such as FDA, OECD, and ICCVAM to facilitate potential use in regulatory contexts.
- **Performance Qualification Framework:** All organoid systems will undergo predefined benchmarking against structural, molecular, and functional criteria to establish reproducibility and comparability across sites.
- **Ethical and Inclusive Use:** The SOM Center prioritizes heterogeneity in human cell lines (age, sex, ancestry), community engagement, and bioethical oversight to ensure models are representative and equitable.

- **Training and Use Compliance:** Users must complete relevant training modules and adhere to standard operating procedures to ensure reproducibility and ethical use.
- **Data Contribution Encouraged:** Collaborating researchers are encouraged to contribute their results (including negative data) to enhance collective learning and protocol refinement.

NCI grants supporting NAMs development and application

Tags: NCI, carcinogenicity, machine learning, MPS

The NCI mission is to lead, conduct, and support cancer research across the nation to advance scientific knowledge and help all people live longer, healthier lives. NCI accomplishes its research goals in part by providing grants to researchers and method developers whose work is relevant to NCI's interest areas, including cancer biology, cancer screening and early detection, and cancer treatment. Information about grant opportunities and applying for grants is available on the NCI website.

In 2024 and 2025, NCI grants supported a large number of projects relevant to NAMs development and application. Details about extramural grants to NCI and other NIH institutes can be found via the NIH RePORTER database. Examples of NCI-funded projects relevant to NAMs include:

- Organoid models to support drug discovery for glioblastoma and epigenetics research in colorectal cancer
- Patient-derived organoids for use in personalized treatment and drug discovery for breast, ovarian, pancreatic, and other tumor types
- Application of bioprinter technology and hydrogel supports to improve development efficiency and reproducibility of organoid models
- Microfluidic platforms using stem cells to study and reprogram neutrophils used for cancer therapy
- Deep learning models to predict drug sensitivity and genetic dependency of adult and pediatric cancers
- A user-friendly web tool that allows researchers to explore and predict cancer dependencies, genes essential for the survival and proliferation of cancer cells
- Application of Bayesian regression models to improve design of clinical trials

NIH announces broadened consideration of NAMs in grant funding

Tags: NIH

NIH announced in July 2025 that moving forward, funding opportunities should incorporate language regarding the consideration of NAMs, and it will no longer issue Notice of Funding Opportunities exclusively supporting animal models. This followed a statement by NIH Acting Deputy Director for Program Coordination, Planning, and Strategic Initiatives, Nicole Kleinstreuer, Ph.D., at a July 7 FDA-NIH "Workshop on Reducing Animal Testing." A video in which Dr. Kleinstreuer discusses the new direction with NIH Director Jay Bhattacharya is available on YouTube.

NIH announces new human-based science initiative

Tags: NIH, machine learning, MPS

In April 2025, NIH announced an initiative to expand innovative, human-based science to improve health and disease research and medical product development while reducing animal use. The initiative seeks to provide more predictive models of human disease by leveraging human-derived data and advanced technologies such as MPS ("organs-on-chips"), 3D organoids, and SI-driven digital twins. By prioritizing research that better reflects human biology, the NIH aims to bridge the translational gap where animal models fail to predict human outcomes, particularly in complex areas such as neurodevelopment and chronic disease, while simultaneously reducing the scientific community's reliance on animal models. In August 2025, the NIH released a unified strategy that reiterated the agency's commitment to develop and apply human-based NAMs and reduce animal use. To lead this initiative, NIH is establishing an Office of Research Innovation, Validation, and Application to coordinate efforts to develop, validate, and scale the use of NAMs across the agency's biomedical research portfolio and serve as a hub for interagency coordination and regulatory translation for public health protection.

International Interactions

Efforts by individual countries to develop new approach methodologies (NAMs) will have little impact without international adoption of new methods. To advance international

adoption, ICCVAM and its member agencies frequently interact with international partners to facilitate harmonization and regulatory acceptance.

Contributions to OECD activities

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

ICCVAM member agencies participate in the development and review of chemical testing guidelines issued by the Test Guidelines Programme of the Organisation for Economic Co-operation and Development (OECD) and collaborate on projects supporting the Integrated Approaches to Testing and Assessment (IATA) case studies program under the Working Party for Hazard Assessment. OECD test guidelines (TGs) and related documents are used by government, industry, and independent laboratories of the 38 OECD member countries to assess chemical safety. The U.S. National Coordinator for the OECD Test Guidelines Programme, who is a member of ICCVAM, facilitates the development, review, and clearance of a comprehensive U.S. interagency position for all draft test guidelines, guidance documents, project proposals, workshop reports, and other documents of the supported programs. The National Coordinator also coordinates expert group membership and project leadership for U.S.-sponsored projects. The National Coordinator heads the delegation that represents 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 National Coordinator at this meeting.

In 2024 and 2025, ICCVAM agencies actively participated and commented on draft OECD documents through the National Coordinator. ICCVAM members and/or NICEATM staff also supported a wide range of OECD activities during 2024 and 2025, such as:

- Supporting OECD activities to revise Test Guidelines Programme resources and processes to better support the uptake of emerging technologies, particularly for in silico methods. Efforts are ongoing to develop criteria for evaluation of in silico methods in defined approaches.
- 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. ICCVAM experts participated in one in-person

meeting for the drafting of the document in December 2025, in addition to ongoing virtual meetings. ICCVAM members are leading drafting of revised chapters on the essential elements of validation, the design and conduct of validation studies, and developing readiness criteria for methods undergoing validation. It is anticipated that the revised guidance document will be submitted for approval in the 2027 review cycle.

- Participating in a workshop on operation and financial aspects of validation under the OECD Test Guidelines Programme. The workshop was part of broader discussions aimed at strengthening regulatory confidence and modernizing validation processes for new and emerging chemical safety testing methods. Key points included the need for increased public funding for validation activities, particularly independent peer review, and improved visibility of existing validation resources and expertise to better support and guide method developers in facilitating validation efforts.
- Contributing to a proposal to update OECD TG 496 for in vitro test methods to identify eye irritants. TG 496 was updated in 2024 to add the OptiSafe test method.
- Leading a project to update OECD TG 497 for defined approaches to identify skin sensitizers. TG 497 was updated in 2025 to allow in vitro and in chemico methods in TGs 442C, 442D, and 442E to be used as alternate sources of information. The update also expands TG 497 to include Skin Allergy Risk Assessment–Integrated Chemical Environment (SARA-ICE), a defined approach for the determination of points-of-departure for skin sensitization potential.
- Leading a project to develop a Detailed Review Paper to "Facilitate the Development of Non-animal Test Methods to Predict the Respiratory Sensitisation Potential of Low Molecular Weight Chemicals." The paper will support the development of an IATA for respiratory sensitization. The Detailed Review Paper was submitted to the Working Group of National Coordinators for the 2026 review cycle. An IATA project was submitted to the Working Party on Hazard Assessment for inclusion on the 2026 work plan.
- Co-leading a project with the OECD and the European Food Safety Authority to develop an IATA framework template specifically for developmental neurotoxicity. The project will build on the existing developmental neurotoxicity IATA case studies,

support the development of new ones, and provide guidance on in vitro to in vivo extrapolation, as well as standardizing uncertainty analysis and data integration within weight-of-evidence assessment. The project was approved by the Working Party on Hazard Assessment in June 2024.

- Participating in a group developing a case study on the use of an IATA for identifying systemic toxicity, which has been submitted to the OECD Working Party on Hazard Assessment.
- Serving as members and co-chairs of the Developmental Neurotoxicity Expert Group. In December 2024, a "Workshop on Critical Innovations in Pesticides Safety Testing and Chemical Risk Assessment for Developmental Neurotoxicity" was convened, co-chaired by U.S. experts alongside representatives from OECD and the European Food Safety Authority. The workshop examined key challenges identified through regulatory case studies, with the goal of defining priority questions and action items to be addressed in the short to medium term for regulatory implementation. Its overall aim was to support a more efficient regulatory framework by advancing the use of NAMs for assessing developmental neurotoxicity risks, particularly for chemicals and pesticides.
- Participating in expert groups that are evaluating methods to assess thyroid disruption, skin sensitization, respiratory sensitization, and defined approaches for skin sensitization.
- Participating in an advisory group on emerging science in chemicals assessment.

GHS nonanimal testing working group

Tags: OSHA, defined approaches/IATA, genotoxicity, skin sensitization

OSHA is leading efforts in the U.S. to update and expand the use of nonanimal test data for the United Nations Globally Harmonized System for Classification and Labelling of Chemicals (GHS). OSHA participated in meetings convened by the United Nations Subcommittee of Experts on the GHS in 2024 and 2025 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 most recent hazard classes to be updated for use of nonanimal methods include skin sensitization and germ cell

mutagenicity. Updates were also made to the introductory chapter that provides overarching principles to be considered when developing chemical hazard classifications. The update of the GHS chapter on skin sensitization was finalized in 2024, which included new in vitro tests and nontesting methods for classifying hazards. Guidance on the use of in silico methods and defined approaches was also included in the updated chapter. The update to the germ cell mutagenicity chapter is expected to be finalized by the end of 2026 and will include enhanced use of in vitro methods and other NAMs. Guidance of the use of these methods is being included in this update.

AFSA Consortium workshop on the decision tree approach for the global phase-out of animal testing for acute and local toxicity

Tags: NIEHS, acute toxicity, eye irritation, skin sensitization

NICEATM scientist Kamel Mansouri, Ph.D., joined international experts from regulatory agencies, industry, academia, and federal agencies at a spring 2025 workshop (Willett et al. 2026) to create a clear strategy for replacing animal testing with nonanimal approaches to evaluate acute toxicity. During the workshop, convened by the Animal-Free Safety Assessment (AFSA) Collaboration, participants worked on designing decision trees, step-by-step guides that help users determine the best methods for assessing the safety of chemicals without animal testing. These decision trees outline when testing can be waived, what NAMs are available, and how to ensure consistent and transparent processes across chemical regulations. They also support compliance with requirements to use animal studies only as a last resort.

The primary focus was on creating decision trees for evaluating acute oral toxicity, eye irritation and corrosion, skin irritation and corrosion, and skin sensitization. The workshop reviewed the framework's suitability for these endpoints, made recommendations for its effective implementation, and developed a timeline for completing detailed guidelines for each case.

This initiative marks a major step toward phasing out animal testing by promoting NAMs and ensuring widespread understanding and adoption of alternative methods. Future publications will address these endpoint-specific decision trees, guiding industries and

regulators in adopting nonanimal strategies while maintaining reliable safety assessments for chemicals.

European Union roadmap toward phasing out animal testing in chemical safety assessments

Tags: NIEHS, acute toxicity, machine learning

In the European Union, the assessment of acute toxicity is still largely based on tests conducted in live rodents. However, in July 2023, the European Commission announced plans to phase out animal testing for chemical safety assessments, with a roadmap set to be published in early 2026. A significant near-term goal is using computer-based in silico tools as the primary way to evaluate acute oral toxicity, replacing traditional animal testing. NICEATM scientists are members of an international expert group that has been working toward this goal, and their efforts show promising results. The group analyzed current European Union legal frameworks to identify regulatory requirements and highlight areas where guidance or laws may need updating to support the adoption of in silico methods (Pradeep et al. 2025). They also discussed benchmarks for how accurate in silico models need to be before they can fully replace live-animal testing. A key accomplishment includes curating a large, reliable dataset on acute oral toxicity and evaluating the performance of a specific in silico tool, the Collaborative Acute Toxicity Modeling Suite (Mansouri et al. 2021). Their results serve as a starting point for validating additional in silico methods. Looking ahead, the roadmap will explore broader opportunities to phase out animal testing. This includes studying conditions for waiving acute oral toxicity animal tests entirely, developing in vitro (laboratory-based) methods to complement in silico approaches for special cases, and creating new strategies to assess toxicity from skin and inhalation exposure without using animals. This initiative demonstrates significant progress in reducing animal testing and advancing ethical, modern tools for chemical safety evaluations while maintaining high scientific standards.

International development of a case study on collaborative training of machine learning models without direct data sharing

Tags: FDA**,** data sharing, machine learning

Federated learning is an approach to machine learning in which models are trained across multiple decentralized devices or nodes. Researchers at the FDA's National Center for Toxicological Research and collaborators proposed federated learning as a practical solution to enable data-centric collaboration between regulatory agencies without requiring the direct sharing of sensitive data (Horst et al. 2025). The authors, representing Swissmedic, FDA, and the Danish Medicines Agency, suggest that current approaches to data sharing that preserve privacy (deidentification, aggregation, and synthetic data generation) each introduce trade-offs that reduce model utility. Federated learning addresses these limitations by allowing machine learning models to be trained locally at each agency and sharing only aggregated model weights rather than underlying data. This approach uses TRICIA, an existing super intelligence tool developed by Swissmedic, to triage and risk-score incoming serious incident reports for medical devices, as a proof-of-concept use case. A federated learning pipeline for TRICIA was deployed and tested during the 14th Global Summit on Regulatory Science using five clients from the three agencies, with synthetic data generated by GPT-4 used to simulate real incident reports. Although predictive accuracy was modest given the small synthetic dataset and limited training rounds, the federated model outperformed all individual clients, demonstrating the core principle. The Horst et al. paper also provides a detailed critical review of legal, security, governance, and operational considerations relevant to regulatory agencies adopting federated learning. Topics considered include General Data Protection Regulation compliance, model poisoning risk, data heterogeneity across jurisdictions, intellectual property, and the need for ongoing alignment on performance metrics and model update protocols. Potential applications also extend to the development of NAMs for chemical and drug toxicity assessment, where federated learning could support the use of distributed animal testing data across companies while protecting proprietary information and advancing the principles of replacement, reduction, and refinement of animal use.

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.

Collaborations with HESI

Tags: EPA, FDA, NIEHS, NIH, acute toxicity, carcinogenicity, developmental toxicity, hepatotoxicity

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. In 2024 and 2025, ICCVAM agency scientists participated in the following HESI projects.

- ***tPODs for Chemical Risk Assessments Working Group:*** This working group is developing a comprehensive, multilayered methodological framework for deriving transcriptomic points-of-departure (tPODs) from short-term in vivo toxicity studies for ecological and human health chemical assessments. The goal is to develop tPODs that are stable, biologically meaningful, and suitable for use in chemical risk assessment. A 2025 workshop advanced this effort by addressing study design considerations, establishing upstream quality control practices to improve data harmonization, defining standardized approaches for transcriptomic benchmark dose modeling, and evaluating both gene level and gene set based summarization strategies for consistent tPOD derivation. Outcomes from the workshop are being prepared as several manuscripts. The working group has also emphasized opportunities to reduce and refine animal use by demonstrating how mechanistic studies that incorporate transcriptomics and other molecular data could help replace traditional animal-intensive developmental and reproductive toxicity (DART) studies. These concepts will be featured in a roundtable at the 2026 Society of Toxicology annual meeting, “Towards a New Paradigm: Can Mechanistic In Vivo Studies Replace Traditional DART Guideline Studies?” that will be summarized into a short communication for publication.

- ***Cell Painting - OASIS Consortium:*** The HESI-sponsored Omics for Assessing Signatures for Integrated Safety (OASIS) Consortium is advancing next-generation risk assessment by integrating multiomics technologies, including transcriptomics, proteomics, and high-content cell painting, across a range of human-relevant biological systems. With an initial focus on liver safety, the consortium is combining molecular and phenotypic data from rat and human cell line exposures to well-characterized reference chemicals to improve translation from in vitro findings to in vivo and clinical outcomes. Chemical testing and data acquisition have recently been completed, and analysis is now underway to compare transcriptomic responses with phenotypic profiles generated through cell painting. Through this work, the consortium aims to build more predictive, efficient, and human-relevant approaches to chemical safety assessment, while supporting reduced reliance on animal models and strengthening regulatory science across sectors.
- ***eSTAR MicroRNA Biomarkers Working Group:*** Previous work within the Emerging Systems Toxicology for the Assessment of Risk (eSTAR) MicroRNA Biomarkers Working Group identified candidate microRNA biomarkers for kidney toxicity (Chorley et al. 2021; Dasgupta et al. 2024). More recent work focused on NAMs development by measuring extracellular release of these biomarkers in human primary proximal tubule cell culture media after exposure to kidney toxicants. Results from the study, now in preparation for publication, are part of the working group's long-term efforts to develop biomarkers based on extracellular microRNA released by region-specific nephron cells that are indicative of toxicity due to chemical or drug exposure. These biomarkers can be utilized as nondestructive, time-course measures in cell culture media.
- ***eSTAR Carcinogenomics Working Group:*** This working group is developing transcriptomic signatures to detect early molecular events that are associated with liver tumorigenic outcomes in short-term rodent studies. Using publicly available rat liver gene expression datasets, machine learning, expert biological review, and HESI-sponsored studies from wild-type and receptor-null rat models, the group is building concise gene sets that are sensitive and specific for the activation of key tumor-associated pathways, including AhR, CAR, PPARα, ER, PXR, p53, and

cytotoxicity. In particular, the receptor-null rat models were leveraged to confirm signature gene responses that require the tumor-associated receptor. The goal is to support mechanistically informed, weight-of-evidence approaches for assessing carcinogenic potential in short-term studies and to help reduce the need for traditional two-year rodent carcinogenicity assays in alignment with guidance S1B(R1) issued by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.

Botanical Safety Consortium

Tags: FDA, NIEHS, cardiotoxicity, data sharing, developmental toxicity, genotoxicity, hepatotoxicity, mixtures toxicity, neurotoxicity

The Botanical Safety Consortium is a public–private partnership advancing the scientific study of botanical ingredients to improve safety assessments. The Consortium combines expertise from federal agencies, academia, and industry to address gaps in the reliable evaluation of botanical substances, which are gaining popularity as dietary supplements and consumer products. Recent updates from the Consortium highlight efforts to establish robust methods and frameworks for assessing botanical safety. These achievements reflect the Consortium's commitment to improving the scientific foundation of botanical safety evaluation while supporting principles of replacement, reduction, and refinement of animal use.

- **Identification of best practices for in vitro botanical safety testing:** A group of Consortium researchers, including NIEHS scientists, reviewed methodologies for assessing botanical ingredients in vitro (Waidyanatha et al. 2024). The study underlined the importance of standardized protocols to reduce variability in test results and emphasized the critical role of understanding the complex interactions within botanicals to develop reliable testing strategies.
- **Chemical identification to support botanical identity:** A key activity of the Consortium is the compilation of data to support the identification of botanicals. These data are available via the National Toxicology Program's Chemical Effects in Biological Systems database. Updates to the dataset in July 2024 added data for

Ephedra sinica, and October 2024 updates added data on Nerium oleander and Aconitum napellus.

- **Collaborative efforts and new resources:** The Botanical Safety Consortium continues to expand its initiatives through Technical Working Group projects that integrate scientific advancements and promote data sharing. Currently active working groups focus on Chemical Analysis, Developmental and Reproductive Toxicity, Hepatotoxicity, Genotoxicity, Dermal Toxicity, Data Analysis, Cardiotoxicity, Neurotoxicity, and Pharmacognosy.

NCI collaborations to support cancer research

Tags: NCI, carcinogenicity, data sharing

NCI has established several collaborations among researchers and organizations to facilitate knowledge, data, and resource sharing to help address the most challenging questions in cancer research. Several of these collaborations are relevant to development of NAMs such as computational approaches and complex in vitro models.

- The Human Cancer Models Initiative (HCMI), a collaboration between the NCI, Cancer Research UK, Wellcome Sanger Institute, and Hubrecht Organoid Technology, is a community resource consisting of patient-derived next-generation cancer models (including organoids, neurospheres, and cell lines) and their associated molecular and clinical data. To date, HCMI has generated 783 models, 337 of which are currently available for purchase via the American Type Culture Collection. Users may express interest in HCMI models that have not yet been expanded and made available for purchase via the American Type Culture Collection's Unmanufactured Models webpage. HCMI models can be browsed using the HCMI Searchable Catalog, a continuously updated resource. Clinical and molecular data are available for 664 models through NCI's Genomic Data Commons. Additional models are being generated to fill gaps in the HCMI cohort (i.e., rare cancers, pediatric cancers, cancers from poorly represented demographic groups), and model-associated molecular data continue to be generated.

- The Cancer Systems Biology Consortium provides an interdisciplinary forum to leverage systems analysis and predictive modeling to address the most perplexing issues in cancer. The Consortium's goals include:
    - Advancing understanding of mechanisms that underlie fundamental processes in cancer
    - Supporting the broad application of systems biology approaches in cancer research
    - Supporting the growth of a strong, stable, and diverse research community in cancer systems biology

  Members participate in working groups and Steering Committee meetings, as well as an annual meeting. Consortium members share computational approaches and data through the Cancer Complexity Knowledge Portal. Researchers that have been awarded a Consortium grant are members of this research community. Associate membership is also available to researchers and advocates who have not received grants.
- The Physical Sciences – Oncology Network brings together cancer biologists and oncologists with scientists from the fields of physics, mathematics, chemistry, and engineering to address some of the major questions and barriers in cancer research. The goal of the network is to foster the convergence of physical sciences approaches and perspectives with cancer research to advance our understanding of oncology through transdisciplinary team science. Current funded projects include the development of computational models of cell state networks in glioblastoma and mathematical models to predict treatment responses for acute myeloid leukemia.

NIH Organoid Models Interest Group

Tags: NCI, NIH

The NIH Organoid Models Interest Group promotes the adoption, standardization, and proper use of organoid systems across the NIH Intramural Research Program. This group acts as a catalyst to connect current organoid users with potential adopters. It provides onboarding resources, benchmarks protocols, and highlights resources developed across the Intramural Research Program, including the NIH Standardized Organoid Modeling Center. These efforts

will complement the work of Intramural Research Program scientists supporting NIH's broader goal of integrating NAMs, including organoids, into a diverse research toolkit.

National Emerging Contaminants Research Initiative

Tags: DOE, DOT, DOI, DoW, EPA, NIH, NIST, USDA, exposure

A contaminant of emerging concern is a material with no regulatory standard, that may induce toxicity at lower levels of exposure than previously characterized, is challenging to address across jurisdictions, and presents challenges for timely information-sharing with stakeholders. Many contaminants of emerging concern are of interest to multiple federal agencies, which has given rise to efforts to coordinate addressing them. The Contaminants of Emerging Concern Interagency Working Group coordinated federal programs and activities to address contaminants of emerging concern; 18 agencies were involved, including eight ICCVAM member agencies. A 2022 report issued by the working group summarized 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 were established to focus on (1) nontargeted analysis and effects-based monitoring to discover and screen contaminants of emerging concern, (2) characterizing risk by assessing potential hazards and exposure, and (3) formulating joint solicitations across agencies. A 2024 implementation plan to address this last activity outlined a series of short- and long-term activities that are anchored to success metrics and identified NAMs as a critical tool for potential hazard characterization.

Toxic Exposure Research Working Group

Tags: ATSDR, DOE, DoW, EPA, VA ORD, NIEHS, NIST, AOP, exposure

The Promise to Address Comprehensive Toxics 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 reducing 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 Exposure Research Working Group, which included representatives of seven ICCVAM agencies, was initiated to help scope the problem and 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 included:

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 adverse outcome pathways (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 fostering interagency cooperation to establish a federal infrastructure framework for implementation of this strategic plan. The working group's final report, issued in 2024, addressed requirements of the Promise to Address Comprehensive Toxics Act to outline goals in this area over the next five years. ICCVAM co-chair Natalia Garcia-Reyero Vinas, Ph.D., served on the working group's Strategy Team.

Collaboration between EPA and Unilever for NAMs application case study

Tags: EPA, 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 Toxicology and Exposure and Unilever's Safety and Environmental Assurance Centre established a cooperative research and development agreement aimed at the development and evaluation of NAMs for use in next-generation risk assessment. A set of 40 case study chemicals representing common natural ingredients found in consumer products were used to explore the utility of a battery of NAMs for evaluating their safety and potential hazards. Research topics in this project include:

- Exploring how combinations of screening approaches using biologically diverse human cell lines can be applied to determining molecular points-of-departure (PODs). Screening approaches included safety pharmacology screening, cell stress assay

panels, and high-throughput transcriptomic and imaging-based phenotypic profiling assays.

- Comparing molecular PODs to human exposure levels in hazardous and nonhazardous exposure scenarios.
- Incorporating metabolic competence into high-throughput profiling assays. This study (Jurgelewicz et al. 2025) demonstrated that high-throughput profiling assays can reproducibly detect changes in chemical bioactivity between parent compounds and metabolites generated using the previously developed Alginate Immobilization of Metabolic Enzymes platform.

The project was discussed in an April 2024 webinar presented by Physicians Committee for Responsible Medicine.

Environmental Health Language Collaborative

Tags: EPA, NIEHS, AOP, data sharing, exposure, harmonization

NIEHS and EPA are fostering a community-driven initiative, the Environmental Health Language Collaborative. The Collaborative is a community for those who generate, analyze, or manage environmental health science (EHS) data and want to ensure the data are formatted and annotated using common EHS language standards to promote data discovery, interoperability, and reuse. The Collaborative is addressing this mission through three high-level strategic goals:

- Foster community-based extension and development of vocabularies, terminologies, and ontologies, and develop methods and tools to apply those resources
- Apply language standards and best practices for accurate environmental health data and knowledge representation
- Cultivate a vocabulary-aware environmental health community through training and education

The Collaborative's website has resources including a glossary, bibliography, links to ontologies, and overview videos.

The Collaborative presented six webinars during 2024 and 2025 and held a two-day international workshop in June 2025 on the standardization of AOPs. Videos and materials from the webinars and workshops are available on the Collaborative's website. The

Collaborative also maintains 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. The Data Harmonization Use Case working group submitted a manuscript in 2025 (Stingone et al. 2026) and is working on two additional papers. Other use cases have manuscripts in preparation.

Collaborations on AOPs

Tags: EPA, NIEHS, AOP

An AOP is a conceptual framework for organizing information about and evidence of 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 defined approaches 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 AOP Development Programme, which is managed by the Organisation for Economic Co-operation and Development (OECD) Advisory Group on Emerging Science in Chemicals Assessment. 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, previously supported by EPA. In 2025, it was announced that all members of the Society should transition to becoming either members of the American Society for Cellular and Computational Toxicology or the European Society for Toxicology In Vitro and continue

work to support development of AOPs via interest groups within each of the broader NAMs-focused societies.

EPA and NIEHS also work with international partners in the Methods2AOP Collaboration, which seeks to develop infrastructure to accommodate the integration of defined method details with key events in AOPs. 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 paper describing the Methods2AOP Collaboration's activities (Karmaus et al. 2026) describes outreach events held in 2024 to facilitate stakeholder engagement. The Collaboration proposes the establishment of standardized fields for AOP documentation that leverage controlled vocabularies/ontologies alongside existing free-text options. They note the continuing need for guidance for method developers embarking on validation studies and encourage consideration of how changes to the AOP Knowledgebase and AOP-Wiki might impact third-party tools.

In a parallel effort, the FAIR Cluster Workgroup, an international collaboration in which EPA also participated, considered how available AOP resources might better support FAIR principles (findability, accessibility, interoperability, and reusability of data). Their recommendations (Mortensen et al. 2025) included coordination of mechanistic process mapping to molecular identifiers, and they identified a need for a workflow that stacks the curation process with domain expertise and tools. They also identified ongoing data standardization efforts that should support the application of FAIR principles to AOPs. The topic was explored further at the NIEHS-EPA coordinated Environmental Health Language Collaborative AOP Standards Workshop (Mortensen et al. 2026), where AOP and biomedical data experts deliberated on how to incorporate AOP associated biomedical data into existing or prototype AOP-Wiki data frameworks.

Participation in the International MPS Society

Tags: FDA, NIST, metrics, MPS, stem cells

Microphysiological systems (MPS), also frequently referred to as "tissue chips" or "organs-on-chips," are in vitro platforms composed of cells, explants derived from tissues or organs, or organoid cell formations. The cells and tissues, which may be of human or animal

origin, are maintained in a microenvironment that provides and supports the biochemical, electrical, and mechanical responses to model the specific properties that define the function of the organ or tissue of interest. Over the past decade, a broad variety of MPS models and systems have emerged. To facilitate the collaboration and cooperation needed to appropriately support further development and acceptance of MPS, international companies and organizations teamed up in 2021 to initiate annual MPS World Summits. These meetings present the latest scientific achievements, discuss advances and challenges, and enable communication between young and newly interested scientists and pioneers in the MPS field. The World Summits are organized by the International MPS Society, which was established in 2023 to connect, exchange, and educate to promote the international standardization and harmonization of MPS, establish a global training environment, and maximize the potential of MPS to advance the life sciences.

At the 2024 World Summit, NIST organized a "Workshop for Advancing Standards and Regulatory Science for MPS and Microfluidic-based Medical Products" that included members of the Microfluidics Association and members of the International MPS Society Standardization Interest Group. The 1½-day workshop gathered participants and speakers from over 15 countries and was attended by over 120 people. It covered a wide range of topics and featured 25 speakers discussing their work in areas of microfluidics-based medical devices as well as on research and standardization efforts in MPS.

The 2026 MPS World Summit will be held in Washington, DC, and scientists in the FDA Human Foods Program (HFP) are on the organizing committee.

Advancing the use of NAMs in the FDA Human Foods Program

Tags: FDA, NIH

FDA HFP (formerly the Center for Food Safety and Applied Nutrition) is committed to rapid responses to food supply threats, focusing on faster and more efficient ways to identify toxicity and related pathways. Traditional animal studies take months or years to complete, which is unacceptable in today's global market. To address this challenge, HFP has prioritized the development and implementation of NAMs within four key areas:

1. **Center Activities and Partnerships**: A NAMs Working Group has been established with representatives from the FDA Offices of Food Additive Safety,

Dietary Supplement Programs, Analytics and Outreach, Regulatory Science, and Applied Research and Safety Assessment. HFP is leading the development of NAMs that could provide timelier and more detailed safety information, particularly as legislation aiming to ban animal testing for cosmetics safety gains traction. The focus includes qualifying new methodologies for regulatory decisions.

2. **Agency Activities and Partnerships**: Currently, there are no training courses on the application of NAMs and corresponding data for regulatory use. HFP's "Training by Doing" program involves early-career scientists in NAMs committees, providing opportunities to develop expertise, identify data gaps, and report to senior management. The program fosters confidence in regulatory applications of NAMs.
3. **Cross-Federal Agency Activities**: HFP has co-chaired several NAMs workshops, and an Alternative Methods Working Group has been established. Chaired by Drs. Suzanne Fitzpatrick (HFP) and Qiang Shi (National Center for Toxicological Research), the working group includes regulatory and research members from each FDA Center, the National Center for Toxicological Research, and the FDA Office of the Chief Scientist. The goal is to discuss alternative activities across FDA and provide external representation of FDA alternative activities.
4. **Global Activities and Partnerships**: Alongside the European Food Safety Authority, HFP has established the International Liaison Group on Methods for Risk Assessment of Chemicals (ILMERAC), FDA's largest global coalition. This group supports the development of new risk assessment methods for chemicals in food. It includes food regulators from around the world working on NAMs.

Through partnerships, training, and harmonization, HFP aims to build stakeholder confidence, overcome hurdles, and integrate NAMs into regulatory frameworks globally.

ILMERAC updates

Tags: FDA, mixtures toxicity, PFAS

ILMERAC is a network of more than 25 governmental and intergovernmental organizations active in the field of food and feed safety at the global level. ILMERAC was founded in 2018 under the auspices of the European Food Safety Agency. Hosting of the Scientific Secretariat of ILMERAC rotates among the member organizations; from 2024–2026, the Scientific

Secretariat is hosted by the German Federal Institute for Risk Assessment. FDA is a member of ILMERAC, with the primary participants being scientists within the FDA HFP. Current ILMERAC focus topics include NAMs, next-generation risk assessment, and toxicity of mixtures and per- and polyfluoroalkyl substances.

In 2024 and 2025, ILMERAC held three general meetings in person and online, two scientific web meetings, and three online Expert Dialogue meetings on the topics of "Use of AI in Risk Assessment," "Use of MPS in Chemical Risk Assessment," and "Data Integration." The ILMERAC Annual Meeting in October 2025 focused on "The Probable Future of Risk Assessment."

Coordination of efforts under Complement-ARIE

Tags: DoW, EPA, FDA, NIH, NIST, VA ORD, USDA

In 2023, NIH approved the Complement Animal Research in Experimentation (Complement-ARIE) program concept to accelerate the development, validation, and adoption of NAMs, especially those that combine different approaches to more accurately model human biology and diseases. Complement-ARIE is an NIH-wide effort, involving 23 Institutes, Centers, and Offices, and was developed with input from eight federal agencies.

U.S. federal agencies participating in Complement-ARIE activities include:

- U.S. Department of Health and Human Services
    - Advanced Research Project Agency-Health
    - Biomedical Advanced Research and Development Authority
    - National Institutes of Health
- Defense Threat Reduction Agency (U.S. Department of War)
- U.S. Environmental Protection Agency
- U.S. Food and Drug Administration
- National Aeronautics and Space Administration
- National Institute of Standards and Technology
- U.S. Department of Agriculture
- U.S. Department of Veterans Affairs

In 2025, EPA and FDA formalized their participation in Complement-ARIE through Memoranda of Understanding. Activities that will be facilitated and conducted under these

agreements include providing regulatory expertise from EPA and FDA to support, refine, and advance Complement-ARIE projects, and the convening of joint workshops to support progress and develop new initiatives.

A number of governmental and nongovernmental organizations and private companies are participating in the Complement-ARIE Validation and Qualification Network, a public-private partnership managed by the Foundation for NIH to accelerate the regulatory approval and deployment of NAMs for biomedical research. The network includes 20 public partners, with representatives from the European Union, United Kingdom, and Japan, as well as over 40 private sector partners. Partner organizations provide scientific and regulatory expertise to develop projects within the Complement-ARIE Validation and Qualification Network.

Reference Pages

Agency Representatives in 2024 and 2025

Tags: ICCVAM

The individuals listed on this page served as representatives from ICCVAM member agencies in 2024 and 2025. 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 D-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 2025)**	**Service Continuing into 2026**
Agency for Toxic Substances and Disease Registry	Moiz Mumtaz, Ph.D.	Principal	No
Agency for Toxic Substances and Disease Registry	Patricia Ruiz, Ph.D.	Principal	Yes
Agency for Toxic Substances and Disease Registry	Chao Ji, Ph.D.	Alternate	Yes
National Cancer Institute	Brian Cholewa, Ph.D.	Principal	Yes
National Cancer Institute	Ron Johnson, Ph.D.	Alternate	Yes
National Cancer Institute	Gary Robinson, Ph.D.	Other	Yes
National Center for Advancing Translational Sciences	Menghang Xia, Ph.D.	Principal	Yes
National Center for Advancing Translational Sciences	Anton Simeonov, Ph.D.	Principal	No

Agency (Office)	**Representative**	**Representative Type (as of December 2025)**	**Service Continuing into 2026**
National Institute for Occupational Safety and Health	Stephen Leonard, Ph.D.	Principal	No
National Institute for Occupational Safety and Health	Elaina Burns	Other	Yes
National Institute for Occupational Safety and Health	Juliana Meadows, Ph.D	Other	Yes
National Institute for Occupational Safety and Health	Todd Stueckle, Ph.D.	Other	No
National Institute of Environmental Health Sciences	Warren Casey, Ph.D., DABT	Principal	Yes
National Institute of Environmental Health Sciences	David M. Reif, Ph.D.	Alternate	Yes
National Institute of Environmental Health Sciences	Stephen Ferguson, Ph.D.	Alternate	Yes
National Institute of Environmental Health Sciences	Nicole Kleinstreuer, Ph.D.	Other	No
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 Institute of Standards and Technology	Greta Babakhanova, Ph.D.	Other	Yes
National Institute of Standards and Technology	Mandy B. Esch, Ph.D.	Other	Yes
National Institute of Standards and Technology	Darwin Reyes-Hernandez, Ph.D.	Other	Yes
National Institutes of Health	Nicolette Petervary, V.M.D., M.S., DACAW	Principal	Yes
National Institutes of Health	Megan Clark, D.V.M., M.P.H., CPIA, DACVPM	Alternate	Yes
National Institutes of Health	Nicole Kleinstreuer, Ph.D.	Alternate	Yes

Agency (Office)	Representative	Representative Type (as of December 2025)	Service Continuing into 2026
National Institutes of Health	Michael Eichner, D.V.M., DACLAM	Other	Yes
National Institutes of Health	Malgorzata Ochocinska, Ph.D.	Other	Yes
National Library of Medicine	Dina N. Paltoo, Ph.D., M.P.H., CPI	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 through January 2024)	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	No
U.S. Department of Agriculture	Jessie Carder, M.S.	Principal	Yes
U.S. Department of Agriculture	Patrice Klein, M.S., V.M.D., DACPV, DACVPM	Alternate	Yes
U.S. Department of Agriculture	Louis DiVincenti, M.S., D.V.M., DACLAM, DACAW	Other	Yes
U.S. Department of Agriculture	Erika Edwards, M.S.	Other	Yes
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 War	Shannon Marko, D.V.M.	Principal	Yes
U.S. Department of War	Saber Hussain, Ph.D., ATS Fellow, AFRL Fellow	Alternate	Yes
U.S. Department of War	Elaine Merrill, Ph.D.	Alternate	Yes
U.S. Department of War	Chris Earnhart, Ph.D	Other	Yes

Agency (Office)	**Representative**	**Representative Type (as of December 2025)**	**Service Continuing into 2026**
U.S. Department of War	Kristen Fulbright, M.S.	Other	Yes
U.S. Department of War	Matthew Grogg, Ph.D.	Other	Yes
U.S. Department of War	Sara Peacock, M.S.	Other	Yes
U.S. Department of War	Donald Cronce, Ph.D.	Other	No
U.S. Department of War	Natalia Garcia-Reyero Vinas, Ph.D., DABT (ICCVAM Co-chair since February 2024)	Alternate	Yes
U.S. Department of Energy	R. Todd Anderson, Ph.D.	Principal	Yes
U.S. Department of the Interior	Barnett A. Rattner, Ph.D.	Principal	Yes
U.S. Department of the Interior	Jason Magnuson, Ph.D.	Alternate	Yes
U.S. Department of the Interior	Jessica Leet, Ph.D.	Alternate	No
U.S. Department of Transportation	Steve Hwang, Ph.D.	Principal	Yes
U.S. Department of Transportation	Rebecca Rothhaas, M.S.	Alternate	Yes
U.S. Department of Transportation	Ryan Vierling, Ph.D.	Alternate	Yes
U.S. Department of Veterans Affairs Office of Research and Development	Cassandra Tansey, D.V.M., DACLAM	Principal	Yes
U.S. Department of Veterans Affairs Office of Research and Development	Holly Krull, Ph.D.	Alternate	Yes
U.S. Department of Veterans Affairs Office of Research and Development	Kristin Mayfield, M.S.	Other	Yes
U.S. Department of Veterans Affairs Office of Research and Development	George Lathrop, D.V.M., M.S., DACLAM	Alternate	No
U.S. Environmental Protection Agency (Office of Pesticide Programs)	William (Bill) Eckel, Ph.D.	Other	Yes

Agency (Office)	**Representative**	**Representative Type (as of December 2025)**	**Service Continuing into 2026**
U.S. Environmental Protection Agency (Office of Pollution Prevention and Toxics)	Anna Lowit, Ph.D. (ICCVAM Co-chair through January 2024)	Principal	Yes
U.S. Environmental Protection Agency (Office of Pollution Prevention and Toxics)	Natalia Garcia-Reyero Vinas, Ph.D., DABT (ICCVAM Co-chair since February 2024)	Other	Yes
U.S. Environmental Protection Agency (Office of Pollution Prevention and Toxics)	Louis (Gino) Scarano, Ph.D.	Other	No
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)	Monique Perron, Ph.D.	Alternate	Yes
U.S. Environmental Protection Agency (Office of Research and Development)	Pamela Noyes, Ph.D.	Alternate	No
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. Environmental Protection Agency (Office of Research and Development)	Cecilia Tan, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Biologics Evaluation and Research)	Leslie Wagner, B.S.	Other	Yes
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	No

Agency (Office)	**Representative**	**Representative Type (as of December 2025)**	**Service Continuing into 2026**
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 M. Dalal-Panguluri, Ph.D.	Other	Yes
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 Drug Evaluation and Research)	Paul C. Brown, Ph.D.	Other	No
U.S. Food and Drug Administration (Human Foods Program)	Omari J. Bandele, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Human Foods Program)	Jessica Camacho, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Human Foods Program)	Danica E. DeGroot, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Human Foods Program)	Suzanne Fitzpatrick, Ph.D., DABT (ICCVAM Co-chair since February 2024)	Principal	Yes
U.S. Food and Drug Administration (Human Foods Program)	Steven Hermansky, Pharm. D., Ph.D., DABT	Other	Yes
U.S. Food and Drug Administration (Human Foods Program)	Shruti Kabadi, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Tobacco Products)	Jueichuan (Connie) Kang, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Veterinary Medicine)	M. Cecilia Aguila, D.V.M.	Other	No

Agency (Office)	Representative	Representative Type (as of December 2025)	Service Continuing into 2026
U.S. Food and Drug Administration (Center for Veterinary Medicine)	Yin Guo, Ph.D, DABT	Other	Yes
U.S. Food and Drug Administration (Center for Veterinary Medicine)	Wesley Hunter, Ph.D.	Other	No
U.S. Food and Drug Administration (Center for Veterinary Medicine)	Li You, Ph.D.	Other	No
U.S. Food and Drug Administration (National Center for Toxicological Research)	Donna Mendrick, Ph.D.	Other	No
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)	Jessica Hawes Oliphant, Ph.D.	Other	Yes
U.S. Food and Drug Administration (National Center for Toxicological Research)	Dana van Bemmel, Ph.D., M.P.H.	Alternate	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)	Tina Morrison, Ph.D.	Other	No
U.S. Food and Drug Administration (Office of the Chief Scientist)	Chad P. Nelson, Ph.D., M.S.P.H.	Other	No
U.S. National Coordinator for OECD Test Guidelines Programme	Charles Kovatch, M.S.	Other	No
U.S. National Coordinator for OECD Test Guidelines Programme	Julie Breeden-Alemi	Other	Yes

NICEATM and ICCVAM Publications, 2024–2025

This page lists publications issued in 2024 and 2025 that describe NICEATM and ICCVAM activities.

NICEATM and ICCVAM reports

Daniel AB, van der Zalm AJ, Allen DG, Clippinger AJ, Kleinstreuer NC. 2025. Addendum to the prospective and retrospective evaluation of the eye irritation potential of agrochemical formulations. Research Triangle Park, NC: National Toxicology Program. https://doi.org/10.22427/NTP-NICEATM-1.

ICCVAM. 2024. Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies. Research Triangle Park (NC): National Institute of Environmental Health Sciences. https://doi.org/10.22427/NICEATM-2.

ICCVAM. 2024. ICCVAM Biennial Progress Report 2022-2023. Research Triangle Park (NC): National Institute of Environmental Health Sciences. https://ntp.niehs.nih.gov/sites/default/files/2024-09/2022-2023_ICCVAMBienRpt_FD_508.pdf.

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

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Journal articles

A full list of NICEATM and ICCVAM publications is available on the National Toxicology Program website.

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Behl M, Karmaus A, Rao M, Lane T, Harris J, Sachs C, Borrel A, Oyetade O, Unnkrishnan A, Hamm J, Hogberg HT. 2025. De-risking seizure liability: integrating adverse outcome pathways (AOPs), new approach methodologies (NAMs), and in silico approaches while highlighting knowledge gaps. Toxicol Sci 207(2):306-319. https://doi.org/10.1093/toxsci/kfaf109.

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Harrill A. 2025. Opportunities to build confidence in use of NAMs for systemic toxicity [Abstract 149]. Presented at the 13th World Congress on Alternatives and Animal Use in the Life Sciences, Rio de Janeiro, Brazil, 31 August–4 September 2025.

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Ji C, Prussia AJ, Mumtaz M, Ruiz P. 2024. Toward the application of in silico tools to chemical mixtures of environmental incidences [Abstract 4320]. Presented at the Annual Meeting of the Society of Toxicology, Salt Lake City, UT, USA, 10–14 March 2024.

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Kleinstreuer N. 2024. Cardiotoxicity NAMs across the translational toxicology pipeline [Abstract]. Presented at the 13th Annual Meeting of the American Society for Cellular and Computational Toxicology, Research Triangle Park, NC, USA, 28–30 October 2024.

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Leary AJ, Patlewicz G, Shah I. 2024. An exploration of the use of hybrid fingerprints in generalized read-across and their impact on predictive performance for selected in vivo toxicity outcomes [Abstract 42]. Presented at the 13th Annual Meeting of the American Society for Cellular and Computational Toxicology, Research Triangle Park, NC, USA, 28–30 October 2024.

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Lin H, Sakolish C, Moyer HL, Ferguson SS, Stanko JP, Carmichael PL, Hewitt P, Hoffmann S, Rusyn I, Chiu WA. 2024. Predicting renal clearance of PFAS with a human kidney proximal tubule tissue chip and a novel physiologically-based kidney model [Abstract 3508]. Presented at the Annual Meeting of the Society of Toxicology, Salt Lake City, UT, USA, 10–14 March 2024.

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Lowit A. 2024. Update on collaborations to support implementation of in vitro and computational approaches from the US EPA Office of Pollution Prevention and Toxics [Abstract 1097]. Presented at the Annual Meeting of the Society of Toxicology, Salt Lake City, UT, USA, 10–14 March 2024.

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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 event: any unexpected or harmful reaction or occurrence experienced by a patient during healthcare interventions.

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.

Air–liquid interface: a boundary where air meets a liquid surface within a biological system.

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.

Ames test: a widely used biological assay that assesses the mutagenic potential of chemical compounds. It utilizes bacteria to determine whether a given chemical can cause mutations in DNA.

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

Bayesian network: a probabilistic graphical model that represents a set of variables and their conditional dependencies. This model is particularly useful for representing and solving problems that involve uncertainty and probabilistic events.

Benchmark concentration/dose: a dose or concentration that produces a predetermined change in the response rate of an adverse effect.

Bioaccumulation: the gradual build-up of substances, such as chemicals or heavy metals, in an organism when intake exceeds the rate of elimination.

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

Bioinformatics: an interdisciplinary field that uses computational tools and methods to analyze, interpret, and manage complex biological data.

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

Biotransformation: the alteration of a substance, typically a drug, within the body.

Carcinogenicity: the capacity of a material to cause cancer.

Cardiotoxicity: toxicity to the heart.

Consensus model: a structured framework used to achieve agreement among participants or standardization across a system, ensuring coordinated decisions or uniform practices.

Context of use: the complete set of circumstances, environments, and conditions in which a model or assay is used.

Controlled vocabulary: a standardized set of predefined terms used to organize, describe, and retrieve information consistently across systems.

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

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

Data annotation: adding metadata or “data about data” to a data item to provide context, provenance, or other useful information to that item.

Data curation: reviewing raw data to ensure that they are of high quality, consistent, relevant to its intended purpose, and interpretable.

Data extraction: transferring data from one information source to another.

Data standardization: reviewing a set of data items to ensure they are consistent with regards to units of measure, terminology, and other features; see also data curation.

Data visualization: converting a set of data into a graph, chart, or other image that effectively communicates commonalities, differences, trends, and other features of the dataset. May be done manually or computationally.

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 neurotoxicity (DNT): effects on the nervous system of offspring that occur as a result of chemical exposures of the pregnant mother.

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

Digital twin: a digital model of a real-world product, system, or process that serves as a digital counterpart for purposes such as simulation, integration, testing, monitoring, and maintenance.

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.

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

Exosome: a small extracellular vesicle ranging from 30 to 150 nanometers in diameter, derived from the endosomal system of cells; exosomes play a crucial role in intercellular communication, acting as natural messengers that facilitate the transfer of biomolecules between cells, thereby influencing various biological processes.

Exposure assessment: the process of evaluating how chemical, physical, or biological agents come into contact with humans or wildlife, and in what amounts.

Fingerprint: compact, fixed-length representation of a molecule that encodes structural or chemical features for computational analysis.

Fit-for-purpose: being suitable and good enough to achieve the intended function or result.

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.

Functional use categories: a standardized classification that describes the primary purpose or function a chemical substance serves in a product or process.

G protein-coupled receptors: 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-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.

Immunotoxicity: toxicity to the immune system, which may result in either a reduced immune response or an inappropriate immune response such as an allergic reaction.

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 nonsex 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.

Large language models: artificial intelligence systems trained on large amounts of text so that they can understand and generate human language.

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.

Local lymph node assay: an nonclinical toxicity test, usually conducted in mice, that measures a cellular response to assess a chemical's skin sensitization potential.

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.

Metadata: additional information about a particular piece of data, helping to organize, manage, and understand the information.

Microfluidics: the study and technology of manipulating small volumes of fluids, typically in the range of nanoliters to picoliters, through micro-sized channels.

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.

Molecular descriptor: a mathematical representation of molecular structure used to predict a chemical’s properties or behavior.

Mutagenicity: the capacity of a substance or agent to induce changes in an organism’s genetic material.

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

Neural network: a computational model inspired by the human brain structure and function, consisting of interconnected nodes or neurons that process and learn from data, enabling tasks such as pattern recognition and decision-making in machine learning.

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.

Nuclear receptor: an intracellular protein that acts as a ligand-activated transcription factor, regulating gene expression in response to hormones, vitamins, and other small molecules.

Ontologies: standardized nomenclature systems**.**

Organoid: a miniature, simplified, three-dimensional version of an organ grown in the lab from stem cells that mimics key structural and functional features of the original organ.

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.

Performance standards: technical specifications used to evaluate whether a test method appropriately evaluates the potential hazards of chemicals within a specific testing application.

Persistence: the enduring presence of substances in environmental or biological systems, resisting breakdown and exerting sustained influence.

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 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.

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.

Potency: the relative amount of a substance that is needed to induce an adverse effect or outcome.

Principal component analysis: a statistical technique that reduces the dimensionality of complex datasets while preserving the most important patterns of variation. In chemical testing, it is especially valuable for analyzing large, correlated datasets from high-throughput measurements.

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

Qualification: a validation framework to ensure that human-relevant models are reliable, reproducible, and suitable for biomedical research and safety testing.

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.

Random forest: a machine learning algorithm that combines the output of multiple decision trees to reach a single result.

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.

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.

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.

S9 fraction: supernatant from liver homogenates containing both microsomal and cytosolic enzymes that is often added to in vitro assays to simulate in vivo metabolism of a chemical.

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, and from corrosion in that the damage is not severe or permanent.

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

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.

Spheroid: a three-dimensional, spherical cell aggregate used to model tissue structure and function in vitro.

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).

Structural alert: feature of an organic molecule that is believed to be responsible for adverse effects and can be used to predict the toxicity of similar compounds.

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).

Transcription factor: a protein that regulates gene expression by binding to specific DNA sequences and controlling whether genes are turned on or off.

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.

Weight-of-evidence approach: a systematic method for evaluating all available information to reach a balanced, scientifically justified conclusion.

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

Acronyms and Abbreviations

3Rs	replacement, reduction, or refinement of animal use
AC50	half-maximal activity concentration
ACC	activity concentration at cutoff
ADME	absorption, distribution, metabolism, and excretion
AFSA	Animal-Free Safety Assessment (Collaboration)
AgNPs	silver nanoparticles
AI	artificial intelligence
AIVIVE	artificial intelligence-aided in vitro to in vivo extrapolation
ALT	alanine aminotransferase

AOP	adverse outcome pathway
API	application programming interface
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
AWIC	Animal Welfare Information Center (U.S. Department of Agriculture)
BCOP	bovine corneal opacity and permeability (assay)
CAAT	Center for Alternatives to Animal Testing (Johns Hopkins University)
CAMERA	Collection of Alternative Methods for Regulatory Application
CATMoS	Collaborative Acute Toxicity Modeling Suite
CBC	Chemical Biological Center (U.S. Army, U.S. Department of War)
CBD	cannabidiol
CDER	Center for Drug Evaluation and Research (U.S. Food and Drug Administration)
cHTS	curated high-throughput screening (data; NIH Integrated Chemical Environment)
Cmax	maximum plasma concentration
CoMPAIT	Collaborative Modeling Project for Acute Inhalation Toxicity
Complement-ARIE	Complement Animal Research in Experimentation (National Institutes of Health)
CPSC	U.S. Consumer Product Safety Commission
CYP	cytochrome P450
DA	defined approach
DART	developmental and reproductive toxicology
DASS	defined approaches for skin sensitization
devTOXqP	devTOX quickPredict assay (Stemina, Inc.)

DILI	drug-induced liver injury
DNT	developmental neurotoxicity
DNT-DIVER	Developmental NeuroToxicity Data Integration and Visualization Enabling Resource (National Institute of Environmental Health Sciences)
DOE	U.S. Department of Energy
DOI	U.S. Department of the Interior
DOT	U.S. Department of Transportation
DoW	U.S. Department of War
DSSTox	Distributed Structure-Searchable Toxicity (U.S. Environmental Protection Agency)
EASA	electrophilic allergen screening assay
ECOTOX	Ecotoxicology Knowledgebase (U.S. Environmental Protection Agency)
ED01	dermal dosage at which there is a 1% chance of inducing sensitization
EHS	environmental health science
ENTRÉE	Engaging NAMs for Toxicant Regulation and Effects (U.S. Food and Drug Administration)
EPA	U.S. Environmental Protection Agency
eSTAR	Emerging Systems Toxicology for the Assessment of Risk (Health and Environmental Sciences Institute)
FAIR	findability, accessibility, interoperability, and reusability (of data)
FDA	U.S. Food and Drug Administration
GHS	United Nations Globally Harmonized System of Classification and Labelling of Chemicals
HCMI	Human Cancer Models Initiative (National Cancer Institute)

HESI	Health and Environmental Sciences Institute
HFP	Human Foods Program (U.S. Food and Drug Administration)
HPPT	human predictive patch test
HTS	high-throughput screening
httk	High-throughput Toxicokinetics (software package, U.S. Environmental Protection Agency)
IATA	Integrated approach to testing and assessment
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ILMERAC	International Liaison Group on Methods for Risk Assessment of Chemicals
InHALES	Independent Holistic Air–Liquid Exposure System (TissUse GmbH)
iPSC	induced pluripotent stem cell
ISTAND	Innovative Science and Technology Approaches for New Drugs (U.S Food and Drug Administration)
IVIVE	in vitro to in vivo extrapolation
JHU	Johns Hopkins University
JP-5	jet fuel formulation
KCC	key characteristic of carcinogens
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
LDH	lactate dehydrogenase
LLNA	local lymph node assay
MPS	microphysiological systems

MRSA	methicillin-resistant Staphylococcus aureus
MSTI	(*E*)-2-(4-mercaptostyryl)-1,3,3-trimethyl-3*H*-indol-1-ium
NAM	new approach methodology
nanoEHS	nano environmental health and safety
NC	Not Classified (GHS classification)
NCATS	National Center for Advancing Translational Sciences
NCBI	National Center for Biotechnology Information (National Library of Medicine)
NCI	National Cancer Institute (National Institutes of Health)
NCTR	National Center for Toxicological Research (U.S. Food and Drug Administration)
NICE	NIH Integrated Chemical Environment
NICEATM	National Interagency Center for the Evaluation of Alternative Test 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
OASIS	Omics for Assessing Signatures for Integrated Safety (Health and Environmental Sciences Institute)
OBO	Open Biological and Biomedical Ontology

OECD	Organisation for Economic Co-operation and Development
OHT	OECD Harmonised Template
OLAW	Office of Laboratory Animal Welfare (National Institutes of Health)
OPERA	Open (Quantitative) Structure–activity/property Relationship App
OPFR	organophosphorus flame retardants
OSHA	Occupational Safety and Health Administration
PBPK	Physiologically based pharmacokinetic
PFAS	per- and polyfluoroalkyl substances
PIANO	paraffin, isoparaffin, aromatic, naphthene, and olefin compounds
POD	point-of-departure
PRT	Predictive Risk Team (U.S. Air Force, U.S. Department of War)
PSCI	PETA Science Consortium International, e.V.
QKAR	quantitative knowledge–activity relationship
QSAR	quantitative structure–activity relationship
QSPR	quantitative structure–property relationship
RXR	retinoid-X receptors
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 Institute of Environmental Health Sciences Division of Translational Toxicology)

SEAZIT-DIVER	SEAZIT Data Integration and Visualization Enabling Resource (National Institute of Environmental Health Sciences Division of Translational Toxicology)
SeqAPASS	Sequence Alignment to Predict Across Species Susceptibility (U.S. Environmental Protection Agency)
SOM	Standardized Organoid Modeling (Center; National Institutes of Health)
STopTox	Systemic and Topical Chemical Toxicity
TEER	transepithelial/endothelial electrical resistance (assay)
TG	test guideline
ToxCast	Toxicity Forecaster (U.S. Environmental Protection Agency)
ToxPi	Toxicological Prioritization Index
tPOD	transcriptomic point-of-departure
TraCe MPS	Translational Centers for Microphysiological Systems (National Center for Advancing Translational Sciences)
U.S.	United States
USAF	U.S Air Force (U.S. Department of War)
USDA	U.S. Department of Agriculture
USGS	U.S. Geological Survey (U.S. Department of the Interior)
VA ORD	U.S. Department of Veterans Affairs Office of Research and Development
VEGF	vascular endothelial growth factor
VQN	Validation and Qualification Network (National Institutes of Health Complement-ARIE program)
Web-ICE	Web-based Interspecies Correlation Estimation (U.S. Environmental Protection Agency)