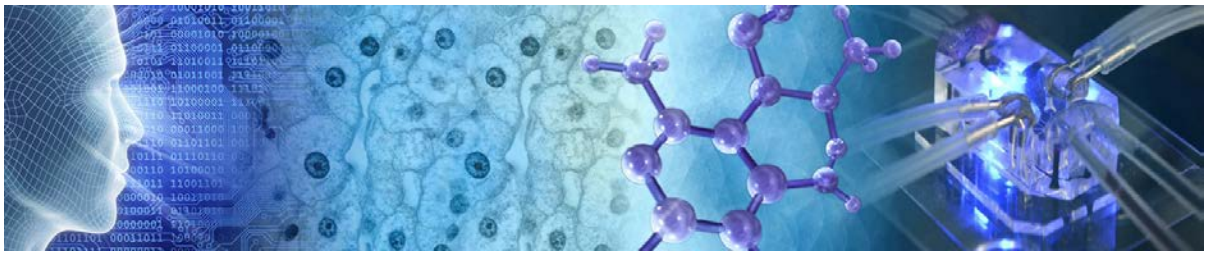
Biennial Progress Report 2020-2021

Interagency Coordinating Committee on the Validation of Alternative Methods



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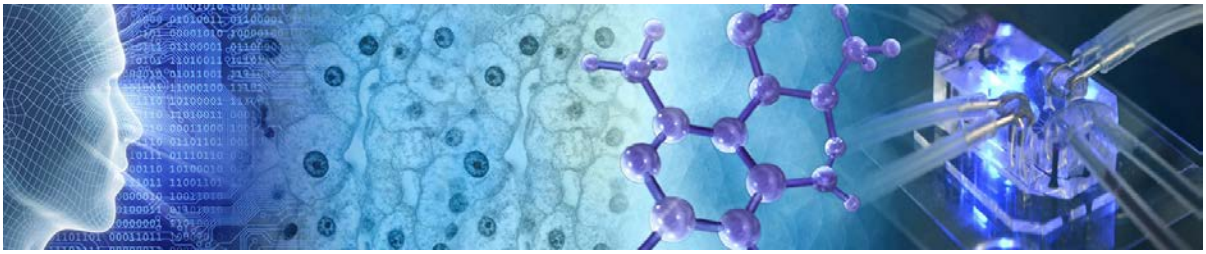

About this Report

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.

In January 2018, ICCVAM published A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States. The roadmap described how ICCVAM agencies will encourage development of new technologies for, support utilization of, and build confidence in new methods. This report summarizes progress toward these goals during 2020–2021.

The ICCVAM Biennial Report is prepared by the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), with support from the NTP Office of Policy, Review, and Outreach.

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Message from NIEHS and NTP

In the 2020-2021 Biennial Report, we are pleased to share with you the many accomplishments of the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), and the 17 ICCVAM agencies that 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.

The Strategic Roadmap has guided broad collaborative efforts to reduce and replace animal use for acute toxicity testing that show promise for having wide-ranging impact. An important milestone in these efforts was realized in 2021 with adoption by the Organisation for Economic Co-operation and Development (OECD) of Guideline 497, which describes use of defined approaches to predict skin sensitization potential without using animals. Development of the new guideline was sponsored by ICCVAM member agencies and led by NICEATM in collaboration with scientists from Canada and the European Union. This guideline represents a new type of product for OECD and will enable the worldwide use of non-animal approaches for identifying potential skin sensitizers. Adoption of Guideline 497 has set the stage for broader acceptance of non-animal defined approaches for predicting eye irritation and other acute endpoints. The Collaborative Acute Toxicity Modeling Suite (CATMoS), which uses computational models to predict rat acute oral toxicity, was further developed during 2020 and 2021, and is being explored by ICCVAM agencies such as the U.S. Department of Defense and the U.S. Environmental Protection Agency as an approach for reducing animal use for specific testing applications. Model predictions, curated reference studies, non-animal assay data, and computational workflows are accessible through the Integrated Chemical Environment, which has been substantially updated over the last two years.

A key area of the Strategic Roadmap is communication and collaboration. Although the COVID-19 pandemic eliminated in-person meetings in 2020 and 2021, NICEATM and ICCVAM continued to effectively connect with stakeholders, as demonstrated by the high attendance and participation at ICCVAM-sponsored virtual public events such as the

Communities of Practice webinars and the Public Forums. In addition, NICEATM and ICCVAM agencies such as the U.S. Environmental Protection Agency and the U.S. Food and Drug Administration sponsored virtual events to educate and engage stakeholders and progress the acceptance and use of alternatives to animal testing. Details and outcomes of many of these events are described herein.

While the COVID-19 pandemic presented significant challenges, it also provided opportunities for the application of human-relevant platforms to understanding disease mechanisms and assessing new therapeutics. As part of the pandemic response, NICEATM established the Microphysiological Systems for COVID Research (MPSCoRe) Working Group to coordinate the use of microphysiological systems in studies of COVID-19 and future emerging infectious diseases. The MPSCoRe group engages researchers, microphysiological systems model developers, therapeutic/vaccine manufacturers, and international regulators to facilitate advancement of promising models and their application to both investigations of disease biology and therapeutic development.

The importance and relevance of ICCVAM's work is reflected in its growth in recent years. Established in 2000 with 15 member agencies, ICCVAM grew to 17 member agencies in October 2020 with the addition of the U.S. Department of Veterans Affairs Office of Research and Development. This growth increases ICCVAM's effectiveness in coordinating efforts across the federal government to reduce, replace, or refine (3Rs) animal use in testing.

We invite you to read the Biennial Report to learn more about all that has been accomplished in the past two years to advance alternative methodologies and the 3Rs.

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Message from NICEATM and ICCVAM

On behalf of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), we are pleased to present the 2020-2021 ICCVAM Biennial Progress Report.

This report provides a comprehensive overview of activities by ICCVAM and its 17 member agencies that support the ICCVAM mission. While these activities vary widely in nature and focus, many notable accomplishments represent advances in the use of computational models. Several articles in the report describe computational approaches for endpoints such as acute systemic toxicity, genotoxicity, skin sensitization, and eye irritation, which are being investigated by ICCVAM agencies to reduce animal use for specific testing applications. Development of both computational and laboratory-based non-animal testing approaches depends on the availability of data that adhere to FAIR data principles: findability, accessibility, interoperability, and reusability. These principles are upheld by the Integrated Chemical Environment resource, which was improved during 2020 and 2021 to update data availability and annotation and launch new tools and associated training materials. These improvements were made in response to evolving ICCVAM agency needs and suggestions by ICCVAM's scientific advisory committee.

A similar spirit of responsiveness to stakeholders underlies the ICCVAM Metrics Workgroup's report, "Measuring U.S. Federal Agency Progress Toward Implementation of Alternative Methods in Toxicity Testing." This document, published in March 2021, was developed in response to the U.S. Government Accountability Office's recommendation that federal agencies establish a workgroup through ICCVAM to propose metrics for assessing progress on the development and promotion of alternative methods. Articles in this report provide insight into how federal agencies are uniquely addressing implementation of alternative methods and appropriate metrics for assessing animal use.

It is becoming increasingly clear that the acceptance and application of new testing approaches that can reduce and replace animal use will require both new approaches to validation and critical evaluation of current test methods. Several projects described in this

report examine variability in reference data from animal studies and consider how such variability could impact the validation of new methods. Other work focuses on human biology-based approaches to method development and validation that move beyond concordance with animal studies.

We would like to acknowledge the contributions of the representatives and interagency workgroup members from the 17 ICCVAM member agencies, NICEATM and its contract staff at Inotiv, the members of ICCVAM's advisory committee, and our many other stakeholders. In particular, we would like to recognize Emily Reinke, Ph.D., who has represented the U.S. Department of Defense (DoD) on ICCVAM since May 2015 and served as ICCVAM co-chair since January 2018. In addition to her expert leadership of the ICCVAM committee and her important contributions to the development of the Strategic Roadmap, Dr. Reinke's engagement and expertise have been key to the success of five ICCVAM workgroups, including those focused on skin sensitization, acute toxicity, and ecotoxicology. Her tenure on ICCVAM ended early in 2022 as she departed DoD to pursue other opportunities. While we will miss interacting with her on ICCVAM, Dr. Reinke plans to remain active in efforts to advance alternatives to animal testing, and we look forward to engaging with her as an ICCVAM stakeholder.

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. We look forward to continued progress and effective interactions with our stakeholders in the coming years.

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Key NICEATM and ICCVAM Accomplishments and Impact

Tags: DoD, EPA, ICCVAM, NIEHS, acute toxicity, COVID-19, metrics, MPS, oral toxicity, QSAR, skin sensitization

Key accomplishments of ICCVAM and ICCVAM member agencies in support of the ICCVAM mission during 2020 and 2021 include:

- Publication of Defined Approach Guideline. In 2021, the Organisation for Economic Co-operation and Development issued Guideline 497, Defined Approaches on Skin Sensitisation. Drafted and sponsored by ICCVAM agency scientists and international partners, Guideline 497 is the first internationally harmonized guideline to describe a non-animal approach that can be used to replace an animal test to identify skin sensitizers.
- Recommendations on Measuring Progress in Promoting Use of Alternative Methods. In March 2021, the ICCVAM Metrics Workgroup published findings and recommendations on the measurement of federal agency progress in promoting the use of alternative toxicological methods. The workgroup recommended that each agency develop its own metrics that are relevant and practical to their unique situation.
- Workgroup on Microphysiological Systems for COVID Research. NICEATM established an international collaborative workgroup to coordinate the use of microphysiological systems to reduce animal use in studies of COVID-19 and future emerging infectious diseases. A key accomplishment of the workgroup was the establishment of a COVID-19 disease portal in an existing microphysiological systems database.
- Further Development of the Collaborative Acute Toxicity Modeling Suite. The ICCVAM Acute Toxicity Workgroup organized a global project that produced the Collaborative Acute Toxicity Modeling Suite (CATMoS), an online resource for in silico screening of organic chemicals for acute oral toxicity. During 2020 and 2021, the utility of CATMoS for predicting acute oral toxicity in research and regulatory

contexts was explored in projects conducted by ICCVAM agencies, including the U.S. Department of Defense and the U.S. Environmental Protection Agency.

- Expansion of NICEATM's Integrated Chemical Environment. NICEATM created the Integrated Chemical Environment to provide curated data and tools for safety assessment of chemicals. Updates of the Integrated Chemical Environment Search tool during 2020 and 2021 enabled search results to be sent to query other data resources. Updates also allowed users to explore similarities among chemicals, find information on chemical use categories, search for structurally similar chemicals, and view and interact with concentration–response curves from curated high-throughput screening data.

Technology

Many ICCVAM member agencies are developing new technologies and resources to replace the use of animals for chemical safety testing. These include new platforms such as microphysiological systems (MPS) and data resources to support the development of predictive models and adverse outcome pathways (AOPs).

Assay Development

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

Two-dimensional cellular and three-dimensional bioprinted skin models to screen topical-use compounds for irritation potential

Tags: NIEHS, NIH, skin irritation

Assessing skin irritation potential is critical for the safety evaluation of topical drugs and other consumer products such as cosmetics. Use of animals for these evaluations is prohibited in some sectors, with advanced in vitro cellular models considered as possible replacements. To compare the utility of different cellular skin models for this purpose, scientists at the National Center for Advancing Translational Sciences (NCATS), the National Eye Institute, and the National Institute of Environmental Health Sciences (NIEHS) assessed irritation potential in different cellular skin models compatible with high-throughput screening (HTS) platforms (Wei et al. 2020). The study tested 46 compounds related to topical products on monolayer keratinocytes, reconstructed human epidermis, and full-thickness skin models, and evaluated performance of the models using generally accepted cellular and molecular indicators of irritant activity. The study indicated that using human cells to generate bioprinted tissue is a quick and reliable method to model human skin in a high-throughput manner, and that this approach could be used as evidence for hazard labeling in a variety of hazard classification systems.

Human neuronal cell line as a model for organophosphorus pesticide-induced neurotoxicity

Tags: AOP, DoD, inhalation toxicity, neurotoxicity

Non-animal screening methods that can rapidly and accurately characterize organophosphorus compound-induced neurotoxicity are needed. Scientists at the U.S. Army Engineer Research and Development Center examined molecular and cellular responses characteristic of this neurotoxicity pathway via an in vitro model using the SYSY5Y human neuroblastoma cell line and the model organophosphorus compound ethyl parathion. In these studies, ethyl parathion was found to increase reactive oxygen species and cell membrane lipid peroxidation and reduce mitochondrial membrane potential, contributing ultimately to cell death. Overall, the mechanistic responses observed in the SYSY5Y cells corresponded closely with in vivo mammalian results, demonstrating potential for this non-animal model to provide accurate neurotoxicology screening for organophosphorus compounds. These investigations also provide data to support development of the AOP for this endpoint. In 2022, the research team will use these methods to investigate time-dynamic neurotoxicity responses to ethyl parathion. They will also begin developing in vitro methods that provide mechanistic toxicology indicators characteristic of mammalian acute ammonia inhalation exposures.

Development of a non-animal-based vaccine test for RHDV2

Tags: NIEHS, USDA, antibodies, biologics

Rabbit hemorrhagic disease is a devastating disease affecting wild, farmed, and companion rabbits. The virus causing the disease is closely related to human norovirus. However, unlike norovirus, which rarely causes severe disease, rabbit hemorrhagic disease causes a syndrome culminating in the death of affected rabbits. The disease was historically not detected in the United States, but in 2020, rabbit hemorrhagic disease virus serotype 2 (RHDV2) was isolated from rabbits in Canada and has since caused significant outbreaks and mortality in the United States. While vaccines are available to prevent the disease, U.S. Department of Agriculture (USDA) licensing of an RHDV2 vaccine requires testing of each lot in live animals to confirm vaccine potency. To provide a non-animal alternative method for testing of a novel RNA-based vaccine, in 2021 NIEHS provided funding to optimize and qualify an in vitro potency assay that uses a monoclonal antibody specific for the RHDV2 target protein. Evaluation of the potency assay will be completed in 2022.

Genetic toxicity screening approach for flavoring chemicals

Tags: FDA, carcinogenicity, genotoxicity, inhalation toxicity, QSAR, machine learning

A variety of compounds are used as flavorings for electronic nicotine delivery systems, or e-cigarettes. Scientists in the U.S. Food and Drug Administration (FDA) Center for Tobacco Products conducted a study (Hung et al. 2020) using alternative methods as prioritization tools to study the genotoxic mode of action of these compounds. The approach specifically examined clastogen-sensitive and aneugen-sensitive biomarkers of DNA damage in human TK6 cells. These biomarkers were aggregated through a supervised three-pronged ensemble machine learning prediction model to prioritize chemicals based on genotoxicity. In addition, in silico quantitative structure–activity relationship (QSAR) models were used to predict genotoxicity and carcinogenic potential. The parallel use of these predictive technologies to clarify modes of action for potential genetic damage might be helpful for screening chemicals in tobacco products.

Integration of transcriptome analysis with pathophysiological endpoints to evaluate cigarette smoke toxicity in an in vitro human airway tissue model

Tags: FDA, carcinogenicity, inhalation toxicity, mixtures toxicity, MPS, genotoxicity

To assess the effects of cigarette smoke on the function and phenotype of airway epithelial cells, scientists at the FDA Center for Tobacco Products and the FDA National Center for Toxicological Research developed a novel repeated-treatment protocol to evaluate the progression of molecular, functional, and structural abnormalities induced by cigarette smoke in a human in vitro air–liquid-interface airway tissue model (Xiong et al. 2021a). Transcriptomics analysis was combined with in vitro measurements to demonstrate cigarette smoke-mediated effects on a variety of cell functions. These measurements revealed effects that were highly consistent with abnormalities observed in airways of smokers. Enrichment analysis on the transcriptomic profiles of the air–liquid-interface cultures revealed key molecular pathways, such as xenobiotic metabolism, oxidative stress, and inflammatory responses, that were perturbed in response to cigarette smoke exposure. A subsequent study (Xiong et al. 2021b) described the transcriptome analyses of these cultures and noted that cultures exposed to the high concentration of cigarette smoke exhibited 5,090 differentially expressed genes and 551 differentially expressed microRNAs. Expression of genes involved

in ciliary development and function were significantly perturbed by repeated cigarette smoke exposures, leading to changes in protein content and cilia beating frequency. In particular, the expression of miR-449a, a conserved miRNA highly enriched in ciliated airway epithelia and implicated in motile ciliogenesis, showed a time-dependent decrease following cigarette smoke, an effect consistent with observations reported in smokers with chronic obstructive pulmonary disease. Investigating the effects of cigarette smoke on the transcriptome profile of human air–liquid-interface cultures may provide both mechanistic insights and potential early biomarkers for harm from cigarette smoke exposure.

In vitro dosimetry analyses for acrolein exposure in human cells

Tags: FDA, AOP, genotoxicity

Establishing accurate dosimetry is important for assessing the toxicity of xenobiotics as well as for comparing responses between different test systems. Scientists at the FDA Center for Tobacco Products and the FDA National Center for Toxicological Research used acrolein as a model toxicant and defined the concentration–response relationships of the key adverse responses in both normal human bronchial epithelial cells and human pulmonary carcinoma cells (Xiong et al. 2021). The researchers developed a new method for indirectly estimating the intracellular uptake of acrolein based on the chemical's key alkylation reactions. Responses, including protein carbonylation, glutathione depletion, and glutathione–acrolein adduct formation, were all linearly correlated with acrolein uptake in both cell types. The study demonstrated that normal human bronchial epithelial cells were more sensitive to acrolein exposure than the pulmonary carcinoma cells and provided mechanistic information about acrolein-induced cytotoxicity. The dosimetric analysis presented in this study may provide useful information for computational modeling and risk assessment of acrolein using different test systems.

Development of cellular and molecular approaches to evaluate effects of environmental contaminants and stressors in fish

Tags: DOI, ecotoxicity, endocrine disruptors, hepatotoxicity

Aquatic exposure to endocrine disruptors, pesticides, and cyanobacteria affects the health of fish in the environment. Most of the affected species are not typically used in the laboratory and their specific responses to such exposure cannot always be inferred from data from

laboratory species. During 2020-2021, scientists in the U.S. Geological Survey (USGS) of the U.S. Department of the Interior (DOI) developed and applied methods to evaluate species-specific cellular and molecular responses to environmental contaminants and thermal stress using primary tissue culture and non-lethal biopsy methods. Specifically, this research focused on brook trout, largemouth bass, and smallmouth bass collected from the upper Chesapeake Bay watershed. Methods used included evaluation of gill biopsy tissue and collection of primary hepatocytes and leukocytes from species that inhabit aquatic ecosystems vulnerable to environmental contamination and thermal stress. Analytical approaches included in vitro exposures, image analysis-based flow cytometry, and transcriptional profiling of gill, hepatic and immune-responsive genes using RNA-seq and nCounter technologies. These approaches are used to investigate specific mechanistic questions in environmentally relevant fishes to minimize the use of vertebrates.

Computational Tools Development

Computationally generated predictions of toxicity endpoints can inform decisions about testing priorities and sometimes eliminate the need for laboratory testing. ICCVAM agencies are developing tools to predict toxicity endpoints such as acute oral toxicity, skin sensitization, and genotoxicity, as well as tools to provide in vivo context to in vitro data through in vitro to in vivo extrapolation (IVIVE).

Collaborative Acute Toxicity Modeling Suite (CATMoS) tool for predicting acute oral toxicity

Tags: ICCVAM, EPA, NIEHS, acute toxicity, oral toxicity, ecotoxicity

There is a pressing need to rapidly and accurately assess the safety of environmental chemicals and reduce the number of animals used in regulatory testing while still protecting wildlife. NICEATM and the ICCVAM Acute Toxicity Workgroup organized a global collaborative project to develop predictive in silico models of acute oral systemic toxicity potential. Participants from 35 international groups submitted a total of 139 models built using a data set of 11,992 chemicals split into training (75%) and evaluation (25%) sets. These crowdsourced models were developed for five endpoints identified as relevant to regulatory decision frameworks: (1) LD50 value, (2) U.S. Environmental Protection Agency (EPA) hazard categories, (3) United Nations Globally Harmonized System of Classification

and Labelling of Chemicals (GHS) hazard categories, (4) very toxic chemicals (LD50 <50 mg/kg), and (5) non-toxic chemicals (LD50 >2,000 mg/kg). Predictions within the applicability domains of the submitted models were evaluated and combined into consensus predictions based on a weight-of-evidence approach. The result, the Collaborative Acute Toxicity Modeling Suite (CATMoS; Mansouri et al. 2021), leverages the strengths and overcomes the limitations of individual modeling approaches. The consensus model predictions are fully reproducible and demonstrate equivalent performance to replicate in vivo data considering the inherent variability, offering a strong potential replacement for animal testing. Based on these results, CATMoS predictions for 178 chemicals are currently being evaluated in comparison to rat acute oral toxicity tests from publicly available ecological risk assessments registered from 1998 to 2020. Findings will inform the potential for using CATMoS estimates to potentially replace data from rat acute oral toxicity studies for assessments of ecological risk (i.e., what assumptions might be made, under what conditions, and which chemicals).

CompTox Chemicals Dashboard

Tags: EPA

The CompTox Chemicals Dashboard is the primary web-based application that provides access to data and algorithms from the EPA Center for Computational Toxicology and Exposure. Since April 2017, a total of 10 releases have increased the data available from an initial release of 560k data points to a total of 906k data points with the December 2021 release. There are now over 310 individual chemical lists available that include substances such as pesticides, disinfectant byproducts, and PFAS. The latest release represents a major architecture and design update, with data management based on a datamart-datahub technology, a rich application programming interface (API), and fresh user interface design. With this architecture, new applications using the same underpinning data and API will be easier to build and maintain long-term. The update also supported significant improvements in performance. For example, batch searches and downloads for 5000 chemicals now generally take less than 5 seconds compared with over a minute in the previous version. Both Generalized Read-Across (GenRA) and Abstract Sifter are now separate modules, allowing their use for querying chemicals beyond those present in the underlying database. The next

release of data, planned for 2022, will expand the number of substances to over 1.2 million chemicals and will include updates to many of the existing chemical lists.

Open (Quantitative) Structure–activity/property Relationship App (OPERA)

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 predicting absorption, distribution, metabolism, and excretion (ADME) endpoints that are important to physiologically based pharmacokinetic (PBPK) modeling and IVIVE studies. The OPERA ADME-related endpoints were added during 2020 and 2021 and include models for octanol/water partition and distribution coefficients, acidic dissociation, fraction of chemical unbound to plasma protein, intrinsic hepatic clearance, and Caco2 permeability. All OPERA models were built using curated data sets split into training and test sets and molecular descriptors developed from standardized QSAR-ready chemical structures. Modeling adhered to the five principles for QSAR model development adopted by the Organisation for Economic Co-operation and Development (OECD). These principles support development of scientifically valid, high accuracy models with minimal complexity that support mechanistic interpretation, when possible.

Existing OPERA models are updated regularly when new experimental data are available. Recently, the models for octanol/water partition coefficient, fraction unbound, and intrinsic clearance were updated with the latest publicly available data sets to improve their predictivity and applicability domain coverage. For consistency and transparency, OPERA also 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. OPERA predictions are available through the EPA CompTox Chemicals Dashboard and the National Toxicology Program's (NTP's) Integrated Chemical Environment. The OPERA application can also be downloaded from the NIEHS GitHub repository as a command-line or graphical user interface for Windows and Linux operating systems.

Integrated Chemical Environment (ICE) tools updates

Tags: NIEHS, Tox21, EPA, IVIVE

The NTP's Integrated Chemical Environment (ICE) provides data and tools to help develop, assess, and interpret chemical safety tests. Updates to ICE during 2020 and 2021 launched several new tools and implemented substantive updates to existing tools.

- The **Search** tool now allows search results to be sent directly to other ICE tools, the EPA CompTox Chemicals Dashboard, and the NTP Chemical Effects in Biological Systems database. Visualization features implemented in March 2021 help users explore query results in more detail.
- Improvements to the **IVIVE** tool allowed users the option to upload their own in vitro data for IVIVE analysis and their own in vivo data for visualizing comparisons to IVIVE predictions, as opposed to being limited to using data available in ICE. An inhalation model was added to the IVIVE tool in October 2020. The tool now also allows assay selection based on mode of action and user specification of whether experimental or predicted data are used for ADME parameters. Results can also be filtered by mode of action or toxicity endpoint annotation.
- New visualization features in the **Chemical Characterization** tool help users better understand the relationships between members of a chemical set and lets users explore chemical use categories from the EPA's Consumer Products database.
- **Curve Surfer**, launched in March 2021, allows users to view and interact with concentration–response curves from curated HTS data.
- The **PBPK** tool, launched in March 2021, uses models from the EPA's httk package to generate predictions of tissue-specific chemical concentration profiles following a dosing event.
- **Chemical Quest**, launched in June 2021, uses Saagar molecular fingerprints (Sedykh et al. 2021) to identify chemicals in the ICE database having similar structures to a query chemical, which can be entered using chemical identifiers or structure drawings.

Improvements to all tools during 2020 and 2021 include organization of data from the EPA Toxicity Forecaster (ToxCast) and the U.S. government's interagency Tox21 programs into query setup menus based on mechanistic targets and modes of action. Other improvements included mapping of ToxCast and Tox21 assays to controlled terms from the National Cancer Institute (NCI) Metathesaurus; new tooltips and information buttons to help users set up queries; and the acceptance of a variety of chemical identifier types as query inputs.

Pred-Skin web portal for predicting human skin sensitizers

Tags: NIEHS, skin sensitization, QSAR

In collaboration with scientists at the University of North Carolina at Chapel Hill and the Universidade Federal de Goiás, NICEATM scientists contributed to the development of a model to predict skin sensitization potential of chemicals for two assays, the human patch test and murine local lymph node assay, and implemented this model in a web portal (Braga et al. 2017). Pred-Skin v. 3.0 (Borba et al. 2021) revised and expanded the freely accessible web tool to integrate multiple QSAR models developed with in vitro, animal in vivo, and human predictive patch test data into a consensus naïve Bayes model that predicts human effects.

Large-scale modeling of multispecies acute toxicity endpoints using consensus of multitask deep learning methods

Tags: NCI, NIEHS, NIH, acute toxicity, machine learning

Scientists from NCATS, NIEHS, and NCI and collaborators developed computational methods to predict chemical activity for 59 acute systemic toxicity endpoints across multiple species, including 36 endpoints for which computational models had not been previously developed. Data used to develop the models were collected and curated from the ChemIDplus database for acute systemic toxicity and represents the largest publicly available such data set, covering over 80,000 compounds. These data were used for developing multiple single- and multitask models utilizing random forest, deep neural networks, convolutional, and graph convolutional neural network approaches. The paper describing the project (Jain et al. 2021) also reports the consensus models based on different multitask approaches. The curated data set and the developed models have been made publicly available to support regulatory and research applications.

Saagar–a new, extensible set of molecular substructures for QSAR/QSPR and read-across predictions

Tags: NIEHS, QSAR

To improve utility and interpretability of molecular structure-based predictive models, NIEHS scientists and collaborators developed a novel set of extensible chemistry-aware substructures, Saagar. The Saagar features were systematically identified based upon open-source literature highlighting relationships among substructural moieties, physicochemical properties, toxicological properties of molecules, and ADME properties. This development approach makes Saagar features more interpretable than standard molecular descriptor libraries. The Saagar substructures were evaluated for their performance in chemical characterization and read-across applications by comparing results with four publicly available fingerprint sets for three benchmark chemical sets including about 145,000 compounds (Sedykh et al. 2021). In 18 of the 20 comparisons, Saagar features performed better than the other fingerprint sets. Saagar features efficiently characterize diverse chemical collections, thus making them a better choice for building interpretable predictive in silico QSAR models and read-across protocols.

Tox21BodyMap: a webtool to map chemical effects on the human body

Tags: EPA, NIEHS, Tox21

To support rapid chemical toxicity assessment and mechanistic hypothesis generation, EPA and NIEHS scientists developed an intuitive webtool allowing a user to identify target organs in the human body where a substance is predicted to be more likely to produce effects (Borrel et al. 2020). This tool, Tox21BodyMap, incorporates results of 9,270 chemicals tested in the United States federal Tox21 research consortium using 971 HTS assays whose targets were mapped onto human organs using organ-specific gene expression data. Via Tox21BodyMap's interactive tools, users can visualize chemical target specificity by organ system and implement different filtering criteria by changing gene expression thresholds and activity concentration parameters. Dynamic network representations, data tables, and plots with comprehensive activity summaries across all Tox21 HTS assay targets provide an overall picture of chemical bioactivity.

Tools to predict chemical-assay interference

Tags: EPA, NIEHS, NIH, Tox21, machine learning

Assays used in Tox21 and other HTS programs rely on luciferase and fluorescence-based readouts. These can be susceptible to signal interference by certain chemical structures resulting in false positive outcomes. However, the Tox21 portfolio includes assays specifically designed to measure interference in the form of luciferase inhibition and autofluorescence via multiple wavelengths and under various conditions. EPA, NIEHS, and NCATS scientists applied multiple machine learning algorithms to predict assay interference based on molecular descriptors and chemical properties (Borrel et al. 2020a). The best performing predictive models were incorporated into a web-based tool called InterPred (Borrel et al. 2020b) that allows users to predict the likelihood of assay interference for any new chemical structure. InterPred increases confidence in HTS data by decreasing false positive testing results.

SARA model for prediction of skin sensitization

Tags: NIEHS, skin sensitization

In May 2021, NICEATM entered into an agreement with consumer products company Unilever to collaboratively test and further develop their Skin Allergy Risk Assessment (SARA) predictive model (Reynolds et al. 2019). SARA is a computational model that uses a variety of input data to estimate a probability that a chemical will cause an allergic skin reaction in humans. NICEATM will test the SARA model using a variety of chemical data sets, including chemicals of interest to U.S. and international regulatory agencies. NICEATM and Unilever will also work together to expand the SARA model to include data generated by the NIEHS Division of the NTP (DNTP). The intent is to make the SARA model openly available for public use along with other NICEATM predictive models. Availability of the SARA model will help further reduce animal use for the endpoint of skin sensitization and will improve upon existing efforts by providing points of departure (PODs) for quantitative human risk assessment.

Computational models for eye irritation classification of mixtures

Tags: NIEHS, eye irritation, mixtures toxicity

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

Incorporating parameter and population variability into PBPK modeling

Tags: NIEHS, IVIVE, QSAR

To identify the potential for a chemical to be of concern to sensitive populations, it is important for new approach methodologies (NAMs) to characterize variations in metabolism that can affect toxicity within a population of interest. NIEHS scientists are incorporating the effects of genetic variability on ADME into pharmacokinetic models. These models can be used to predict a tissue concentration from an external exposure (forward dosimetry) or estimate an external exposure that would result in a plasma or tissue concentration equivalent to an effective concentration in an in vitro assay (reverse dosimetry). The models use inputs from ADMET Predictor software (Simulations Plus, Inc.) to characterize what enzymes might be involved in a chemical's metabolism and the structure and proportions of metabolites produced. Work is ongoing to identify polymorphisms in the genes coding for enzymes that might affect metabolism, characterize the polymorphisms' prevalence within populations, and incorporate this information into IVIVE and PBPK models. The OPERA QSAR modeling suite will then be used to predict a range of resulting toxicities for chemical metabolites.

Web application to apply skin sensitization defined approaches to user data

Tags: NIEHS, skin sensitization

In June 2021, OECD issued Guideline 497, Defined Approaches on Skin Sensitisation, the first internationally harmonized guideline to describe a non-animal approach to predict skin sensitization potential. NICEATM scientists are developing an interactive web application that computationally applies the defined approaches outlined in Guideline 497 through a user-friendly interface. The user uploads data from the test methods used in the defined approach to the web application, which then generates skin sensitization predictions for the user's chemicals of interest. The user selects the analysis variables, and the application dynamically provides feedback about the user's data set to identify problematic data values. The application is still in development and will be available on the NTP website in 2022.

Development of a human intestinal cell permeability model

Tags: NIEHS

Intestinal absorption plays a role in the toxicity of chemicals. The human Caco-2 cell line, derived from human colon epithelial cancer cells, is currently used in an in vitro model of human intestinal absorption. However, there are several limitations to this method, including long cell culture times and inability to conduct HTS. NIEHS scientists are developing computational models for chemical absorption in human intestines that would allow for screening a wide range of chemicals for potential intestinal permeability, which would assist in evaluating the toxicity potential of these chemicals. To date, a data set containing over 4,500 unique chemicals has been compiled and prepared; development of a model is planned for 2022.

Investigating DNA adduct formation by flavor chemicals and tobacco byproducts in e-cigarettes using in silico approaches

Tags: FDA, carcinogenicity, inhalation toxicity, mixtures toxicity, genotoxicity, skin sensitization, QSAR

The potential harms from inhaling flavoring chemicals used in e-cigarettes and byproducts of those chemicals have not been extensively studied. One mechanism of interest is DNA adduct formation, which may lead to carcinogenesis. Scientists at the FDA Center for Tobacco Products (Kang and Valerio 2020) determined that flavoring chemicals and their

byproducts include alkenylbenzenes and aldehydes, both of which are known to form DNA adducts. Using in silico toxicology approaches, they conducted a structural similarity analysis and generated in silico model predictions of these chemicals for genotoxicity, mutagenicity, carcinogenicity, and skin sensitization. Good concordance was observed between DNA adduct formation and models predicting mammalian mutagenicity and skin sensitization for both chemical classes. On the other hand, different prediction profiles were observed for the two chemical classes for the endpoints of unscheduled DNA synthesis and bacterial mutagenicity. These results are likely due to the different mode of action between the two chemical classes, as aldehydes are direct acting agents, while alkenylbenzenes require bioactivation to form the electrophilic intermediates that in turn form DNA adducts. This study suggests that in silico prediction for the mouse lymphoma assay may serve as a surrogate endpoint to help predict DNA adduct formation for chemicals found in tobacco products such as flavors and byproducts.

Using in silico toxicology tools to predict mutagenic potential of tobacco products

Tags: FDA, carcinogenicity, inhalation toxicity, mixtures toxicity, QSAR, genotoxicity, metrics

Scientists at the FDA Center for Tobacco Products (Goel and Valerio 2020) utilized in silico tools to predict the mutagenic potential of chemicals in tobacco products and tobacco smoke in a validation test and in a separate screening test. Publicly available QSAR models were validated against 900 chemicals relevant to tobacco products for which experimental Ames mutagenicity data were available from public sources. The predictive performance of the individual and combined QSAR models was evaluated using various performance metrics. All the models performed well, predicting mutagens and nonmutagens with 75%-95% accuracy, 66%-94% sensitivity, and 73%-97% specificity. Subsequently, in a screening test, a combination of complementary structure–activity relationship and QSAR models was used to predict the mutagenicity of 6,820 chemicals cataloged in tobacco products and/or tobacco smoke. More than 1,200 of these chemicals were predicted to have mutagenic potential, with 900 potential mutagens in tobacco smoke. This research demonstrates the validity of in silico QSAR tools to predict mutagenicity of chemicals in tobacco products or tobacco smoke, and

suggests QSAR models may be useful as screening tools for hazard identification to inform tobacco regulatory science.

Using in silico approaches to explore the potential neurotoxicity of vaping vitamin E or vitamin E acetate

Tags: FDA, carcinogenicity, inhalation toxicity, mixtures toxicity, QSAR, neurotoxicity

Serious adverse health effects have been reported with the use of vaping products, including neurologic disorders and e-cigarette or vaping product use-associated lung injury. Vitamin E acetate, often added as a diluent to cannabis-containing products, has been linked to lung injury. FDA scientists explored the relationship of such additives to neurotoxicity. Literature searches were performed on vitamin E and vitamin E acetate-associated neurotoxicity. The literature review showed that the neurotoxic potential of inhalation exposures to these compounds in humans is unknown. The blood-brain barrier penetration potential of vitamin E and vitamin E acetate were evaluated using cheminformatic techniques. Physicochemical properties suggest that these compounds are lipophilic, and molecular weights indicate vitamin E and vitamin E acetate have the potential for blood-brain barrier permeability. Computational models also predict both compounds may cross the blood-brain barrier via passive diffusion. The literature search found no experimental nonclinical studies or clinical information on the neurotoxic potential of vitamin E via inhalation. However, neurotoxic effects of phenyl acetate, a pyrolysis by-product structurally analogous to vitamin E acetate, suggest that vitamin E acetate has potential for central nervous system impairment. Cheminformatic model predictions provide a theoretical basis for potential central nervous system permeability of these inhaled dietary ingredients, suggesting that they should be prioritized to evaluate for potential central nervous system hazard.

Development of a nicotinic acetylcholine receptor binding activity prediction model

Tags: FDA, carcinogenicity, inhalation toxicity, mixtures toxicity

Addiction to nicotine found in tobacco products causes difficulty in quitting among users. Nicotinic acetylcholine receptors (nAChRs) are the physiological targets of nicotine and facilitate addiction to tobacco products, primarily the nAChR-α7 subtype. Therefore, predicting the binding activity of tobacco constituents to nAChR-α7 is an important component for assessing addictive potential of tobacco constituents. Scientists at the FDA

Center for Tobacco Products developed a predictive computational model for nAChR-α7 binding activity. The model was trained on data from 843 chemicals with human nAChR-α7 binding activity extracted from PubChem and the European Union's ChEMBL chemicals database (Sakkiah et al. 2020). The model was tested using 1,215 chemicals with rat nAChR-α7 binding activity data from the same databases. The developed model was then used to predict the potential human nAChR-α7 binding activity for 5,275 tobacco constituents. The human binding activity data for 84 of the 5,275 tobacco constituents were experimentally measured to validate the prediction results. This model of human nAChR-α7 binding may be a useful tool for screening of potentially addictive tobacco constituents.

Deep learning image analysis of high-throughput toxicology assay images

Tags: NIEHS, machine learning

HTS (high-throughput screening) approaches often employ microscopy to capture photomicrographs from multi-well cell culture plates, generating thousands of images that require manual human analysis. To automate this subjective and time-consuming process, NIEHS scientists and collaborators developed a method that uses deep learning to automatically classify digital assay images (Tandon et al. 2021). A convolutional neural network was trained to perform binary and multiclass classification. The binary classifier accurately binned assay images into healthy (comparable to untreated controls) and altered (not comparable to untreated controls) classes, while the multiclass classifier accurately assigned "Healthy," "Intermediate," and "Altered" labels to assay images. The study results indicated a strong correspondence between dosage and classifier-predicted scores, suggesting that these scores might be useful in further characterizing benchmark dose. Together, these results clearly demonstrate that deep learning-based automated image classification of cell morphology changes upon chemical-induced stress can yield highly accurate and reproducible assessments of cytotoxicity across a variety of cell types.

Derek Nexus QSAR software for predicting skin sensitization and EC3 values

Tags: DoD, skin sensitization, QSAR

Traditionally, skin sensitization potential of chemicals has been determined using in vivo methods. One of these methods, the mouse lymph node assay, expresses the potential of a chemical to cause skin sensitization as an EC3 value: the concentration of a chemical

required to elicit a threshold positive response. Scientists at the U.S. Air Force Research Laboratory explored approaches that use in silico and in vitro assays (Naumann and Arnold 2019) to calculate EC3 values and develop safe surface levels for chemicals used in fabrication and maintenance shops. A list of 125 relevant chemicals was compiled for evaluation of their skin sensitization potential using this approach. Of those chemicals, 21 known sensitizers have been identified, and an additional 58 chemicals were identified as having insufficient information available to determine sensitization potential. To support the analysis, additional in vitro testing was completed on a number of chemicals using two assays for skin sensitization potential, the direct peptide reactivity assay and the KeratinoSens assay. QSAR analysis using a nearest-neighbors approach was performed on the known sensitizers and measured or predicted EC3 values were obtained using Derek Nexus software (Lhasa Limited). Of the 58 chemicals lacking sensitization or toxicity data, predictions of sensitizing potential were also obtained using both Derek Nexus and publicly available OECD QSAR Toolbox software and results compared with six chemicals identified as potential sensitizers. Very few differences in sensitizer vs. nonsensitizer predictions of sensitization potential were found. The EC3 values derived from these efforts will be used to calculate surface levels. In vitro test results generated for the project will also be made available to update current in silico models of skin sensitization.

Continued development of httk

Tags: DoD, EPA, NIEHS, developmental toxicity, inhalation toxicity, IVIVE

To fully characterize the potential human health risk of a substance, data are often needed on that substance's toxicokinetics: how a substance is absorbed, distributed, metabolized, and eliminated in the body. Traditional approaches for obtaining toxicokinetics data use animals, but alternative approaches are being developed to computationally estimate toxicokinetic parameters. Scientists within EPA and the U.S. Air Force Research Laboratory are developing such an approach to generate high-throughput toxicokinetic estimates of chemical inhalation exposures (Linakis et al. 2020). This approach has been implemented in an inhalation component for the R-based high-throughput toxicokinetics (httk) package. In total, 142 exposure scenarios across 41 volatile organic chemicals were modeled and compared to published data. Ongoing work is focused on development of inhalation models for aerosols

and mixed exposures. To better model and evaluate fetal exposure to environmental chemicals, EPA scientists have also developed a gestational model component for httk, which estimates the chemical concentrations in multiple fetal tissues (Kapraun et al. 2019, Kapraun et al. 2022). The gestational model has been used for IVIVE analysis by NICEATM to evaluate fetal exposures that cause developmental toxicity risk. NICEATM scientists have also actively participated in testing of httk and review of its code, data, and documentation.

Evaluation of QSAR models for predicting metabolic parameters

Tags: DoD, mixtures toxicity, QSAR

To fully characterize the potential human health risk of a substance, data are often needed about a substance's toxicokinetics to understand how it is absorbed, distributed, metabolized, and eliminated in the body. Traditional approaches for obtaining toxicokinetics data use animals, but alternative approaches are being developed to computationally estimate toxicokinetic parameters in silico using QSAR models. Scientists at the U.S. Air Force Research Laboratory (Sweeney and Sterner 2022) evaluated QSAR models that use chemical structure or property information to predict two toxicokinetic parameters: the maximal capacity for metabolism (Vmax) and the half-maximal concentration for metabolism (KM). None of the evaluated QSAR models in their published forms could be fully validated. Literature review, use of graphical information, and other strategies allowed the deficiencies to be addressed for QSAR predictions of these parameters for alkylbenzenes, volatile organic chemicals, and substrates of alcohol dehydrogenase, aldehyde dehydrogenase, cytochrome P450, and flavin-containing monooxygenases. The updated QSAR expressions for smaller data sets tended to show greater accuracy in Vmax and KM prediction than larger data sets, and Vmax was generally more accurately predicted than KM. In a feasibility case study, the QSAR models were used to estimate toxicokinetic parameters of jet fuel components to determine the potential utility of this approach for investigation of mixture toxicokinetics. Results suggested that the models performed better to predict toxicokinetic parameters of volatile organic chemicals and alkylbenzenes as compared to cytochrome P450 substrates, likely due to the physicochemical properties of the chemicals used in the models' development.

Molecular docking and deep learning to predict binding and activity of compounds on neurological targets

Tags: DoD, neurotoxicity, machine learning

Airmen are exposed to hundreds of chemicals in the operational environment. Some of these chemicals can interact with proteins in the brain and cause neurotoxic effects impairing memory and cognitive performance. To address the need for rapid assessment of neurotoxicity of chemicals, scientists at the U.S. Air Force Research Laboratory developed an in silico tool that uses a reverse molecular docking approach to identify receptor targets for chemicals (McCarthy et al. 2022). They also developed deep learning computational models that predict activity at the neurological targets. The chemical assessment process was automated via a Python-based graphical user interface that prepares all necessary files and performs docking and deep learning modeling. This is a novel tool that can potentially be used to screen and assess chemicals of interest for possible neurotoxic effects that can impact cognitive performance of airmen.

Structure-based QSAR models to predict repeat-dose toxicity points of departure

Tags: EPA, QSAR

Data gap filling techniques, such as QSAR models based on chemical structure information, can predict potential hazards of chemicals that have little experimental data. Risk assessment requires identification of a quantitative POD value, the lowest dose or concentration at which a treatment-related response is observed; effects of treatment by lower doses must be estimated by low-dose extrapolation. A study by EPA scientists (Pradeep et al. 2020) describes two sets of QSAR models to predict POD values for repeat-dose toxicity. The first set of QSAR models predicts point estimates of POD values using structural and physicochemical descriptors for repeat-dose study types and species combinations. The second set of QSAR models predicts the 95% confidence intervals for PODs using a constructed POD distribution based on previously published typical study-to-study variability that may lead to uncertainty in model predictions (Pham et al. 2020). Enrichment analysis to evaluate the accuracy of the predicted PODs showed that 80% of the 5% most potent chemicals were found in the top 20% of the most potent chemical predictions. This suggests

that these repeat-dose POD QSAR models may help inform screening-level human health risk assessments in the absence of other data.

Data Resources

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

Semi-automated extraction of literature data using machine learning methods

Tags: DOE, FDA, NIEHS, developmental toxicity, machine learning, endocrine disruptors

NICEATM, other scientists within DNTP, the Oak Ridge National Laboratory (U.S. Department of Energy [DOE]), and FDA are collaborating to automate the process of identifying high-quality developmental toxicity studies in the published scientific literature. The approach applies natural language processing and machine learning methods to identify specific data elements in the full text of scientific publications using both unsupervised and supervised approaches.

Preliminary models were trained using a uterotrophic database (Kleinstreuer et al. 2016) built for the EPA Endocrine Disruptor Screening Program. The models leveraged natural language processing and multivariate machine learning models to identify papers that meet minimum criteria to be considered guideline-like studies (Herrmannova et al. 2018). Supervised and unsupervised approaches were developed to automatically extract text features that correspond to study descriptors and classify papers based on their adherence to minimum criteria derived from regulatory guideline studies. These methods demonstrated high cross-validated performance on the uterotrophic training set.

This work is being extended and applied to automate the identification of high-quality prenatal developmental toxicity studies in the literature, in collaboration with the ICCVAM Developmental and Reproductive Toxicity Expert Group. A publication describing this work is being drafted for submission in 2022.

Extraction and annotation of legacy developmental toxicity study data

Tags: NIEHS, developmental toxicity

To support the evaluation of non-animal approaches for developmental toxicity assessment, NICEATM scientists extracted information from more than 100 legacy NTP prenatal developmental toxicity animal studies and a subset of about 50 studies submitted to the European Chemicals Agency that were deemed high-quality by NTP subject matter experts. Study details extracted included species, strain, administration route, dosing duration, and treatment-related effects.

The extracted data were standardized by applying controlled vocabularies and ontologies to facilitate computational analyses and integration with other structured databases such as EPA's Toxicity Reference Database (ToxRefDB). Elements of three controlled vocabularies (the Unified Medical Language coding system, the German Institute for Risk Assessment DevToxDB ontology, and the OECD Harmonised Template 74 terminologies) were combined with automation code to programmatically standardize primary source language of extracted developmental toxicology endpoints. This work aims to reduce manual labor, facilitate further analyses (e.g., systematic review, model-building, NAM validation), and uphold findability, accessibility, interoperability, and reusability (FAIR) principles. A poster describing this work (Foster et al.) was presented at the 11th World Congress on Alternatives and Animal Use in the Life Sciences, and a publication is being drafted for submission in 2022.

Compilation of human skin sensitization data

Tags: CPSC, FDA, NIEHS, skin sensitization

Appropriate evaluation of NAMs requires reference data for assessing a NAM's ability to predict an outcome of interest. Human data provide the most relevant basis for such comparisons, but they are rarely available due to obvious ethical issues associated with toxicology testing in humans. One exception is data from skin sensitization tests that are routinely conducted using a wide range of materials. For this project, U.S. Consumer Product Safety Commission (CPSC), FDA, and NICEATM scientists and collaborators collected data from 2,277 human predictive patch tests conducted under two protocols: the human repeat insult patch test and the human maximization test. Data were collected from more than 1,500 publications. The data collection process also captured protocol elements and positive or negative outcomes, calculated traditional and non-traditional dose metrics, and developed a

scoring system to evaluate each test for reliability. The resulting database, which contains information for 1,366 unique substances, was characterized for physicochemical properties, chemical structure categories, and protein binding mechanisms. A description of the database (Strickland et al.) was presented at the 2021 annual meeting of the American Society for Cellular and Computational Toxicology, and a publication is being drafted for submission in 2022. The data are publicly available via ICE to serve as a resource for the development and evaluation of NAMs for skin sensitization testing.

Integrated Chemical Environment data updates

Tags: NIEHS, Tox21, metrics, carcinogenicity, developmental toxicity, genotoxicity, skin irritation, skin sensitization

NICEATM's Integrated Chemical Environment (ICE) provides data and tools to help develop, assess, and interpret chemical safety tests. Updates to ICE data sets during 2020 and 2021 provided additional metadata in downloads of query results, updated curated HTS data, and new data in these areas:

- Skin irritation: in vivo and in vitro data.
- Cancer/genotoxicity: reference chemical lists and in vitro and in vivo data.
- Developmental and reproductive toxicity: in vivo data.
- Skin sensitization: data from human predictive patch tests.
- Chemical property predictions from OPERA.

Variability analysis of in vivo skin irritation data to use in establishing confidence for alternative methods

Tags: NIEHS, skin irritation

A limiting factor in identifying a complete in vitro replacement for the standard in vivo skin irritation test could be the variability inherent to the subjective scoring of endpoints in the in vivo test. This is particularly relevant for mild and moderate irritants, where interindividual differences in scoring are most likely to occur. To characterize the reproducibility of the in vivo assay, NICEATM assessed variability in study results from substances tested multiple times (Rooney et al. 2021). A set of 2,624 test records was compiled and curated,

representing 990 unique mono-constituent substances, each tested at least twice. Conditional probabilities were used to evaluate the reproducibility of the in vivo method in identification of EPA or GHS hazard categories. Chemicals classified as moderate irritants at least once were classified as mild irritants or non-irritants at least 40% of the time when tested repeatedly. Variability was greatest between mild and moderate irritants, for which each type of substance had less than a 50% likelihood of its classification being replicated. This analysis indicates that variability of the rabbit skin irritation test should be considered when evaluating the performance of non-animal alternative methods as potential replacements. The analysis was used as a case study in a review (Alves et al. 2021) highlighting the importance of data curation in developing data sets used as inputs for artificial intelligence models.

Variability analysis of in vivo acute oral systemic toxicity data

Tags: EPA, NIEHS, acute toxicity, oral toxicity

There is a pressing need to develop reliable and robust reference acute systemic toxicity data sets to contextualize results, to set expectations regarding NAM performance, and to train and evaluate computational models. To meet these needs, EPA and NICEATM compiled and curated rat acute oral LD50 data from multiple databases (Karmaus et al. 2022). More than 2,000 chemicals with LD50 values from at least two independently conducted rat acute oral systemic toxicity assays were subjected to comprehensive manual review to curate all data. Variability could not be attributed to any chemical-specific characteristics, and thus it was concluded that inherent biological or protocol variability is likely underlying the variance in the results. Understanding the challenges with reproducibility of the rat acute systemic toxicity test helps to better inform appropriate evaluation of future NAM performance assessments.

Variability analysis of human skin sensitization data to use in establishing confidence for alternative methods

Tags: CPSC, FDA, NIEHS, skin sensitization

Because humans are the primary subject of interest for regulatory safety testing, it is advantageous to have human reference data available for evaluation of NAMs for assessing chemical safety. Scientists with CPSC, FDA, and NIEHS and collaborators compiled such a data set for human skin sensitization potential by collecting data from the scientific literature

for human predictive patch tests that used the human maximization or human repeated insult patch test protocols. They then assessed the variability of these data to determine the potential impact on concordance with NAMs. The data collection identified 2,255 tests that were deemed to be sufficiently reliable for the analysis, including reports for 232 substances with at least two test results. The substances included anilines, amines, aldehydes, esters, and other chemical classes. For 68 substances, all tests were positive (at least one sensitized subject in a study); for 126 substances, all tests were negative (no sensitized subjects); and for the remaining 38 substances, both positive and negative results were obtained. None of the protocol variables such as test type, skin patch size, sample size, or dose applied were associated with high or low variability. There was also no detected association with variability for any of the 10 physicochemical properties examined. The effect of variation in vehicle used could not be analyzed because the majority of tests used a single vehicle. Future work will examine the variability of potency estimates, measured as the dose per skin area that sensitizes one subject. This characterization provides context for defining benchmarks for the evaluation of NAMs for skin sensitization assessments. A poster describing the data set (Strickland et al.) will be presented at the 2022 annual meeting of the Society of Toxicology.

Implementation of ontologies for zebrafish developmental toxicity screening

Tags: DoD, EPA, NIEHS, developmental toxicity

Toxicological evaluation of chemicals using early life stage zebrafish (Danio rerio) involves the observation and recording of altered phenotypes. Variability has been observed among researchers in phenotypes reported from similar studies. This variation and a lack of consistent data annotation indicate a need for harmonization of both terminology and data. When examined from a data science perspective, many apparent differences can be parsed into the same or similar endpoints whose measurements differ only in time, methodology, or nomenclature. Standardized nomenclature systems known as ontologies can be leveraged to integrate diverse data sets. Building on this premise, the NTP's Systematic Evaluation of the Application of Zebrafish in Toxicology program coordinated a collaborative exercise to evaluate how the application of standardized phenotype terminology improved data consistency (Thessen et al. 2022). Zebrafish researchers were asked to assess images of

zebrafish larvae for morphological malformations in two surveys. In the first survey, researchers were asked to annotate observed malformations using their own terminology. In the second survey, researchers were asked to annotate the images from a list of terms and definitions from the Zebrafish Phenotype Ontology. Analysis of the results suggested that the use of ontology terms increased consistency and decreased ambiguity, and that utilizing a common data standard should reduce the heterogeneity of reported terms and potentially increase agreement and repeatability between different laboratories.

Quantification of variance in data from systemic in vivo toxicology studies

Tags: EPA

NAMs for chemical hazard assessment are often evaluated in comparison to animal studies. However, variability in animal study data represents a barrier to an objective evaluation of NAM accuracy. Data available in EPA's ToxRefDB enable consideration of such variability in effect levels, measured as the lowest effect level (LEL) for a treatment-related effect and the lowest observable adverse effect level (LOAEL) defined by expert review. These data are available from subacute, subchronic, chronic, and multi-generational reproductive and developmental toxicity studies. EPA scientists reviewed ToxRefDB data to quantify the variance within systemic LEL and LOAEL values and to estimate the upper limit of NAM prediction accuracy (Pham et al. 2020). The analysis enabled both a quantification of the total variance in systemic LEL and LOAEL values and an estimate of the unexplained variance in LEL and LOAEL values. These findings suggest quantitative considerations for building scientific confidence in NAM-based systemic toxicity predictions.

Relocation of ALTBIB to NTP website

Tags: NIEHS, NLM

ALTBIB is the Bibliography on Alternatives to the Use of Live Vertebrates in Biomedical Research and Testing. The National Library of Medicine (NLM) developed ALTBIB to provide access to PubMed citations for users seeking information on alternatives to animal testing. Many citations provide access to free full text.

In December 2020, ALTBIB was revised and relocated to the NICEATM section of the NTP website. The search strategy on the new ALTBIB site was updated to capture key topics of

current interest such as MPS and QSAR models. The content was reorganized to provide additional user support, and more links were added to the "Additional Resources" list. Users can still edit the ALTBIB search strategy to broaden or narrow searches. A list of keywords related to specific topics that was available on the NLM ALTBIB site is being updated and revised, and the list will be added back to the NICEATM website when that work is complete.

Development of MPS-Db data portal

Tags: DoD, NIEHS, NIH, COVID-19, MPS

NICEATM and the UK National Centre for the 3Rs (NC3Rs) are partnering with the National Institute of Allergy and Infectious Diseases, the U.S. Army Combat Capabilities Development Command Chemical Biological Center, and NCATS to direct the MPS for COVID Research (MPSCoRe) working group. This group is coordinating the use of MPS to reduce animal use in studies of COVID-19 and future emerging infectious diseases.

One key activity of MPSCoRe is supporting the expansion of the MPS Database (MPS-Db) to include a COVID-19 disease portal, which went live in April 2021. Through this portal, researchers can share experimental data, analytic tools, model designs, and study components to accelerate the development and adoption of human MPS for testing therapies and improving disease understanding. The portal has links to further information and resources to support the development and application of MPS that can recapitulate the pathophysiology of COVID-19 in various organ systems. Details of commercially available MPS, as well as components used in designing and implementing SARS-CoV-2/COVID-19 studies, have been uploaded to the platform to support access to existing MPS and the development of new models.

In the next phase of development of the MPS-Db, MPSCoRe members will upload and share their own COVID-19 MPS models and study data generated by them. Model details and data collected in the portal include model schematics, cell sources/types, key references, model variations, study designs, and assay data and associated metadata generated in response to various stimuli. The primary user can specify data access permissions so that other users of the database can access these data and use the in-built modeling capabilities to reanalyze them, maximizing the potential impact of each individual study. The development of the

COVID-19 disease portal and the creation of a comprehensive centralized hub for COVID-19 infection and pathogenesis in the MPS-Db will potentially improve the speed and efficiency with which researchers obtain the information required to inform the design, development, and application of human MPS experimental models for therapeutic development.

Annotation and visualization of high-throughput in vitro data

Tags: NIEHS, Tox21

Linking in vitro HTS data from programs such as ToxCast and Tox21 to regulatory endpoints remains a challenge and requires both detailed information about the assays and an understanding of their biological context. For example, while information may be provided about an in vitro assay's technology platform, design, and gene target information, it remains a challenge to interpret this information in a toxicological context for potential regulatory applications. NICEATM scientists developed a mapping approach for HTS assay endpoints that provides a robust assay grouping schema applicable beyond HTS data sets in a toxicological endpoint-based framework. This expert-led curation and annotation is available in ICE and is described in Abedini et al. 2021. The annotations map HTS assays to regulatory toxicological endpoints of interest through modes of action, which use structured vocabularies to allow data to be searched, grouped, and visualized. The annotations further increase accessibility for those unfamiliar with individual assays by defining mechanistic targets that provide context for in vitro assays to facilitate data interpretation. By leveraging these annotations, users of ICE can better identify data gaps, gain insight into mechanistic plausibility, and investigate endpoints of regulatory relevance. ICE also provides data visualization to aid review of a chemical's potential activity based on selected mechanistic targets or modes of action contributing to regulatory endpoints.

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.

To more broadly address evolving challenges in toxicology, Tox21 partners developed a new strategic and operational plan (Thomas et al. 2018) that expands the focus of the program's research activities. New areas of focus are:

- Develop alternative test systems that are predictive of human toxicity and dose response.
- Address key technical limitations of current in vitro test systems.
- Curate and characterize legacy in vivo toxicity studies.
- Establish scientific confidence in integrated assay batteries and in vitro test systems.
- Refine and deploy in vitro methods for characterizing pharmacokinetics and in vitro disposition.

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

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

Tox21 projects and projects using Tox21 data are described below and throughout this report.

Predictive modeling of developmental toxicity with human pluripotent stem cells

Tags: EPA, FDA, NIEHS, developmental toxicity, IVIVE, stem cells, Tox21

EPA, FDA, and NICEATM scientists and collaborators applied IVIVE to evaluate the impact of pharmacokinetic models on predicting relevant external exposure from in vitro developmental toxicity potential concentrations derived from an in vitro human-induced pluripotent stem cell (iPSC)-based assay. Previous work showed that the devTOX quickPredict assay ranked the developmental toxicity potential of valproate analogues in a manner that was consistent with observed developmental toxicity potency in vivo. The IVIVE analysis in this project estimated equivalent administered doses that would result in maternal and fetal blood concentrations equivalent to the developmental toxicity potential and cytotoxic in vitro concentrations (Chang et al. 2022). The estimated equivalent

administered doses were compared to published lowest effect levels from in vivo developmental toxicity studies. Preliminary results of this analysis showed that equivalent administered doses for the valproate analogues based on different pharmacokinetic and PBPK models were quantitatively similar to in vivo data from both rats and humans, where available. The impact of in vitro kinetics on equivalent administered dose estimates was chemical-dependent. Equivalent administered doses from this study were within the range of predicted doses from other in vitro and model organism data. This suggested that the devTOX quickPredict assay and IVIVE approaches can quantitatively assess a chemical's developmental toxicity potential.

Incorporating genetic susceptibility into developmental neurotoxicity screening via population diversity

Tags: EPA, FDA, NIEHS, developmental toxicity, neurotoxicity, Tox21

The potential for neurotoxicity in children following exposure to environmental chemicals is of concern due to recent increases in the prevalence of neurological disorders such as attention deficit hyperactivity disorder and autism. Neurotoxicity risk for an individual can be influenced both by genetic background and by exposures to neurotoxic chemicals in the environment. To investigate the role of genetic diversity in susceptibility to neurotoxicity, scientists at NIEHS, EPA, and FDA are using a genetically diverse set of cells to evaluate a curated set of chemicals with neurotoxic potential. Neural progenitor cells were derived from a set of mice bred to maximize genetic diversity. An initial set of 200 genetically different cell lines from male and female mice was narrowed down to 135 cell lines, considered to be the minimum number of cell lines needed to quantitatively assess diversity in population responses. The panel of cell lines was exposed to 8 concentrations of a 12-chemical test set. The intracellular morphometry of each treated cell was visualized using a high-content imaging assay called cell painting, which uses six fluorescent dyes to quantitatively describe cell features such as cell membranes, mitochondria, DNA and RNA, cytoskeleton, and Golgi bodies (Bray et al. 2016). Data analysis is ongoing, and data will be used to derive PODs for chemical-induced intracellular morphometric endpoints and characterize the variability in these PODs across cell lines for each chemical. Data will also be analyzed to explore differences in toxicity mode of action that may differ across lines and therefore be genetically

linked. These data will inform data-driven uncertainty factors that account for interindividual variability, allowing for adequate protection of genetically sensitive subpopulations. A poster describing the project (Harrill et al.) will be presented at the 2022 annual meeting of the Society of Toxicology, with a talk planned for an accepted symposium at the 2023 meeting. A paper describing the project is planned for submission in 2023.

Automation of reference chemical generation

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

To use data generated by HTS initiatives such as Tox21 and ToxCast in regulatory applications, the assays and models built from the assays must be validated based on their performance against the biological targets they query. This requires developing sets of reference chemicals that consistently yield reproducible results when assayed for these biological targets. Furthermore, the development of reference chemical sets needs to be streamlined and rapid enough to manage the tens to hundreds of assays that can help inform regulatory toxicity endpoints. To address this need, scientists at EPA and NIEHS developed a process to identify reference chemicals that consistently produce positive or negative results when tested in defined assays (Judson et al. 2019). Work under this project conducted in partnership with NCATS seeks to automate the generation of reference chemical lists without the need for new experimental tests or upfront literature review. The targets being examined for this project are androgen receptor, estrogen receptor, glucocorticoid receptor, peroxisome proliferator-activated receptor gamma, progesterone receptor, retinoic acid receptors, thyroid hormone receptor, tumor protein P53, mitochondria toxicity, and cell stress pathways. The list of assays and reference chemicals generated through this process will be used to validate new assays or AOPs, which will improve chemical screening abilities when predicting toxicity. The project will be described in one or more papers to be submitted for publication in 2022 and 2023.

Retrofitting existing Tox21 HTS assays with metabolic capability

Tags: EPA, NIEHS, NIH, Tox21

The HTS assays that have been run in the Tox21 testing program to date generally lack the metabolic activity found in living systems, which can potentially increase or decrease the toxicity of chemicals. As a result, HTS results may not accurately reflect in vivo activity.

Scientists at EPA, NCATS, and NIEHS are using several approaches to address this problem: adding human or rat liver microsomes into the existing assays, transfecting cells with mRNAs encoding human metabolic enzymes, or using metabolically capable human HepaRG cells. Work in 2020 and 2021 (Ooka et al. 2022) focused on three types of assays for which data have already been generated: cellular stress-related assays, endocrine disruption assays, and CYP450 enzyme inhibition assays. The retrofitted assays were used to screen the Tox21 10K chemical library to identify chemicals that are either bioactivated or detoxified by metabolic activity.

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

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

To date, Tox21 HTS assays have focused primarily on selected nuclear receptor and stress response pathways. This relatively limited focus suggests that activity in other toxicity pathways has not been adequately assessed; it is likely that some unexplored pathways relate to unanticipated adverse drug effects. Therefore, expanding the coverage of biological responses by adding assays that probe under-represented pathways in the current Tox21 assay portfolio may improve the predictivity of Tox21 data. Scientists at FDA, NIEHS, and NCATS are systematically identifying these under-represented pathways in a data-driven approach and nominating assays for development and Tox21 chemical screening. The data generated will be used to build models for human toxicity prediction, focusing on common adverse drug effects such as drug-induced liver injury and cardiotoxicity. The initial survey identified targets and pathways of interest, including cytochrome P450 metabolic enzymes and G protein-coupled receptors. HTS assays were optimized and validated for the proposed new targets and pathways, and these assays will be used to screen the Tox21 10K chemical library in 2022. Models are being built that could use HTS assay data to predict human toxicity. Predictions from the models will be validated in relevant assays. A paper describing the project and the prediction models will be prepared in late 2022.

Profiling activity of acetylcholinesterase inhibitors

Tags: EPA, FDA, NIEHS, NIH, neurotoxicity, Tox21

Acetylcholinesterase inhibitors cause a variety of adverse effects in the nervous system. Some acetylcholinesterase inhibitors serve as drugs, while others are used as pesticides or found in natural products. Scientists at FDA, NIEHS, and NCATS and collaborators developed acetylcholinesterase inhibition assays (Li et al. 2017, 2019). These assays were then used to screen the Tox21 10K chemical library to identify environmental chemicals that inhibit acetylcholinesterase activity (Li et al. 2021). The screening study, which included NIEHS scientists and other collaborators, identified both known and not previously reported acetylcholinesterase inhibitors, which were further characterized in lower-throughput cell-based assays, enzyme-based activity assays, and molecular docking studies. Specifically, this study identified 18 potential novel acetylcholinesterase inhibitors, most of them clinically approved drugs. Cmax values, which represent the highest concentration of a drug in the blood or target organ after a dose, were found from the literature for 14 drugs. These were compared with half-maximal inhibitory concentration (IC50) values generated from this study (Santillo and Xia, 2022). The ratio of IC50 and Cmax can be used to prioritize the compounds for further study. This represents a robust and reliable approach to evaluating large sets of environmental compounds for their acetylcholinesterase inhibitory activity.

Development of high-throughput assays to detect potential sensitizers or irritants

Tags: NIEHS, NIH, skin irritation, skin sensitization, Tox21

Assessing the sensitization or irritation potential is a key element in the safety evaluation of topically applied or exposed chemicals and drugs. European legislation now mandates the use of alternative test methods for these compounds, instead of testing them on animals. Developing HTS assays for these compounds would provide a faster and cheaper alternative testing method than current in vitro assays. Scientists from NIEHS and NCATS collaborated to explore development of HTS approaches to identify potential irritants and sensitizers. Studies described in a 2020 publication (Wei et al. 2020) found that HTS-compatible 2D cellular and 3D tissue skin models could be combined with irritation-relevant activity endpoints to assess the irritation effects of topical-use compounds and identify potential hazards. To identify potential skin sensitizers, the Tox21 10K compound library was screened for potential sensitizers using the KeratinoSens assay. Substances identified as active were further tested using a high-throughput version of the direct peptide reactivity

assay (Wei et al. 2021), an interleukin-8 assay, and the human cell line activation test. Analysis of these data are ongoing, and the results will be reported in a paper to be published in 2022.

Predictive toxicology of the retinoid signaling pathway

Tags: EPA, FDA, NIEHS, NIH, AOP, developmental toxicity, IVIVE, 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. For example, a computational model that linked chemical disruption of the retinoic acid signaling pathway with fetal skeletal defects has been aligned with a provisional AOP framework for craniofacial, vertebral, and/or appendicular phenotypes (Knudsen et al. 2021; Pierro et al. submitted). Papers in preparation related to this project describe the use of these data as input for IVIVE models to predict relevant exposure levels and to develop candidate reference chemicals for in vitro retinoid pathway assays. Participants in the project contributed to a "Detailed Review Paper on the Retinoid System" issued by OECD in 2021, and described findings in a poster presented at the 2021 annual meeting of the Society of Toxicology (Pierro et al.), a platform presentation at the 2021 annual meeting of the Society for Birth Defects Research and Prevention (Pierro et al.), and a workshop session at the 11th World Congress on Alternatives and Animal Use in the Life Sciences (Knudsen et al. 2021).

Investigation of environmental determinants of pubertal timing in girls

Tags: NIH, NIEHS, 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. While

some studies (e.g., Bandera et al. 2011) have suggested a relationship between the presence of endocrine-disrupting chemicals in girls and pubertal timing, more evidence is needed to develop clear associations. Scientists at NIEHS and NCATS are examining the potential effects of endocrine-disrupting chemicals on important components of the biochemical pathway responsible for pubertal timing. Chemicals in the Tox21 10K collection are being tested in human cell-based assays that measure activation or inhibition of the gonadotropin-releasing hormone receptor and kisspeptin receptor. These receptors are expressed in the hypothalamus and are important players in the control of pubertal timing. Chemicals that exhibit activity will be tested further in cell-based assays or possibly in animals to confirm the biological relevance of the identified activity and determine the chemical's mechanism of action.

Confidence

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

Assay Application

ICCVAM and its member agencies conduct, support, and evaluate studies investigating the suitability of new laboratory methods for specific purposes. Methods evaluated during 2020 and 2021 addressed endpoints including inhalation toxicity, skin sensitization, eye irritation, and developmental toxicity.

Electrophilic allergen screening assay validation study

Tags: ICCVAM, skin sensitization, CPSC, DoD, FDA, NIST, NIOSH

Binding of a chemical to skin proteins is the first step in the development of allergic contact dermatitis. The electrophilic allergen screening assay is a chemical assay that measures light absorbance or a fluorescent signal in proportion to a chemical's tendency to bind to proteins.

The electrophilic allergen screening assay, developed by scientists at the National Institute for Occupational Safety and Health, was nominated to ICCVAM to evaluate its usefulness for identifying potential skin sensitizers. Five ICCVAM member agencies are participating in an ongoing validation study of the assay. Testing of 10 chemicals during 2018 showed that the method has sufficiently good reproducibility and accuracy rates to support further evaluation. In 2019, scientists at CPSC and the National Institute of Standards and Technology (NIST) modified the assay to a 96-well format to increase throughput and accessibility of the assay; details of this process are described in an abstract (Gordon et al.) presented at the SOT 2020 annual meeting. Testing of 20 chemicals will be completed during 2022 using the 96-well assay.

OptiSafe validation study

Tags: ICCVAM, NIEHS, eye irritation, acute toxicity

NICEATM coordinated a multi-laboratory validation study to determine the reliability and relevance of the OptiSafe test method. In this method, a test substance is applied to a semi-permeable membrane to assess the substance's potential to cause eye irritation.

The study was completed in 2018 and a report on the study has been published (Choksi et al. 2020). The study demonstrated that the OptiSafe method is useful for identifying non-surfactant substances that do not require classification for eye irritation and thus can reduce the use of animals for this type of testing.

Developing a defined approach for eye irritation testing

Tags: EPA, NIEHS, eye irritation, IATA, mixtures toxicity

NICEATM, PETA Science Consortium International e.V., EPA, and CropLife America member companies are collaborating to develop an in vitro defined approach for hazard classification of eye irritation potential of agrochemical formulations. A three-phased prospective evaluation was designed to (1) assess the applicability of seven in vitro eye irritation/corrosion protocols to agrochemical formulations and (2) develop a defined approach for agrochemical formulations testing for prediction of U.S. and international irritancy classifications. Sixteen formulations were tested in the bovine corneal opacity and permeability, neutral red release, isolated chicken eye, EpiOcular, and porcine cornea reversibility test methods. Results were compared to the hazard classification assigned based on the in vivo rabbit test (Choksi et al. 2021). For each test method, at least one formulation was discordant with the in vivo rabbit classification, but none of the methods yielded discordant results for all tested formulations. Initial results indicate that certain test method combinations may be used to predict in vivo outcomes. Additional analyses will focus on physicochemical properties and composition of tested formulations to determine if there are any common features that impact in vitro test method performance. Analysis results were presented in a poster (Choksi et al.) at the 11th World Congress on Alternatives and Animal Use in the Life Sciences. These data will be considered in the context of a recent review of eye anatomy and mechanisms of chemically induced eye irritation in humans and other species (Clippinger et al. 2021), which supported reduced reliance on comparisons to rabbit data to show the validity of other methods in favor of a greater focus on human relevance and assay reliability.

Interlaboratory study to examine effects of key protocol elements for zebrafish developmental toxicity studies

Tags: NIEHS, developmental toxicity

To enable broader adoption of zebrafish for toxicological screening, NTP established the Systematic Evaluation of the Application of Zebrafish in Toxicology (SEAZIT) program.

An initial information-gathering phase of the SEAZIT program identified a need for an interlaboratory study to examine the effects of variation in key protocol elements in developmental toxicity studies. The study was designed to determine the effect of chorion removal and exposure media renewal on study outcomes. Participating laboratories will use in-house protocols to test a defined chemical set while varying these two protocol elements. The chemical set, which was designed to provide overlap with other NTP studies, includes chemicals with a range of physicochemical properties and developmental effects. Many of the chemicals have in vivo reference data available from rodent and other zebrafish studies. The interlaboratory study also includes a pilot study of chemical kinetics in support of future studies of ADME in zebrafish. Dose range-finding experiments study began in 2019, although progress over the last 2 years has been delayed due to COVID-related laboratory closures.

In addition to advancing broader adoption of zebrafish for toxicological screening, SEAZIT is also supporting development of best practices for data analysis. To this end, the data generated in this study will be made publicly available, so that all study data may be used by investigators to estimate consensus toxicity values for each chemical.

Testing to expand the applicability domain of three in vitro skin sensitization assays

Tags: NIEHS, CPSC, EPA, FDA, skin sensitization, IATA, mixtures toxicity

In 2021, OECD issued Guideline 497, Defined Approaches on Skin Sensitisation. Drafted and sponsored by ICCVAM agency scientists and international partners, Guideline 497 is the first internationally harmonized guideline to describe a non-animal approach that can be used to replace an animal test to identify skin sensitizers.

To assess and expand the potential applicability of these defined approaches and those accepted by EPA to a broader range of chemical types, ICCVAM agencies nominated more

than 200 chemicals for additional testing in the direct peptide reactivity assay, the KeratinoSens assay, and the human cell line activation test. Chemicals tested included pesticide ingredients and formulations, industrial chemicals, and personal care product ingredients. NTP completed this testing in 2020; reports are being prepared for publication in 2022. A poster describing performance of these test methods individually and in the defined approach for testing of 27 agrochemical formulations (Strickland et al.) was presented at the 11th World Congress on Alternatives and Animal Use in the Life Sciences.

Application of the monocyte activation test for medical device pyrogen testing

Tags: FDA, NIEHS

Pyrogens are substances that can produce fever when present as contaminants in a drug or medical device. Most pyrogens are biological substances derived from bacteria, fungi, and viruses; material-mediated pyrogens, while less common, may also be present. Drugs for injection and medical device products for implantation or other systemic exposure should meet pyrogen limit specifications before they are marketed.

Animal-based pyrogen tests are often conducted to investigate the presence of pyrogens. Non-animal monocyte activation tests (MAT) are widely available but infrequently used for pyrogen testing. To review MAT and discuss ongoing challenges to its widespread implementation for medical device testing, NICEATM and PETA Science Consortium International e.V. co-organized a September 2018 workshop. A workshop report has been published in ALTEX (Brown et al. 2021).

Workshop participants explored how the FDA Medical Device Development Tools Program could be used to qualify MAT as a standalone pyrogen test for specific medical device contexts of use. Attendees discussed practical aspects of pyrogen testing and the evidence needed to support qualification of MAT as a replacement for animal-based pyrogen tests. There was general agreement that MAT could be qualified as acceptable for batch-release testing for microbial-based pyrogens. However, additional studies were recommended to demonstrate its ability to detect known material-mediated pyrogens. This testing would determine whether the assay can be used for both biocompatibility and sterility or if other information on material-mediated pyrogens would be needed to address biocompatibility. Participants also discussed information gaps on material-mediated pyrogens, potential test

controls, and other challenges and opportunities for implementing the use of MAT as a comprehensive pyrogen test.

Human-relevant approaches to assess eye corrosion/irritation potential

Tags: NIEHS, EPA, eye irritation, IATA, mixtures toxicity

Although multiple internationally harmonized test guidelines describe in vitro and ex vivo eye irritation and corrosion test methods for regulatory use, these methods have not been widely adopted for testing agrochemical formulations due to a lack of concordance with parallel results from the rabbit eye test. The inherent variability of the rabbit test, differences in the anatomy of the rabbit and human eyes, and differences in modeling exposures in rabbit eyes relative to human eyes contribute to this lack of concordance. Because the regulatory purpose for these tests is protection of human health, there is a need for a testing approach based on human biology. A paper coauthored by EPA and NICEATM (Clippinger et al. 2021) reviews the available in vivo, in vitro, and ex vivo test methods with respect to their relevance to human ocular anatomy, anticipated exposure scenarios, and the mechanisms of eye irritation/corrosion in humans. Consideration of the mechanisms of eye irritation and the strengths and limitations of the in vivo, in vitro, and ex vivo test methods show that the in vitro and ex vivo methods are as or more reflective of human biology and less variable than the currently used rabbit approach. The paper suggests approaches to further optimize the most promising methods to distinguish between severe (corrosive), moderate, mild, and non-irritants and provide information about the reversibility of effects. It also considers the utility of including additional information such as physicochemical properties in a hazard assessment, consistent with accepted guidance (OECD 2019) on integrated approaches to testing and assessment (IATA) for potential eye irritation.

Selecting a minimal set of androgen receptor assays for screening chemicals

Tags: EPA, NIEHS, endocrine disruptors, AOP, IATA

Screening certain environmental chemicals for their ability to interact with endocrine targets, including the androgen receptor, is an important global concern. EPA and NICEATM previously developed a model using a battery of 11 in vitro androgen receptor assays to predict in vivo androgen receptor activity (Kleinstreuer et al. 2017). Follow-up work completed in 2020 (Judson et al. 2020) revised the modeling approach to incorporate data

from newly available assays and demonstrate that subsets of assays can provide close to the same level of predictivity. These subset models were evaluated against the full 11-assay model using 1,820 chemicals, as well as in vitro and in vivo reference chemicals from the literature. Agonist batteries of as few as six assays and antagonist batteries of as few as five assays can yield balanced accuracies of 95% or better relative to the full model. Balanced accuracy of the subset batteries for predicting reference chemicals was 100%. The paper also outlines an approach for researchers to develop their own subset batteries to accurately detect androgen receptor activity using assays that map to the pathway of key molecular and cellular events involved in chemical-mediated androgen receptor activation and transcriptional activity. This work indicates that in vitro bioactivity and in silico predictions mapping to the androgen receptor pathway could be used in an IATA for identifying chemicals that interact directly with the mammalian androgen receptors.

An evaluation framework for NAMs for human health safety assessment

Tags: FDA, NIEHS

Increased interest in using NAMs for safety assessment has resulted in an explosion of initiatives by numerous organizations. For the most part, these have been carried out independently and are not coordinated in any meaningful way. To help remedy this situation, a multistakeholder group of industry, academic, and regulatory experts including FDA and NIEHS scientists developed a framework that presents a consistent set of criteria, universal across initiatives, to evaluate a NAM's fit-for-purpose (Parish et al. 2020). This framework will provide a structure to collect relevant, confidence-building information that will accelerate, facilitate, and encourage development of new NAMs for use within the appropriate regulatory contexts. In addition, this framework provides a systematic approach to evaluate currently available NAMs and determine their suitability for potential regulatory application. This three-step evaluation framework, along with the demonstrated application with case studies, will help build confidence in the scientific understanding of these methods and their value for chemical assessment and regulatory decision-making.

Agencies partner with NASA to provide support for MPS research

Tags: FDA, NCI, NIH, MPS

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

FDA pilot program to support novel approaches to drug development

Tags: FDA, MPS

In November 2020, FDA announced establishment of the Innovative Science and Technology Approaches for New Drugs pilot program. This program is designed to encourage innovation of drug development tools that are out of scope for existing qualification programs but may still be useful for drug development. Drug development tools are methods, materials, or measures that have the potential to facilitate drug development. Approaches that could be considered under the pilot program include using MPS to assess safety or efficacy questions; developing novel nonclinical pharmacology or toxicology assays; or using artificial intelligence-based algorithms to evaluate patients, develop novel endpoints, or inform study design.

Optimization and validation of an in vitro botulinum neurotoxin assay

Tags: DOI, NIEHS, biologics, ecotoxicity, neurotoxicity

Tests to detect and measure botulinum neurotoxin are required by multiple federal agencies for a variety of purposes, such as detecting toxin in possibly contaminated food or wildlife. Currently, the standard test for these endpoints is a mouse lethality assay that can use large numbers of animals. NIEHS supported the optimization and validation of enzyme-linked immunosorbent assays (ELISAs) that replace animal-based methods for diagnosing suspected avian botulism samples. Methods were developed for determining the presence or absence of botulinum neurotoxin serotypes C-D (chimeric toxin) and E in field-collected samples from a wide range of bird species. There was good correlation between botulinum intoxication diagnoses and the ELISAs in the optimization phases, but occurrences of false

positives and false negatives in specific scenarios were identified as opportunities for improvement. To address these potentially confounding factors, the USGS National Wildlife Health Center (DOI) is accumulating data and setting sample quality standards. Progress on testing these samples in the ELISA assay has been hampered over the last 2 years due to the pandemic but will progress in 2022 as conditions permit.

Application of defined approaches to evaluating skin sensitization potential of agrochemicals

Tags: NIEHS, skin sensitization, IATA, mixtures toxicity

Skin sensitization testing is a regulatory requirement for safety evaluations of pesticides in multiple countries. Globally harmonized test guidelines that include in chemico and in vitro methods reduce animal use, but no single assay is recommended as a complete replacement for animal tests. Defined approaches that integrate data from multiple non-animal methods are internationally accepted, specifically via OECD Guideline 497. However, these defined approaches were evaluated with mono-constituent substances, which may limit their applicability to multi-constituent substances such as pesticides. An analysis by NIEHS scientists and collaborators evaluated rule-based defined approaches for hazard and/or potency categorization of skin sensitization for agrochemical formulations. The data set for the analysis included 27 formulations, each tested using the direct peptide reactivity assay, the KeratinoSens™ assay, and the human cell line activation test. The KeratinoSens assay had the highest performance for predicting in vivo hazard outcomes and performed better than any of the defined approaches. These results were presented in a poster (Strickland et al.) at the 2021 annual meeting of the American Society for Cellular and Computational Toxicology. The analysis demonstrates that non-animal test methods have utility for evaluating the skin sensitization potential of agrochemical formulations. Further investigation will be required to determine whether defined approaches can outperform individual assays for predicting in vivo sensitization hazard of pesticide formulations in general.

In vitro strategy for screening environmental chemicals and mixtures

Tags: DOI, ecotoxicity, mixtures toxicity

USGS (DOI) is collaborating with McGill University and Environment and Climate Change Canada to evaluate and validate the use of an avian embryo early life stage test in

conjunction with transcriptomic assessment using the avian EcoToxChip (Farhat et al. 2019) for screening environmental chemicals and mixtures. The early life stage test is a more biologically realistic alternative to cell-based in vitro testing methods because metabolism and multi-organ effects can be studied. Other advantages of the early life stage protocol are its low cost and rapidity, its reduced animal usage, and its ability to be applied to non-model or wildlife species to improve cross-species toxicity assessments. The avian EcoToxChip, developed initially for Japanese quail, is a targeted quantitative polymerase chain reaction array of about 360 genes. It includes genes representing about 20 biological pathways of regulatory relevance, including immune and endocrine systems and xenobiotic metabolism. In tandem with its partnered data evaluation tool, this focused transcriptomic approach will permit more rapid and less expensive characterization, prioritization, and management of environmental chemicals, reducing animal use.

Use of cause-and-effect analysis to optimize reliability of in vitro inhalation toxicity assays

Tags: CPSC, NIST, inhalation toxicity

Human-relevant in vitro inhalation toxicology methods are increasingly being used to replace animal testing for research and regulatory purposes. However, these methods use a variety of biological test systems, exposure platforms and conditions, substances tested, and endpoints. These differences represent a major challenge for use of the methods in regulatory testing. Additionally, there is a need to systematically account for variability and maximize the reliability of these methods, especially methods that use cells cultured at an air–liquid interface. One tool that has been used to evaluate the robustness of in vitro test methods is cause-and-effect analysis, a conceptual approach to analyze key sources of potential variability in a test method. Identified sources of variability can then be evaluated using robustness testing and potentially incorporated into in-process control measurements in the assay protocol. A paper by CPSC and NIST scientists and collaborators (Petersen et al. 2021) describes how cause-and-effect analysis can be applied using a modular approach, based on the idea that shared components of different test methods have similar sources of variability even though other components may differ. Cause-and-effect analyses of different in vitro inhalation methods revealed a common set of recommended exposure systems and biological

in-process control measurements. This approach, when applied in conjunction with Good Laboratory Practice criteria, should help improve the inter- and intralaboratory agreement of in vitro inhalation test results, leading to increased confidence in these methods for regulatory and research purposes.

Quantitative analysis of in vitro assay data to rank mutagenic potency of cigarette products

Tags: FDA, carcinogenicity, inhalation toxicity, mixtures toxicity, genotoxicity

Short-term in vitro genotoxicity assays are useful tools to assess whether new and emerging tobacco products potentially have reduced toxicity. Scientists at the FDA Center for Tobacco Products and the FDA National Center for Toxicological Research used quantitative analysis of data from two types of in vitro tests to rank mutagenic potency of cigarette whole-smoke solutions.

Meng et al. (2021) used the bacterial reverse mutation (Ames) test to analyze whole-smoke solution samples generated from two commercial cigarette brands under different smoking machine regimens. Benchmark dose modeling analysis was used to rank the mutagenic potency of the products. The quantitative approaches resulted in a similar rank order of mutagenic potency for the Ames test for both frameshift mutations and base-pair substitution. Under the conditions of this study, these results indicate that quantitative analysis of the Ames test data can discriminate between the mutagenic potencies of whole-smoke solution samples on the basis of smoking machine regimen and differences in smoke chemistry.

Mittelstaedt et al. (2021) evaluated dose-response modeling of in vitro micronucleus test data to determine if this test can discriminate among different tobacco products. Micronucleus responses were generated in mouse lymphoma and human lymphoblastoid cells from a series of whole-smoke solutions expected to have different levels of genotoxicity based on differences in their machine-generated smoke constituents. Eight whole-smoke solutions were prepared by machine-smoking different numbers of two commercial cigarettes under two different smoking machine regimens and tested in the two cell lines with and without rat liver S9 activation. The S9-mediated dose-response data were evaluated with PROAST software (developed by the Netherlands National Institute for Public Health and the Environment) and benchmark doses and upper and lower confidence intervals generated. The

response differed based on the number and type of cigarettes smoked and smoking machine regimen. Responses produced in mouse lymphoma cells generally were greater than in the human lymphoblastoid cells, but the ability of the two cell types to differentiate between whole-smoke solutions was similar. The results indicate that benchmark dose potency ranking is useful for differentiating between in vitro micronucleus test responses.

Chemical and in vitro bioactivity analysis of cigarillos

Tags: FDA, carcinogenicity, inhalation toxicity, mixtures toxicity, genotoxicity

There has been limited toxicity testing of cigarillos, including comparisons to cigarettes. A study by scientists at the FDA Center for Tobacco Products and collaborators (Crosby et al. 2021) compared the smoke chemistry and the cytotoxic and genotoxic potential of 10 conventional cigarettes and 10 cigarillos based on the greatest market share. Tobacco-specific nitrosamines, carbonyls, and polycyclic aromatic hydrocarbons were measured using gas chromatography-mass spectrometry. Bioactivity of total particulate matter smoke extracts was evaluated using several in vitro assays. Cytotoxicity was assessed in continuously cultured human bronchial epithelial cell lines using the neutral red uptake assay. Genotoxic potential was assessed using the micronucleus (continuously cultured human lung adenocarcinoma cell line), Ames, and thymidine kinase assays. The study found that the tested U.S.-marketed cigarillos have greater tobacco constituent levels, cytotoxicity, and genotoxicity than the compared cigarettes. These findings are important for understanding the human health toxicity from the use of cigarillos relative to cigarettes and for building upon knowledge regarding harm from cigarillos to inform risk mitigation strategies.

Evaluation of farnesoid X receptor (FXR)-active chemicals identified from Tox21 screening

Tags: NIEHS, Tox21

One of the primary goals of Tox21 is using in vitro HTS assays to prioritize toxicity evaluations for large numbers of chemicals in commercial use for which little or no toxicity data are available. Chemicals that can disrupt nuclear receptor signaling are a particular area of interest for Tox21 because such activity can have profound biological impacts. The farnesoid X receptor (FXR) is a nuclear receptor that can affect bile acid homeostasis, glucose metabolism, lipid homeostasis and hepatic regeneration. NIEHS scientists and

collaborators evaluated FXR agonists and antagonists identified through Tox21 screening using in vitro cell-based assays, in silico modeling approaches, and in vivo assessments using a fish model. These studies demonstrated the molecular complexity of FXR–ligand interactions and confirmed the ability of diverse ligands to modulate FXR, facilitate differential coregulator recruitment, and activate or repress receptor-mediated transcription. A paper describing these studies will be published in 2022.

Optimization and prequalification of a splenocyte-based assay for potency testing of vaccines

Tags: NIEHS, biologics

Potency assays for vaccines are often limited to animal-based methods in which animals are immunized, challenged with the disease vector, and evaluated for symptoms or mortality. NIEHS has supported the evaluation of a novel relative potency assay that measures immunological response in vitro. This method uses splenocytes from vaccine-immunized animals to determine the ability of increasing doses of a vaccine to elicit a reactivation response in vitro. Therefore, this method has potential to both reduce and refine animal use for vaccine potency testing. The test method developer has also established a cryopreservation procedure to allow long-term storage of splenocytes. The goals of this study are to (1) determine whether a splenocyte-based assay can reliably determine the relative potency of a test sample, and (2) demonstrate that cryopreserved cells can be used in the proposed potency method.

Use of fish embryo toxicity tests for prioritizing testing of environmental contaminants

Tags: DOI, cardiotoxicity, developmental toxicity, ecotoxicity

As part of ongoing assessments of wildlife health, DOI is investigating potential cardiovascular effects on fish from pesticides and pharmaceuticals frequently detected in surface waters and fish tissues. The USGS Columbia Environmental Research Center conducts high-content screening of compounds to formulate hypotheses and prioritize compounds for further toxicity testing. This approach reduces animal use, test compound needed, and waste by utilizing pre-feeding fish embryos in a microtiter plate format. This approach is also being used to better characterize toxicity of polycyclic aromatic

hydrocarbons (PAHs) and oxygenated PAHs from a subsurface oil spill by assaying groundwater samples from different trophic levels. These assays can provide evidence to justify larger-scale studies to determine actual risk versus perceived risk of contaminants.

The USGS Center's current high-content screening assay is a developmental cardiotoxicity assay that assesses total body length, pericardial area, intersegmental vessel area, circulation, and heart rate after a 72-hour exposure to a test substance. This array of endpoints allows for a targeted assessment of toxicity. In addition to an LC50 estimate, the assay provides a rapid approach to inform on mode of action, allowing formation of hypotheses on sublethal impacts of contaminants. Data derived from these studies on acute toxicity and mode of action for pesticides, pharmaceuticals, PAHs, and oxygenated PAHs will support a better understanding of potential effects on wildlife species.

Alternative approaches to evaluating bioactivity in surface waters

Tags: DOI, carcinogenicity, ecotoxicity, endocrine disruptors, genotoxicity

Chemical contaminants are introduced to environmental waters via many sources, and many of these contaminants have the potential to adversely affect organisms living in these waters. Recognized adverse effects include the induction of cancer via genotoxic mechanisms, endocrine disruption via the derailment of normal hormone signaling pathways, and outright toxicity leading to disease or death. Over the past decade, USGS scientists (DOI) have established or adapted water collection, extraction, and in vitro screening assays to evaluate the bioactivity of surface water samples. These approaches circumvent the need to utilize vertebrates and minimize endpoint variability in bioactivity measures. Data from these assays are incorporated into predictive modeling analyses to identify land uses associated with predicted biological disruption. They are also applied to responsibly inform site selection for comprehensive environmental sampling. Notably, it has been found that these approaches are better predictors of the impact of various land use scenarios on wildlife health than sentinel vertebrates sampled at the same locations. During 2020-2021, these assays were applied to augment USGS and other collaborator data sets collected from environmental surface and well waters collected in the eastern and midwestern United States.

Integrated selection and screening process for potential fish toxicants

Tags: DOI, acute toxicity, ecotoxicity, QSAR

Fisheries managers use pesticides, specifically referred to as management chemicals, to control invasive and undesirable fish species. Effective management chemicals should show heightened toxicity to target invasive or undesirable species while posing a minimal hazard to native species. The USGS Upper Midwest Environmental Science Center (DOI) has developed a two-phase screening process to minimize the use of animal testing during the development of new management chemicals. Although in vivo testing continues to be utilized in the development of new management chemicals, the screening process minimizes the number of animal toxicity studies necessary during research and development.

In the first phase, an in silico analysis is conducted using ecotoxicity QSAR modeling that incorporates species sensitivity distributions and the EPA tool Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS). The ecotoxicity modeling determines species-specific responses to chemical exposures using existing toxicity data and chemical physicochemical properties. These in silico assessment methods can be used to prioritize candidate compounds and estimate cytotoxicity. Models utilize more than 3,000 endpoints from over 600 taxa that are reported for more than 9,000 chemicals, and a publicly available interactive model prototype is in development.

In the second phase, promising chemical candidates identified by ecotoxicity modeling are tested using fish cell line cytotoxicity assays to examine target versus non-target species sensitivity. Cell lines developed from multiple tissue types (e.g., gill tissue) were established at the U.S. Fish and Wildlife Service Midwest Fishery Center; species represented include bluegill, fathead minnow, lake sturgeon, paddlefish, gizzard shad, silver carp, bighead carp, grass carp, and trout. Multiple species-specific chemical candidates have been identified and screened through cytotoxicity assays, with only the most promising candidates evaluated using in vivo toxicity studies.

Activities of the Developmental Neurotoxicity Health Effects Innovation Program

Tags: NIEHS, developmental toxicity, neurotoxicity

Exposure to environmental chemicals is a contributing factor to the significant increase in prevalence of neurodevelopmental disorders in children. However, there is limited developmental neurotoxicity information on chemicals in use due to challenges and limitations with current in vivo guidelines. To address these challenges, an in vitro battery of

promising NAMs was developed through an international collaboration (Crofton and Mundy 2021). An OECD guidance document with specific IATA case studies is being developed that informs on the application and interpretation of the testing battery, and will be published in 2022.

The Developmental Neurotoxicity Health Effects Innovation Program (DNT HEI) is a program within NIEHS DNTP that aims to generate and analyze screening-level information on chemicals using the in vitro battery. DNTP developed a unified data analysis pipeline to combine data from the individual assays in the in vitro battery (Behl et al 2019). In 2021, a set of 115 chemicals (Phase 1 chemicals) was selected and distributed for testing in the in vitro battery. Data have been received from most of the assay developers and are currently being analyzed by the DNT HEI, with results to be published in early 2023. A second set of chemicals has been selected by the DNT HEI based on nominations from various stakeholders.

In addition, DNTP led the development of a case study focused on organophosphorus flame retardants for use of a developmental neurotoxicity IATA to prioritize chemicals within a class of compounds. The case study will be included in the final OECD guidance document described above.

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, ATSDR, EPA, NIEHS, antibodies, mixtures toxicity

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

- The sixth of these webinars, presented on Jan. 21, 2020, was titled Use of Animal-free Affinity Reagents. In this webinar, Alejandra Solache, Ph.D., of Abcam presented an overview of production, use, and characteristics of monoclonal and polyclonal antibodies. Rebecca Clewell, Ph.D., of 21st Century Tox Consulting summarized a recent review by a working group of the Scientific Advisory Committee of the European Union Reference Laboratory for Alternatives to Animal Testing on the scientific validity and benefits of animal-free technologies to produce affinity reagents.
- The Jan. 26, 2021, Communities of Practice webinar focused on Non-animal Approaches for Mixtures Assessment. In this event, three ICCVAM agency scientists described approaches being used to assess human risk of toxicity from mixtures. Cynthia Rider, Ph.D., NIEHS, discussed assessing safety of botanicals. Patricia Ruiz, Ph.D., Agency for Toxic Substances and Disease Registry (ATSDR), described computational approaches to predict toxicity of mixtures. Kristin Isaacs, Ph.D., EPA, presented a data mining approach that uses purchasing data to estimate exposure to consumer product ingredients.

ICCVAM Public Forums

Tags: ICCVAM

Traditionally held in person, the ICCVAM public forum meetings in 2020 and 2021 were held virtually because of restrictions on public gatherings due to COVID-19. However, both meetings provided opportunities for public interaction with representatives from ICCVAM member agencies.

- View materials from the 2020 Public Forum
- View materials from the 2021 Public Forum

ICCVAM held its seventh public forum on May 21, 2020, which was viewed by about 400 individuals. Representatives of ICCVAM member agencies described work both to advance new approaches to safety testing of chemicals and medical products and to reduce the amount of testing required. Commenters at the forum commended the agencies' progress and noted the importance of federal agencies taking leadership roles in reducing animal testing. In

addition to government agencies, viewers represented pharmaceutical and chemical companies, animal welfare organizations, and test method developers.

The 2021 public forum was held on May 27 and attracted nearly 200 viewers. The program featured 13 presentations from nine of the 17 ICCVAM member agencies. Representatives described efforts to reduce animal use within their agencies and by their stakeholders, as well as research activities that advance new non-animal approaches. Stakeholders expressed particular interest in activities that advance alternatives to animal use in the context of regulatory requirements. Such activities were outlined by CPSC and two EPA offices. A request to the public for comments to be presented during the meeting produced a robust response, with nine commenters presenting during the meeting and an additional four written comments received.

ICCVAM advisory committee meetings

Tags: ICCVAM, antibodies, ecotoxicity

The Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) is a federally chartered advisory group that advises NICEATM, ICCVAM, and the Director of NIEHS about ICCVAM activities. Traditionally held in person, the 2020 and 2021 public meetings of SACATM were held virtually because of restrictions on gatherings due to COVID-19.

More than 200 people viewed or participated in the Sept. 2-3, 2020, SACATM meeting, making it one of the most broadly viewed SACATM meetings ever. Committee members praised advances in use of computational methods to reduce animal use for chemical safety testing. They suggested resources and approaches that could be developed to further advance these tools and identified testing areas where they could be applied. Committee members also encouraged ICCVAM member agencies to increase efforts to advance non-animal alternatives for identifying substances that could cause cancer or other chronic toxicity outcomes, and to promote strategies for reducing animal use for vaccine testing and antibody production.

About 160 people viewed the Sept. 28-29, 2021, SACATM meeting. This event focused on detailed discussion of two topics of current interest to ICCVAM: reducing or replacing

animal use for ecotoxicity testing and new approaches to validation of new testing methods. The committee urged ICCVAM member agencies to support greater communication among regulators, regulated industry, and test method developers. ICCVAM members also were encouraged to engage groups focused on environmental justice and climate change.

ICCVAM agency-sponsored workshops and webinars

Tags: EPA, FDA, NIEHS, NIH, acute toxicity, antibodies, biologics, carcinogenicity, COVID-19, eye irritation, IATA, inhalation toxicity, MPS, nanomaterials, neurotoxicity, skin sensitization

ICCVAM agencies convened workshops and webinars during 2020 and 2021, summarized in the table below, to foster collaboration and provide information about alternative testing methods. All meetings took place online.

Meeting Date	**Sponsoring Agency (and partner organization)**	**Meeting Title (with link to page if available)**	**Meeting Summary**
April 17, 2020	NIEHS	NIEHS SBIR/STTR Town Hall: Development of New Approach Methodologies to Reduce Animal Use in Toxicity Testing	This online town hall meeting was presented to facilitate improved communication between method developers and end-users, providing an opportunity for assay developers to hear from ICCVAM stakeholders on the desired characteristics and requirements for NAMs.
May 14, 2020	FDA	Artificial Intelligence for Regulatory Science Research	This presentation summarized current thinking and ongoing efforts at the FDA's National Center for Toxicological Research in artificial intelligence, with examples from drug and food safety, natural language processing of regulatory documents, and biomarker discovery and development. The presentation also discussed guiding principles and best practices for applying artificial intelligence in regulatory science research.

Meeting Date	**Sponsoring Agency (and partner organization)**	**Meeting Title (with link to page if available)**	**Meeting Summary**
Jan. 22, 2020-Nov. 3, 2021	EPA (PETA Science Consortium International eV., Physicians Committee for Responsible Medicine)	Webinar Series on the Use of New Approach Methodologies (NAMs) in Risk Assessment	These webinars discussed computational models for acute toxicity and carcinogenicity prediction, and skin sensitization and eye irritation testing. A September 2020 event presented a proposed NAM nomination form for Toxic Substances Control Act consideration. The series will continue in 2022.
Ongoing; initiated August 2020	FDA	Alternative Methods Webinar Series	This FDA internal webinar series, organized by the FDA Alternative Methods Working Group, provides test method developers with the opportunity to introduce their new technology to FDA and give individual FDA programs the option to contact them for further information.
Feb. 19, 2020-Dec. 1, 2021	FDA (Society of Toxicology)	Colloquia on Emerging Toxicological Science: Challenges in Food and Ingredient Safety	Since 2014, the Society of Toxicology and the FDA Center for Food Safety and Applied Nutrition have presented colloquia on high-quality, cutting-edge, future-oriented toxicological science topics. Many of the colloquia focus on alternatives to animal use. The series is continuing into 2022.
July 23-Nov. 12, 2020	NIEHS (PETA Science Consortium International eV., EURL ECVAM)	Antibody Webinar Series	Attendees learned about the applications and benefits of animal-free recombinant antibodies.
Sep. 15-17, 2020	EPA	Use of NAMs to Derive Extrapolation Factors and Evaluate Developmental Neurotoxicity for Human Health Risk Assessment	This public meeting of the Federal Insecticide, Fungicide, and Rodenticide Scientific Advisory Panel considered and reviewed the use of NAMs to derive extrapolation factors and evaluate developmental

Meeting Date	Sponsoring Agency (and partner organization)	Meeting Title (with link to page if available)	Meeting Summary
			neurotoxicity for human health risk assessment.
Sep. 30, 2020	EPA, NIEHS (Health and Environmental Sciences Institute)	Opportunities and Challenges in Using the Kinetically Derived Maximum Dose Concept to Refine Risk Assessment	This symposium highlighted issues commonly raised in application of the kinetically derived maximum dose for design and interpretation of animal toxicity studies, and provided the background information needed to develop more consistent, transparent approaches to support broader application in risk assessment.
Oct. 19-20, 2020	EPA	Conference on the State of Science on Development and Use of NAMs for Chemical Safety Testing	This was the second in a series of EPA conferences to highlight the state of the science on the development and use of NAMs for chemical safety testing. The next conference will be held in 2022.
May 19-June 2, 2021	EPA (PETA Science Consortium International eV., Unilever, Syngenta)	Inhalation Toxicity Testing	These three webinars focused on using in silico and in vitro approaches for next generation risk assessment of potential respiratory toxicants.
May 26-27, 2021	FDA	FDA Science Forum	This event allowed the public to view scientific research and collaborative efforts of FDA scientists. Several topic areas focused on the application of big data and predictive toxicology to inform FDA decision-making and drive innovation.
June 24-Sep. 10, 2021	NIEHS	Environmental Health Language Collaborative Webinars	These interactive events helped set the stage, begin discussions, and draft plans for how to make progress on the Collaborative's goals.
Sep. 9, 2021	NIH	Bioinformatics and Computational Biology Symposium	The program for this event included a keynote presentation on "Big data for

Meeting Date	Sponsoring Agency (and partner organization)	Meeting Title (with link to page if available)	Meeting Summary
			health and disease" and presentations on DNA structure, proteomic tumor analysis, data integration to study COVID-19, single-cell sequencing, and genomics.
Sep. 21-Dec. 8, 2021	NIEHS (Swiss Centre for Applied Human Toxicology, Swiss State Secretariat for Economic Affairs)	Current Concepts in Quantitative Risk Assessment for Skin Sensitization	This webinar series provides an overview of the current state of the science in this rapidly developing field. The webinars will continue in 2022 and are intended as background for a planned workshop on quantitative risk assessment of skin sensitizing pesticides.
Oct. 25, 2021	EPA (Johns Hopkins University Center for Alternatives to Animal Testing)	Challenges and Opportunities for Overcoming Dog Use in Agrochemical Evaluation and Registration	At this virtual workshop, presenters reviewed the role the 90-day dog study has played in the regulation of agrochemicals in both the U.S. and Europe during the past 20 years and what approaches may be employed to substantially reduce its use.

Animal Welfare Information Center resources

Tags: USDA, metrics, ecotoxicity

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

AWIC provides information to the toxicology community on the NAL website related to the development and use of alternatives to toxicity testing, as well as information and guidelines on mandatory and regulatory testing set by various agencies. These web pages highlight peer-

reviewed publications and other online resources that discuss ways to replace, reduce, and refine animal use in toxicity testing. For example, AWIC provides links and selected citations (updated in 2021) on 3Rs testing methods used in ecotoxicity testing.

During 2020 and 2021, 256,208 people visited the AWIC website. These visits generated a total of 512,253 page views. AWIC also provides information on animal use alternatives through outlets such as presentations and posters, workshops, webinars, and conference exhibits. Through these outlets, AWIC presented to over 3,590 participants at 67 different outreach events in 2020 and 2021, collectively. At these outreach events, AWIC described best practices for searching and finding current literature on methods to reduce, refine, or replace animal use in testing and experimentation.

FDA reports on progress in advancing alternatives

Tags: FDA, metrics, Tox21

In January 2021, FDA published "Advancing New Alternative Methodologies at FDA," which provided updates on activities to advance development of alternatives to animal use and their application to regulatory decision-making. The report was developed by the FDA Alternative Methods Working Group to highlight the progress made by FDA scientists in laying the groundwork to integrate alternatives to animal testing into FDA regulatory programs. The report described activities within eight FDA offices and centers, as well as FDA working groups and interagency partnerships such as ICCVAM and Tox21. The report also summarized FDA engagement to advance alternatives to animal testing in international venues such as OECD and the International Cooperation on Cosmetic Regulation.

New agency webpages summarizing alternative methods activities

Tags: EPA, FDA, metrics

During 2020 and 2021, several ICCVAM agencies published new webpages summarizing activities to advance alternative methods.

- In May 2020, FDA published "Advancing Alternative Methods at FDA," which highlights activities of its Alternative Methods Working Group. The working group was established to facilitate interactions with global regulatory bodies interested in implementing alternative methods in toxicology. Additionally, the group examines

opportunities and viable ways by which emerging methods and new technologies can support regulatory review of risk, safety, and efficacy of FDA-regulated products. Resources available from the page include links to relevant reports, publications, and FDA webpages and contact information for the working group.

- In October 2020, EPA published "Strategic Vision for Adopting New Approach Methodologies," which describes activities, partnerships, and goals of several EPA offices to reduce animal testing. Resources available from this page include links to EPA webpages that provide more details on activities within the EPA Offices of Pesticide Programs, Pollution Prevention and Toxics, Science and Policy Coordination, and Research & Development.
  A key resource within the EPA website is the webpage, "Adopting 21st Century Science Methodologies – Metrics." This page includes data compiled by the Hazard and Science Policy Council, the Chemistry and Acute Toxicology Science Advisory Council, and other groups about how EPA activities and policies have reduced the number of animals used in testing and saved EPA and stakeholder resources.

Computational Tools Applications

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

CATMoS and additivity approaches to predict toxicity of mixtures

Tags: DoD, acute toxicity, oral toxicity, mixtures toxicity

The majority of existing toxicity data and tools to assess toxicity are for single compounds. However, humans are exposed to complex chemical mixtures in a variety of scenarios. Of particular interest to the U.S. Air Force are exposures to chemical mixtures experienced by airmen. Scientists at the U.S. Air Force Research Laboratory have developed a computational approach to help assess the acute oral toxicity of mixtures by using an additivity formula that estimates toxicity of a mixture based on the acute toxicity estimate of individual ingredients. Acute toxicity estimates for single chemicals were collected from existing databases or

calculated using CATMoS (Chushak et al. 2021). The proposed approach was implemented in a standalone Python-based app that allows users to rapidly assess the acute toxicity for mixtures related to U.S. Air Force needs and to identify chemicals that can impact physiological performance of airmen.

Analysis of the performance of the GHS Mixtures Equation to predict acute oral toxicity of formulations

Tags: EPA, NIEHS, acute toxicity, oral toxicity, mixtures toxicity

The majority of pesticide registration applications require product-specific acute toxicity data. Thus, an alternative to in vivo testing for this purpose could greatly reduce animal testing. However, predicting toxicity using NAMs is challenging for mixtures such as pesticides. The GHS Mixtures Equation estimates the acute toxicity of mixtures using the toxicities of mixture components. EPA and NIEHS scientists conducted a study (Hamm et al. 2021) to evaluate the concordance of hazard classifications predicted using the GHS Mixtures Equation with classifications based on in vivo test results. The results of the analysis suggested that the GHS Mixtures Equation can help predict the acute oral toxicity of mixtures, particularly those with lower toxicity.

Use of IVIVE and data from a stem cell-based assay to predict developmental toxicity potential

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

To support implementation of NAMs for regulatory decision-making on developmental toxicity, FDA and NIEHS scientists and collaborators evaluated the performance of the devTOX quickPredict assay for predicting lowest effect levels in rat developmental toxicity studies. Studies conducted during 2020 and 2021 focused on developmental toxicity potential of valproic acid and analogues (Chang et al. 2022) and 186 chemicals from the Tox21 program. Developmental toxicity potential (dTP) concentrations from the devTOX quickPredict assay were used as inputs to IVIVE analyses to estimate equivalent administered doses that would result in the maximum plasma concentrations equivalent to dTP concentrations. Results suggested that the devTOX quickPredict assay can quantitatively predict developmental toxicity potential at concentrations relevant to human exposure and in

some cases may provide a more conservative hazard estimate than animal studies for use in risk assessment.

In silico screening approaches for assessing cardiovascular safety

Tags: NIEHS, cardiotoxicity, endocrine disruptors, machine learning

Mounting evidence supports the contribution of environmental chemicals to cardiovascular disease burden. NIEHS scientists evaluated chemicals in the Tox21 chemical library for cardiotoxicity potential by focusing on HTS assays whose targets are associated with adverse events related to cardiovascular failure modes (Krishna et al. 2021). The objective of the evaluation was to develop new hypotheses around environmental chemicals of potential interest for adverse cardiovascular outcomes using Tox21 and ToxCast HTS data. Bioactivity signatures relevant to cardiotoxicity were identified for 40 targets measured in 314 assays and used to prioritize 1,138 Tox21 chemicals. The approach identified drugs with known cardiotoxic effects in a variety of use classes including estrogenic modulators, anti-arrhythmic drugs, and antipsychotic drugs like chlorpromazine. Several classes of environmental chemicals such as organotins, bisphenol-like chemicals, pesticides, and quaternary ammonium compounds demonstrated strong bioactivity against cardiovascular targets. Screening outcomes were added to existing data from literature studies using cultured heart cells, animals, or human epidemiological approaches to prioritize these chemicals for further testing.

A number of chemicals identified in the initial Tox21 screen were found to inhibit potassium channels important to cardiac rhythm regulation (Krishna et al. 2022). A set of molecular descriptors was applied to characterize these chemicals. Machine learning approaches were then applied to build robust statistical models that can predict the probability of any new chemical to cause cardiotoxicity via this mechanism.

Application of CATMoS to predict acute mammalian toxicity for pesticide hazard and risk assessment

Tags: EPA, NIEHS, acute toxicity, oral toxicity

CATMoS is a set of predictive in silico models of acute oral systemic toxicity potential. The consensus model predictions are fully reproducible and demonstrate equivalent performance to in vivo data. This offers an opportunity for a potential replacement to animal testing for

applications such as regulatory evaluations of pesticide toxicity. EPA and NIEHS scientists are evaluating CATMoS predictions for 178 chemicals in comparison to rat LD50 tests from publicly available ecological risk assessments registered from 1998 to 2020. Findings from this study will help in understanding the applicability of CATMoS estimates as a potential replacement of the rat acute single oral dose study for establishing the effects endpoint in ecological risk assessments.

Utilization

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

Assessments of Agency Needs and Practices

The 2018 ICCVAM Strategic Roadmap identified the definition of agency information needs as a key step in its implementation. ICCVAM member agencies are collaborating both within ICCVAM workgroups and independently to define agency information needs and make those needs known to stakeholders. Areas considered in 2020 and 2021 included carcinogenicity, liver and kidney toxicity, ecotoxicity, and nanomaterials toxicity.

FDA/CDER perspective on use of new approach methodologies (NAMs) in drug development

Tags: FDA

Nonclinical testing of human pharmaceuticals is conducted to assess the safety of compounds to be studied in human clinical trials and for marketing of new drugs. Recent advances in science have led to the emergence of numerous NAMs for nonclinical testing that are currently being used in various aspects of drug development. A paper by scientists within the FDA Center for Drug Evaluation and Research (CDER) and the FDA National Center for Toxicological Research (Avila et al. 2020) discusses CDER's view on the opportunities and challenges of using NAMs in drug development, especially for regulatory purposes. The paper includes examples where NAMs are currently being used in nonclinical safety assessments and where they may supplement or enhance current testing methods.

In silico approaches in carcinogenicity hazard assessment: Current status and future needs

Tags: FDA, NIEHS, carcinogenicity

Historically, identifying carcinogens has relied primarily on tumor studies in rodents, which require enormous resources in both money and time. In silico models have been developed for predicting rodent carcinogens but have not yet found general regulatory acceptance, in

part due to the lack of a generally accepted protocol for performing such an assessment as well as limitations in predictive performance and scope. There remains a need for additional, improved in silico carcinogenicity models, especially ones that are more human-relevant, for use in research and regulatory decision-making. As part of an international effort to develop in silico toxicological protocols, FDA and NIEHS scientists participated in a consortium of toxicologists, computational scientists, and regulatory scientists across several industries and governmental agencies to evaluate the extent to which in silico models exist for each of the recently defined 10 key characteristics of carcinogens. A position paper developed by the group (Tice et al. 2021) summarizes the current status of in silico tools for the assessment of each key characteristic and identifies the data gaps that need to be addressed before a comprehensive in silico carcinogenicity protocol can be developed for regulatory use.

In silico approaches in organ toxicity hazard assessment: Current status and future needs in predicting liver toxicity

Tags: ATSDR, FDA, NIEHS, hepatotoxicity

Hepatotoxicity is one of the most frequently observed adverse effects resulting from exposure to a xenobiotic, and one of the major reasons for drug withdrawals, clinical failures, and discontinuation of drug candidates. Faster and cheaper methods to assess hepatotoxicity that are both more sustainable and more informative are critically needed. A review (Bassan et al. 2021) coauthored by ATSDR, FDA, and NIEHS scientists summarizes biological mechanisms and processes underpinning hepatotoxicity and describes experimental approaches to support the prediction of hepatotoxicity, including toxicokinetic considerations. The paper describes the increasingly important role of in silico approaches and highlights challenges to the adoption of these methods, including the lack of a commonly agreed upon protocol for performing such an assessment and the need for in silico solutions that take dose into consideration. A proposed framework for the integration of in silico and experimental information is provided, along with a case study describing how computational methods have been used to successfully respond to a regulatory question concerning non-genotoxic impurities in chemically synthesized pharmaceuticals.

In silico approaches in organ toxicity hazard assessment: Current status and future needs for predicting heart, kidney, and lung toxicities

Tags: ATSDR, FDA, NIEHS, hepatotoxicity, cardiotoxicity, inhalation toxicity

The kidneys, heart, and lungs are vital organ systems evaluated as part of acute or chronic toxicity assessments. New methodologies are being developed to predict adverse effects based on in vitro and in silico approaches. A review coauthored by ATSDR, FDA, and NIEHS scientists considered the current state of the art in predicting these organ toxicities (Bassan et al. 2021). The review outlines the biological basis, processes, and endpoints for kidney toxicity, pulmonary toxicity, respiratory irritation and sensitization, and functional and structural cardiac toxicities. The review also covers current experimental approaches, including off-target panels from secondary pharmacology batteries. Current in silico approaches for prediction of these effects and mechanisms are described as well as obstacles to the use of in silico methods. Ultimately, a commonly accepted protocol for performing in silico assessments would be a valuable resource to expand the use of such approaches across different regulatory and industrial applications. The review identifies factors impeding the widespread deployment of in silico approaches, including lack of a comprehensive understanding of the mechanisms of toxicity, limited in vitro testing approaches, limited in vivo databases suitable for modeling, a limited understanding of how to incorporate ADME considerations into the overall process, a lack of in silico models designed to predict a safe dose, and a need for an accepted framework for organizing the key characteristics of these organ toxicants.

Assessment of U.S. federal agency ecotoxicity information and testing needs

Tags: ICCVAM, DoD, DOI, EPA, FDA, NIEHS, NIST, USDA, ecotoxicity

U.S. regulatory and research agencies use ecotoxicity test data to assess the hazards and risks associated with substances that may be released into the environment, including but not limited to industrial chemicals, pharmaceuticals, pesticides, food additives, and cosmetics. These data are used to conduct hazard assessments and evaluate potential risks to non-target animals and plants. To identify opportunities for regulatory uses of non-animal replacements for ecotoxicity tests, the needs and uses for these types of test data must first be clarified. The ICCVAM Ecotoxicology Workgroup prepared a review (Ceger et al. 2022) that identifies the ecotoxicity test data relied upon by U.S. federal agencies. The review describes the standards, test guidelines, guidance documents, and endpoints that are used to address each of the responding agencies' regulatory and research needs regarding ecotoxicity testing in the

context of their application to decision-making. This information will be useful for coordinating efforts to develop and implement alternative test methods to reduce, refine, or replace animal use in chemical safety evaluations.

U.S. federal agency interests and key considerations for new approach methodologies for nanomaterials

Tags: ICCVAM, CPSC, DoD, EPA, FDA, NIEHS, NIOSH, NIST, nanomaterials

Engineered nanomaterials (ENMs) come in a wide array of shapes, sizes, surface coatings, and compositions, and often possess novel or enhanced properties compared to larger-sized particles of the same elemental composition. To ensure the safe commercialization of products containing ENMs, it is important to thoroughly understand their potential risks. Given that ENMs can be created in an almost infinite number of variations, it is not feasible to conduct in vivo testing on each type of ENM. Instead, NAMs such as in vitro or in chemico test methods may be needed, given their capacity for high-throughput testing, lower cost, and ability to provide information on toxicological mechanisms. However, the different behaviors of ENMs compared to dissolved chemicals may challenge safety testing of these substances using NAMs. The ICCVAM Nanomaterials Workgroup queried ICCVAM member agencies about what types of ENMs are of agency interest and whether there is agency-specific guidance for ENMs toxicity testing (Petersen et al. 2021). To support the ability of NAMs to provide robust results in ENM testing, two key issues in the usage of NAMs, namely dosimetry and interference/bias controls, are thoroughly discussed.

Applying IVIVE to facilitate the use of in vitro toxicity data in risk assessment and decision-making

Tags: ICCVAM, ATSDR, CPSC, DoD, EPA, FDA, NIEHS, NIST, NLM, IVIVE

In vitro toxicity assays are being applied to transform toxicology from an observational to a predictive science, improve throughput, and reduce costs. The qualitative linkage between in vitro and in vivo toxicity endpoints can be strengthened via application of IVIVE, which relates an in vitro concentration associated with bioactivity to an external exposure level. In some contexts, applications of IVIVE have advanced past the exploratory research stage and are beginning to gain acceptance for risk assessment of chemicals. The ICCVAM IVIVE Workgroup requested information from ICCVAM member agencies regarding the extent and

context of their use of IVIVE. Surveyed agencies were also asked about programmatic needs, data gaps, and agency-specific guidance documents or publications related to IVIVE, as well as for information about modeling tools or software they had used or may use for facilitating IVIVE analysis and decision-making. This information was compiled into a review summarizing the workgroup's findings, current challenges, and future needs (Chang et al. 2022). The review also proposes operational definitions for IVIVE, presents literature examples for several common toxicity endpoints, and highlights implications of IVIVE use in decision-making processes.

Request for information on efficacy testing of ectoparasiticide products

Tags: NIEHS

In a December 2021 Federal Register notice (86 FR 72251), NICEATM requested available data and information on approaches and/or technologies currently used for efficacy testing of ectoparasiticide products, such as products to prevent flea and tick infestations on dogs and cats. Submitted information will be used to assess the state of the science and determine technical needs for non-animal test methods used to evaluate the efficacy of ectoparasiticides on dogs and cats and to facilitate their incorporation into a testing strategy for regulatory purposes.

Initiatives to Replace or Reduce Animal Use

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

EPA New Approach Methods Work Plan

Tags: EPA, metrics

In June 2020, EPA released its New Approach Methods Work Plan, which was created in response to the EPA Administrator's 2019 directive to prioritize activities that will reduce the use of animal testing while continuing to protect human health and the environment. The work plan was developed by experts across the agency to set the objectives and strategies for

using new approach methods to meet the ambitious goals set out in the Administrator's directive.

An update of the work plan was released in December 2021. The updated work plan addresses recommendations made by the ICCVAM Metrics Workgroup in its report, "Measuring U.S. Federal Agency Progress Toward Implementation of Alternative Methods in Toxicity Testing." In its updated work plan, EPA commits to reporting summary metrics on reducing vertebrate animal testing and use annually on its website beginning in the fourth quarter of 2022.

EPA guidance for waiving of toxicity tests on animal skin

Tags: EPA, acute toxicity, metrics

In December 2020, EPA published final guidance that will allow researchers to forego testing chemicals for acute systemic toxicity on animal skin in certain circumstances. The "Final Guidance for Waiving Acute Dermal Toxicity Tests for Pesticide Technical Chemicals and Supporting Retrospective Analysis" allows for data waivers for acute dermal systemic toxicity studies for single active ingredients used to develop end-use products other than fumigants and rodenticides. The document includes a policy statement to waive all acute lethality dermal studies for single active ingredients in many cases.

This guidance supports EPA efforts to identify opportunities for test waivers that can reduce animal use for testing required for pesticide registration. It was based on a retrospective analysis conducted by EPA and collaborators, which concluded that such studies provide little to no added value in regulatory decision-making when an acute oral toxicity study has been conducted. This guidance is expected to save up to 750 test animals annually from unnecessary testing, as well as EPA, industry, and laboratory resources.

Retrospective analysis of triple pack studies for dermal absorption

Tags: EPA, NIEHS, acute toxicity

Substances can cause acute systemic toxicity when absorbed through the skin. Dermal systemic toxicity is in turn driven by the ability of a substance to penetrate the skin. Dermal absorption can be estimated using the "triple pack," a study design that combines in vivo rat,

in vitro rat, and in vitro human data to calculate an estimated human dermal absorption factor.

To assess the feasibility of deriving a dermal absorption factor using only in vitro data, NICEATM and EPA conducted a retrospective evaluation of agrochemical formulations to compare the dermal absorption factors derived from each method (Allen et al. 2021). The dermal absorption factor derived from the human in vitro study was also compared to the dermal absorption factor generated from the triple pack approach. Absorption through in vitro human skin was found to be similar to or less than that observed in rat skin for all formulations. For most of the formulations evaluated:

- The in vitro rat method generated a similar or higher dermal absorption factor value than the in vivo method.
- The human in vitro method provided a similar or higher estimate of dermal absorption than the triple pack approach.

This analysis supports potentially using in vitro data alone for dermal absorption factor derivation for human health risk assessment of pesticides. While it is preferable to use data from human skin for human health risk assessments, human in vitro data are not always available. The analysis also demonstrated that estimates of dermal absorption based on in vitro rat data are at least as protective as in vivo rat data, and thus could also be considered adequate for use in establishing dermal absorption factors.

Replacement of in vivo leptospirosis vaccine potency testing in the United States

Tags: NIEHS, USDA, biologics

A 2012 workshop co-organized by NIEHS, USDA, and partner organizations sought to develop an international strategy to replace the use of animals in tests required during the assessment of veterinary leptospirosis vaccines (Johnson et al. 2013). Workshop participants gave special attention to the barriers remaining to replace the in vivo vaccine potency test in hamsters. At the time of the workshop, in vitro methods were not widely used as replacements for the in vivo potency test, but advances in both technology and regulatory arenas have since increased their acceptance and use. NIEHS and USDA scientists prepared a review of recent progress (Rogers et al. 2022) to reduce the use of hamsters in leptospirosis

vaccine batch potency testing, as well as obstacles remaining to completely replace the use of hamsters with accepted in vitro methods.

EPA evaluation of avian toxicity tests for pesticide registration

Tags: EPA, acute toxicity, ecotoxicity, metrics, oral toxicity

In a February 2020 news release, EPA announced that it had finalized guidance to reduce testing of pesticides on birds when registering conventional outdoor pesticides. The guidance describes the results and implications of a retrospective study conducted by EPA and People for the Ethical Treatment of Animals (Hilton et al. 2019). The study explored the quantitative and qualitative contributions of risk assessment methods using single oral dose and subacute dietary toxicity endpoints to the overall conclusions of acute avian risk. The analysis indicated that, in most cases, the subacute dietary results had little impact on risk conclusions arrived upon by use of acute oral data alone. This finding is expected to reduce the number of animals tested by a total of 60 birds per test, for a total projected animal savings of over 700 animals per year based on a typical number of new chemicals registered.

Policies and Guidance for Implementation of Alternative Methods

To encourage adoption and use of NAMs, the 2018 ICCVAM Strategic Roadmap called on agencies to provide clear guidance on use and acceptance of data from these approaches. During 2020 and 2021, agencies issued guidance for use of NAMs in the areas of testing for skin sensitization, carcinogenicity, and developmental and reproductive toxicity, as well as for testing of medical devices, vaccines, cancer drugs, pesticides, and consumer products.

Measuring progress toward implementation of alternative methods in toxicity testing

Tags: ICCVAM, CPSC, DoD, DOI, DOT, EPA, FDA, NIEHS, NIH, USDA, metrics

In September 2019, the U.S. Government Accountability Office issued "Animal Use in Research: Federal Agencies Should Assess and Report on Their Efforts to Develop and Promote Alternatives." This report describes how the U.S. Department of Health and Human Services, USDA, and EPA ensure researchers consider the use of alternatives to animals and examines the steps the agencies have taken to facilitate the use of alternative research methods and to assess the effect of their efforts on animal use. The report recommended that

ICCVAM establish a workgroup to develop metrics that ICCVAM member agencies could use to assess progress made toward reducing, refining, or replacing animal use in testing. Furthermore, the report recommended that such metrics be incorporated into ICCVAM Biennial Progress Reports. In response, ICCVAM established its Metrics Workgroup in early 2020. The workgroup included members from nine ICCVAM agencies. Its charge was to determine how agencies can best address the Government Accountability Office report's recommendations within the context of the ICCVAM Authorization Act.

In March 2021, the ICCVAM Metrics Workgroup published "Measuring U.S. Federal Agency Progress Toward Implementation of Alternative Methods in Toxicity Testing," which describes its findings and recommendations. The workgroup's key finding was that no one set of metrics can be used by all ICCVAM member agencies to assess progress toward reducing, refining, or replacing animal use in testing. The workgroup instead recommended that each agency develop its own metrics that are relevant and practical to their unique situation. This document describes the recommendations of the ICCVAM Metrics Workgroup along with references and other materials that can be used to follow federal agency progress in promoting the use of alternative toxicological methods.

In response to the Metrics Workgroup's recommendations, ICCVAM agencies have developed webpages to inform their stakeholders about progress on adoption of alternatives and reduction of animal use. Links to those pages are available on the NTP website. Activities to assess progress toward reducing, refining, or replacing animal use in testing are also described throughout this report.

Characteristics to consider when selecting a positive control material for an in vitro assay

Tags: CPSC, NIEHS, NIST

The use of in vitro assays to inform decision-making requires robust and reproducible results across studies, laboratories, and time. Experiments using positive control materials are integral to demonstrating the extent to which a measurement system is performing as expected. A paper by CPSC, NIEHS, and NIST scientists and collaborators (Petersen et al. 2021) reviewed 10 characteristics that should be considered when selecting a positive control material for an in vitro assay. These include: (1) the biological mechanism of action, (2) ease

of preparation, (3) chemical purity, (4) verifiable physical properties, (5) stability, (6) ability to generate responses spanning the dynamic range of the assay, (7) technical or biological interference, (8) commercial availability, (9) user toxicity, and (10) disposability. The paper presented examples and a case study of the monocyte activation test to demonstrate the application of these characteristics for identification and selection of potential positive control materials. Because specific positive control materials are often written into testing standards for in vitro assays, selection of the positive control material based on these characteristics can aid in ensuring the long-term relevance and usability of these standards.

Draft guidance on carcinogenicity testing

Tags: FDA, carcinogenicity

In an October 2021 Federal Register notice (86 FR 54982), FDA announced draft guidance for industry, "S1B(R1) Addendum to S1B Testing for Carcinogenicity of Pharmaceuticals." The draft guidance expands the testing scheme for assessing human carcinogenic risk of small molecule pharmaceuticals. It introduces an integrative approach that provides specific weight-of-evidence criteria that inform whether a 2-year rat study adds value in completing a human carcinogenicity risk assessment. The draft guidance also adds a plasma exposure ratio-based approach for setting the high dose in the rasH2-Tg mouse model. FDA accepted comment on the draft guidance through December 2021.

Adoption of OECD guideline on defined approaches for skin sensitization testing

Tags: EPA, FDA, NIEHS, skin sensitization, ICCVAM

In June 2021, OECD issued Guideline 497, Defined Approaches on Skin Sensitisation. Drafted and sponsored by ICCVAM agency scientists and international partners, Guideline 497 is the first internationally harmonized guideline to describe a non-animal defined approach that can be used to replace an animal test to identify skin sensitizers. Validation against a curated set of human data indicates that this approach predicts human skin sensitization hazard better than the accepted animal test. NICEATM and ICCVAM are collaborating with stakeholder groups to convene events to raise awareness about the guideline and promote best practices for implementation of defined approaches to skin sensitization testing and assessment.

Draft risk assessment for chlorothalonil

Tags: EPA, inhalation toxicity

In a May 2021 Federal Register notice (86 FR 27593), EPA announced availability of draft human health and/or ecological risk assessments for several pesticides. EPA accepted public comment on the risk assessments through September 2021.

One of the pesticides covered by this announcement was the fungicide chlorothalonil, which was the subject of a case study to use an in vitro model to develop an inhalation risk assessment. The case study will be published in an OECD guidance document in 2022. Data from the in vitro model were used in conjunction with human dosimetry modeling to evaluate human inhalation risk, which is included in the document "Chlorothalonil: Revised Human Health Draft Risk Assessment for Registration Review." This and other documents relevant to the chlorothalonil evaluation are available in a docket on Regulations.gov.

Proposed guidance for industry for Federal Hazardous Substances Act testing

Tags: CPSC

In a March 2021 Federal Register notice (86 FR 16704), CPSC requested comment on its "Proposed Guidance for Industry and Test Method Developers: CPSC Staff Evaluation of Alternative Test Methods and Integrated Testing Approaches and Data Generated from Such Methods to Support Federal Hazardous Substances Act (FHSA) Labeling Requirements." CPSC has developed this guidance, building on its Animal Testing Policy, to assist stakeholders in determining what test methods are deemed reliable for determining compliance with the labeling requirements under the Federal Hazardous Substances Act. This includes clarification of CPSC informational requirements and a process for evaluating NAMs and IATA. CPSC accepted comments on the draft guidance through June 2021 and will release the final guidance in 2022.

EPA list of NAMs for TSCA information requirements

Tags: EPA, acute toxicity, carcinogenicity

A 2016 update of the Toxic Substances Control Act (TSCA) required EPA to issue a list of methods and approaches that do not use vertebrate animals (i.e., NAMs) to develop new data or information required under TSCA. In February 2021, EPA updated the list of NAMs that

the agency will consider for the purpose of satisfying information requirements under TSCA. Some updates reflected changes made to OECD test guidelines, while other updates considered guidance on acute systemic toxicity testing waivers issued by the EPA Office of Pesticide Programs and availability of a new expert system to predict carcinogenicity of organic chemicals, fibers, metals, and polymers.

The NAMs list is an element of EPA's 2018 "Strategic Plan to Promote the Development and Implementation of Alternative Test Methods Within the TSCA Program." Information about the NAMs list and the Strategic Plan is available on the EPA website.

Draft risk assessment for isothiazolinones

Tags: ICCVAM, EPA, NIEHS, skin sensitization

In May 2020, EPA requested comment (85 FR 28944) on draft human health and ecological risk assessments for a group of antimicrobial chemicals known as isothiazolinones. EPA accepted comments on the draft risk assessments through November 2020.

Isothiazolinones are used in a variety of products including plastics, household cleaners, and laundry detergents. They frequently cause skin sensitization. EPA requested comment on the use of an in vitro and artificial neural network-based defined approach instead of using laboratory animal data to evaluate skin sensitization risks for products containing isothiazolinones. This is the first consideration of this type of defined approach in regulatory risk assessment.

The draft risk assessments rely heavily on work done by NIEHS and ICCVAM. The in vitro testing was conducted by the Toxicology Branch of DNTP. NICEATM analyzed the in vitro data and ran the artificial neural network-based defined approach to provide quantitative potency predictions used to determine PODs. The ICCVAM Skin Sensitization Expert Group reviewed the DNTP testing report and the NICEATM analyses before data were provided to EPA for development of the risk assessments.

Guidance on nonclinical evaluation of immunotoxic potential

Tags: FDA, skin sensitization, biologics

In February 2020, FDA issued draft guidance on "Nonclinical Safety Evaluation of the Immunotoxic Potential of Drugs and Biologics." This guidance supplements previously

issued recommendations on nonclinical evaluations of immunotoxic potential and is intended to assist sponsors in such evaluations. The guidance includes several specific recommendations on assessing potential for dermal sensitization:

- FDA no longer recommends that sponsors conduct the murine local lymph node assay to assess the sensitization potential of topical drug products due to the limitations of the assay.
- As an alternative screen for skin sensitization for individual chemicals, FDA will consider a battery of in silico, in chemico, and in vitro studies that have been shown to adequately predict human skin sensitization with an accuracy similar to existing in vivo methods.

Guidance on in vitro drug interaction studies

Tags: FDA

In January 2020, FDA published "In Vitro Drug Interaction Studies – Cytochrome P450 Enzyme- and Transporter-mediated Drug Interactions Guidance for Industry." This guidance is intended to help drug developers plan and evaluate studies to determine the drug-drug interaction potential of an investigational drug product. It focuses on in vitro approaches to evaluate the interaction potential between investigational drugs with cytochrome P450 enzymes and transporters, as well as how in vitro results can inform future clinical drug-drug interaction studies. The appendices of this guidance include factors to consider when choosing in vitro experimental systems, key issues regarding in vitro experimental conditions, and more detailed explanations regarding model-based drug-drug interaction prediction strategies.

Guidance on reproductive and developmental toxicity studies for human pharmaceuticals

Tags: FDA, developmental toxicity

In May 2021, FDA published "S5(R3) Detection of Reproductive and Developmental Toxicity for Human Pharmaceuticals - Guidance for Industry" This guidance is intended to help drug developers plan and evaluate nonclinical developmental and reproductive toxicity studies necessary to support human clinical trials and marketing authorization for

pharmaceuticals. The 2021 revisions to this guidance describe testing strategies utilizing alternative assays for the assessment of malformations and embryofetal lethality. The guidance also provides basic principles that will assist in the development, qualification, and potential regulatory use of alternative assays for evaluating adverse effects on embryofetal development.

Amendment of Hazardous Materials Regulations to consider in vitro methods for classification of corrosives

Tags: DOT

In August 2021, the Pipeline and Hazardous Materials Safety Administration (PHMSA) of the U.S. Department of Transportation published a Notice of Proposed Rulemaking (86 FR 43844) to amend certain sections of the Hazardous Materials Regulations (49 CFR parts 171 to 180). The purpose of the amendments is to maintain alignment with international regulations and standards relevant to the transportation of hazardous materials. The proposed amendments build on international collaborations of PHMSA with the United Nations to update the classification criteria for Class 8 (corrosive) substances to include in vitro non-animal test methods. The rulemaking incorporates the 2016 version of OECD Test No. 431, In Vitro Skin Corrosion: Reconstructed Human Epidermis (RHE) Test Method, used to assign a packing group to Class 8 materials. The rulemaking also provides an opportunity to assign certain Class 8 materials to a more conservative packing group in lieu of additional in vivo testing, reducing the need for animal testing to transport corrosive materials safely. The public comment period for the proposed rulemaking ended in October 2021 and the final rule is now being reviewed.

Leadership

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

Resources for Alternative Methods Development

To support developers of new testing approaches, ICCVAM is drafting a new document describing criteria and processes for validation and regulatory acceptance of toxicological testing methods. During 2020 and 2021, 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 models to advance COVID-19 research. They also supported development of platforms such as iPSCs and in vitro organoids, computational approaches, and methods to incorporate genetic diversity into NAMs.

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

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

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

In 2021, ICCVAM established a Validation Workgroup to consider this issue. The workgroup, which has members from nine ICCVAM agencies, is applying the advances of the last two decades to a new document describing criteria and processes for validation and regulatory acceptance of toxicological testing methods. In addition to supporting

development of flexible validation practices that consider context of use, the new document will address the need to align validation approaches in a manner that encourages international harmonization and incorporates best practices for quality and quality systems development. The document will draw upon recent well-established validation publications to ensure alignment of approaches.

As of December 2021, drafting of the new document was in progress, and it is envisioned that it will be made available for public comment sometime in 2022.

Small business grants to support alternative methods development

Tags: NIEHS, Tox21, machine learning

Throughout 2020 and 2021, NIEHS provided funding for small businesses developing technologies of interest to the Tox21 program. The funding was offered as part of the 2020 and 2021 Omnibus Solicitations of the National Institutes of Health (NIH), Centers for Disease Control, and FDA for small business grant applications to support development and commercialization of innovative technologies. Technologies supported by NIEHS included improved or expanded testing methods for toxicity screening, computational approaches for predictive toxicology, and other technologies such as alternative or improved methods for fixing and preserving tissues.

In addition to funding offered via the Omnibus Solicitations, NIEHS offers grants to support development of specific types of technologies targeting specific endpoints. Funding offered in 2020 and 2021 supported development of:

- Chemical testing resources and approaches that reflect the genetic diversity among human populations, including panels of human or rodent cells or cell lines, lower organism strains with well-characterized genetic backgrounds, or in silico approaches to enhance the ability to characterize the effects of genetic variation in toxicity testing (December 2019, submissions open through February 2020).
- Application of artificial intelligence and machine learning for advancing environmental health sciences (December 2020)

Funding to develop machine learning tools

Tags: DOE, machine learning

In March 2021, DOE announced availability of $29 million in grants to develop new tools to analyze massive amounts of scientific information. The grants were intended to support development of tools that use technologies such as artificial intelligence, machine learning, and advanced algorithms. They focused on two specific areas, data-intensive scientific machine learning and analysis to help detect patterns in data, and randomized algorithms for extreme-scale science to make large data sets easier to understand.

Grants to develop in vitro models of SARS-CoV-2 infection and treatment

Tags: FDA, NIH, COVID-19, MPS, biologics

In April 2020, NCATS issued two Notices of Special Interest to support research in collecting and examining data on the risks and outcomes for COVID-19 infections using MPS. Such work is expected to advance the translation of research findings into diagnostics, therapeutics, and vaccines. Applications for administrative supplements and competitive revisions for existing grants to advance COVID-19 research were accepted through January 2022.

In a separate effort announced in September 2020, FDA awarded over $5 million to a global collaboration to inform the development of treatments and vaccines for coronavirus infections. The collaboration, led by the University of Liverpool, will sequence and analyze samples from humans and animals to create profiles of various coronaviruses, including SARS-CoV-2, which causes COVID-19. As part of this three-year project, investigators will evaluate how in vitro models of coronavirus infection, including human organs-on-chips, compare to in vivo responses in animal models and humans. Ultimately, these in vitro models may be employed in the development of biologics, drugs, or devices to treat COVID-19.

Grants to support development of PBPK models

Tags: FDA, IVIVE

In January 2021, FDA CDER announced availability of grants to support development of PBPK models for bioequivalence studies for new generic drugs. CDER requested that proposals include scientifically justified IVIVE strategies, discuss how the models will address population variability, and describe a plan for how models would be verified and validated. The intent was to award a total of $600,000 over 2 years to one or two awardees.

Challenge to develop ecotoxicity gene expression assay

Tags: DoD, EPA, ecotoxicity

In March 2020, EPA announced a partnership with DoD, international governments, and industry to sponsor the EcoTox TARGET innovation challenge. The challenge would award $300,000 to the applicant who successfully developed a low-cost high-throughput technology for measuring global gene expression in samples from four common aquatic toxicity test organisms. The winner will be announced in spring 2022.

International Interactions

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

Contributions to OECD activities

Tags: ICCVAM, IATA, skin sensitization, eye irritation, endocrine disruptors, developmental toxicity, neurotoxicity

ICCVAM member agencies participate in the development and review of chemical testing guidelines issued by the OECD Test Guidelines Programme. OECD test guidelines are used by government, industry, and independent laboratories of the 38 OECD member countries to assess chemical safety. The U.S. National Coordinators for the OECD Test Guidelines Programme, who are members of ICCVAM, solicit and collate U.S. comments on draft test guidelines and other documents of the Test Guidelines Programme. The National Coordinators represent the United States at the annual meeting of the Working Group of National Coordinators and in other test guideline development activities. One or more ICCVAM subject matter experts may join the U.S. National Coordinators at this meeting. In 2020 and 2021, ICCVAM agencies commented on draft OECD documents through the U.S. National Coordinators. ICCVAM members and/or NICEATM staff also supported the Test Guidelines Programme during 2020 and 2021 by:

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

- Serving on an expert group developing a guideline for defined approaches for skin sensitization. This group supported the development of Guideline 497, Defined Approaches for Skin Sensitisation, issued in 2021. Guideline 497 is the first internationally harmonized guideline to describe a non-animal defined approach that can be used to replace an animal test to identify skin sensitizers. NICEATM and ICCVAM scientists are currently contributing to two proposals to update Guideline 497 to include new information sources for existing defined approaches, and new defined approaches for quantitative risk assessment.
- Participating in a group developing a Case Study on the Use of an IATA for Identifying Androgen Receptor Active Chemicals, which has been submitted to the OECD Working Party on Hazard Assessment.
- Contributing to a retrospective review of available data and information to support an OECD test guideline for a human reconstructed epidermis model for phototoxicity testing. Test Guideline 498, In Vitro Phototoxicity - Reconstructed Human Epidermis Phototoxicity Test Method, was adopted in June 2021.
- Serving on the Validation Management Group – Non-animal, which focuses on evaluation of new methods for identifying endocrine disruptors.
- Serving on an expert group considering test batteries for developmental neurotoxicity.

Participation in the International Cooperation on Alternative Test Methods

Tags: ICCVAM, skin sensitization, IATA, antibodies, IVIVE, eye irritation, acute toxicity

The International Cooperation on Alternative Test Methods (ICATM) was created to foster dialogue among national validation organizations. This dialogue facilitates international cooperation in the critical areas of validation studies, independent peer review, and development of harmonized recommendations. ICATM includes member organizations from the European Union, United States, Japan, Canada, and South Korea. Brazil and China have been participating in ICATM since 2015 as observers.

ICATM Participant Organizations

- **ICCVAM** is an interagency committee of the U.S. government that coordinates technical reviews of alternative test methods and cross-agency activities relating to validation, acceptance, and harmonization of test methods. NICEATM administers ICCVAM and provides scientific support for its activities.
- **EURL ECVAM** (European Union Reference Laboratory for Alternatives to Animal Testing) is a unit within the Institute of Health and Consumer Protection in the European Union's Joint Research Centre. EURL ECVAM coordinates the validation of alternative test methods in the European Union.
- **JaCVAM** (Japanese Center for the Validation of Alternative Methods) coordinates the evaluation of alternative test methods for the Japanese National Institute of Health Sciences, its parent organization.
- **Health Canada's** Environmental Health Science and Research Bureau coordinates the evaluation of alternative test methods in Canada. The Canadian Centre for Alternatives to Animal Methods and its subsidiary, the Canadian Centre for the Validation of Alternative Methods, participate as partners with Health Canada in ICATM activities.
- **KoCVAM** (Korean Center for the Validation of Alternative Methods) is part of the National Institute of Food and Drug Safety Evaluation of the South Korean Food and Drug Administration.
- **BraCVAM** (Brazilian Center for the Validation of Alternative Methods) functions as the focal point within Brazil to identify or receive requests for test method validation. It works to implement appropriate validation studies with the National Network of Alternative Methods to the Use of Animals within the Brazilian Ministry of Science, Technology, Innovations, and Communications.

An ICATM coordination meeting was hosted virtually by JaCVAM in October 2020. Representatives from the United States, European Union, Canada, Japan, South Korea, and Taiwan presented updates on recent activities. Discussion topics included.

- The concept of standards and approaches to validation that could replace multi-laboratory studies.

- Activities to address European recommendations on use of non-animal derived antibodies.
- Development of detailed review papers and IATA.
- Human-relevant approaches to assessing the eye irritation potential of agrochemical formulations.

In 2020, EURL ECVAM issued recommendations on use of non-animal derived antibodies. The EURL ECVAM recommendations were based on a scientific advisory committee review that found that:

- Animal-free technologies are able to produce affinity reagents with equal or better quality than that offered by antibodies produced using the conventional animal-based methods.
- Use of animal-free affinity reagents provides scientific benefits.

Use of animal-free affinity reagents was discussed at the 2020 ICCVAM Communities of Practice webinar. A webinar series co-organized by NICEATM, EURL ECVAM, and the PETA Science Consortium International e.V. and presented in July-November 2020 explored international activities in this area in more detail.

ICATM partner representatives from the United States, Japan, and the European Union presented updates at the October 2021 "International Symposium on Alternatives to Animal Testing in Taiwan."

ICCVAM member agency scientists serve on management teams or peer review panels for test method validation studies conducted by ICATM partners. Since 2018, a NICEATM scientist has been serving on the management team for a validation study led by JaCVAM. The validation management team has been evaluating the sensitivity and specificity of luciferase-based assays for the detection of relevant cytokines as individual components of the multi-immunotox assay. The multi-immunotox assay is intended to be used as a comprehensive screening assay to identify potentially immunotoxic chemicals by assaying the activity of T cells and dendritic cells.

The following ICCVAM workgroups had ICATM member liaison representatives during 2020 and 2021.

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

Participation on ICH

Tags: FDA, carcinogenicity, developmental toxicity

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

FDA solicited comment in October 2021 on a draft addendum to ICH guidance on carcinogenicity testing and published guidance aligned with ICH documents on evaluation of developmental and reproductive toxicity as discussed elsewhere in this report.

Accelerating the Pace of Chemical Risk Assessment

Tags: EPA

The advent of NAMs for generating safety information on chemicals provides an opportunity to assess what chemical risk assessment should look like in the 21st century. EPA played a leadership role in Accelerating the Pace of Chemical Risk Assessment (Kavlock et al. 2018),

an international activity to bring together regulators from agencies such as the European Chemicals Agency and Health Canada to discuss progress in applying new tools to prioritization, screening, and application to quantitative risk assessment of differing levels of complexity. During 2020 and 2021, the collaboration developed case studies on the development and applications of NAMs for chemical risk assessment, one of which was described in a paper published by EPA scientists and collaborators (Paul Friedman et al. 2020). These collaborations examined how NAMs might transform regulatory evaluation of chemicals and help overcome barriers to acceptance by increasing confidence in the use and acceptance of NAMs in regulatory chemical risk assessment.

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

Tags: FDA, NIEHS, biologics

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

FDA and NIEHS are supporting a project led by the World Health Organization and NC3Rs to carry out an independent and comprehensive review of animal use requirements within World Health Organization guidelines for biologics and to make recommendations for where non-animal approaches can be adopted. The project is currently in an information-gathering phase. In 2021, a survey of biologics manufacturers was conducted to better understand the opportunities and barriers to adoption of replacement, reduction, and refinement alternatives and use of non-animal approaches in quality control, batch, and lot release testing of biologics. A similar survey of national regulatory authorities and national control laboratories will be conducted in early 2022. Data collected will be anonymized and published in an appropriate scientific peer-reviewed journal or as a report on the NC3Rs website, and/or presented at scientific meetings or webinars.

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.

Activities of the Tri-Services Toxicology Consortium

Tags: DoD, VA ORD, inhalation toxicity, skin sensitization, QSAR, mixtures toxicity

The mission of the Tri-Services Toxicology Consortium is to communicate, coordinate, and optimize toxicology needs throughout DoD by providing a central point of contact to DoD toxicology resources. The Consortium currently includes over 200 individuals from 11 organizations within DoD, seven of which have laboratories. Given the shared missions, individuals from the Veterans Administration and Department of Homeland Security are also invited to participate.

The Consortium convened four meetings during 2020 and 2021. These meetings began with brief presentations of new work including efforts in toxicity of novel compounds in various in vitro and in vivo systems, improving in silico predictions of molecular targets, characterization of toxicity using evidence integration techniques, advances in PBPK modeling and development, use of NAMs within phased approaches to toxicity data collection and hazard assessment, and new developments in understanding the mechanism for toxicity for chemicals of military interest. Significant recent efforts included:

- Substance-specific technical reviews and publications concerning chromium, lead, trichloroethylene, and selected PFAS.
- Identification of toxicity and biomarkers of exposure to diesel exhaust using a circulated human multi-tissue organoid platform.
- Investigations into biomarkers of pulmonary toxicants.
- Evaluation of fluorine-free aqueous-film forming foam formulations.
- Use of in silico methods, including QSAR models, to predict skin sensitization, to address mixtures, and to derive metabolic parameters from in vitro data.
- Continued DoD technical representation on relevant government and scientific committees.

Botanical Safety Consortium

Tags: FDA, NIEHS, cardiotoxicity, mixtures toxicity

In 2019, FDA, NIEHS, and the non-profit Health and Environmental Sciences Institute signed a memorandum of understanding establishing the Botanical Safety Consortium. The Consortium includes over 20 participants from industry, academia, and government to promote scientific advances in evaluating the safety of botanical ingredients and mixtures in dietary supplements. This group will look at novel ways to use cutting-edge toxicology tools, including alternatives to animal testing, to promote the goals of safety and effectiveness.

The Botanical Safety Consortium held virtual annual meetings in 2020 and 2021. The 2020 meeting provided a forum to articulate the needs for a scientific approach to assessing botanicals safety and refine the goals of the Consortium. Presenters at the 2021 meeting described a project to characterize cardiotoxicity of botanicals and provided overviews of applications of in silico modeling and NAMs to predict toxicity of botanicals.

FDA Alternative Methods Working Group

Tags: FDA

In 2019, FDA chartered an Alternative Methods Working Group with representatives from all of FDA. The goals of this working group are to:

- Strengthen FDA's long commitment to promoting the development and use of new technologies and to reduce animal testing.
- Discuss new alternative in vitro/in silico/in vivo methods across FDA.
- Interact with federal government partners and other global stakeholders to facilitate discussion and development of draft performance criteria for such assays.

During 2020 and 2021, the working group coordinated an internal webinar series on alternative methods that provided test method developers the opportunity to present their new methods to FDA scientists.

The 2021 FDA report, Advancing Regulatory Science at FDA: Focus Areas of Regulatory Science, outlined areas needing continued targeted investment in regulatory science research to foster the development of innovative products, provide data and methods to inform

regulatory decision-making, and improve guidance to sponsors. One of those priority areas was "Advancing Novel Technologies to Improve Predictivity of Non-clinical Studies and Replace, Reduce, and Refine Reliance on Animal Testing."

MPSCoRe Working Group

Tags: DoD, NIEHS, NIH, COVID-19, MPS

In January 2021, NICEATM, the National Institute of Allergy and Infectious Diseases, the U.S. Army Combat Capabilities Development Command Chemical Biological Center, and NCATS established the MPS for COVID Research (MPSCoRe) Working Group in collaboration with NC3Rs. The MPSCoRe Working Group coordinates the use of MPS to reduce animal use in studies of COVID-19 and future emerging infectious diseases. Members include researchers, MPS model developers, therapeutic/vaccine manufacturers, and international regulators. A July 2021 feature article in Drug Discovery Today (Kleinstreuer and Holmes 2021) reviews MPSCoRe Working Group activities in the context of the broader effort to apply MPS to the development of therapies for COVID-19.

Activities of the MPSCoRe Working Group have included:

- An organizational meeting in January 2021.
- A workshop in April 2021 that featured presentations on the themes of testing therapeutics and understanding disease mechanisms.
- A tutorial in May 2021 on how to use the University of Pittsburgh Microphysiological Systems Database to find and share data on COVID-19 projects.
- A series of four webinars from June-September 2021 in which working group members described applications of MPS technologies to understanding the mechanisms of COVID-19 disease and assessing the safety and efficacy of potential novel therapeutics.
- A meeting with representatives of the World Health Organization in December 2021 to discuss research needs relevant to the biology and treatment of the Omicron SARS-CoV-2 variant and the potential for MPS to provide human-relevant platforms for rapidly addressing those needs.

The Tox21 compound library: collaborative chemistry advancing toxicology

Tags: EPA, FDA, NIEHS, NIH, Tox21, metrics

Since 2009, the Tox21 project has screened about 8,500 chemicals in more than 70 high-throughput assays, generating upward of 100 million data points, with all data publicly available. Underpinning this public effort is the largest compound library ever constructed specifically for improving understanding of the chemical basis of toxicity across research and regulatory domains. The different programmatic objectives of the Tox21 partners led to three distinct, overlapping compound libraries that, when combined, not only covered a diversity of chemical structures, use categories, and properties but also incorporated many types of compound replicates. A 2021 publication (Richard et al. 2021) describes the history of development of the Tox21 "10K" chemical library and data workflows implemented to ensure quality chemical annotations and allow for various reproducibility assessments. The paper presents cheminformatics profiles that demonstrate how the three partner libraries complement one another to expand the reach of each individual library, as reflected in coverage of regulatory lists, predicted toxicity endpoints, and physicochemical properties.

National Academies Panel on variability and relevance of mammalian toxicity tests

Tags: EPA, NIEHS, NIST

ICCVAM members from NIEHS and NIST are serving on a committee convened by the National Academies of Sciences, Engineering, and Medicine for the consensus study, "Variability and Relevance of Current Laboratory Mammalian Toxicity Tests and Expectations for New Approach Methods (NAMs) for use in Human Health Risk Assessment." This study was undertaken by the National Academies in response to a request from EPA. It will review the variability and relevance of existing mammalian toxicity tests, specifically in the context of human health risk assessment. The review will support the establishment of data-driven and science-based expectations for NAMs based on the variability and relevance of the traditional toxicity testing models.

The committee was established in 2021 and has held two public meetings. The first of these on September 23 included discussions with EPA staff regarding the statement of task, reasons for the committee's study, and description of the types of committee recommendations that would be most useful, as well as a brief public comment period. A

workshop on December 9 featured expert presentations addressing several questions relevant to the study:

- How are traditional toxicity studies used in informing chemical safety decisions?
- What do we know about the variability and concordance of traditional mammalian toxicity studies?
- What are the needs and expectations of different stakeholders?

Proceedings from this workshop will be published in 2022. The committee will hold additional public meetings in 2022 and expects to issue a report with recommendations late in the year.

Environmental Health Language Collaborative

Tags: NIEHS, EPA

NIEHS and EPA are fostering a community-driven initiative, the Environmental Health Language Collaborative, to advance integrative environmental health research by developing and promoting adoption of a harmonized language. This initiative will facilitate answering large-scale complex research questions that require integration of multiple disparate data sources by developing language standards for describing data and biomedical knowledge. Several webinar events were held during 2021 to help set the stage, begin discussions, and draft plans for how to make progress on the Collaborative's goals. The Collaborative welcomes diverse representation of expertise, needs, and scientific interests to make this a successful and sustainable community, and those interested are invited to join its email distribution list.

FDA partnership to apply lung chips to safety evaluation of COVID-19 vaccines and therapies

Tags: FDA, COVID-19, MPS

In October 2020, FDA entered into a Cooperative Research and Development Agreement with Emulate, Inc. to enable multiple studies using Emulate's Organ-Chips across FDA offices in priority research areas. Organ-Chips are in vitro systems that recreate the natural physiology of specific human tissues and organs. Some projects will evaluate COVID-19 vaccines or investigate human immune response against SARS-CoV-2, the virus that causes

COVID-19. Under the agreement, FDA will use a range of Organ-Chips to study the safety, efficacy, and mechanisms of action of drugs regulated by the FDA. This new agreement follows the successful completion of the first Emulate Cooperative Research and Development Agreement with the FDA initiated in 2017 that focused on toxicity studies using Emulate's Liver-Chip.

U.S. Department of Veterans Affairs Office of Research and Development joins ICCVAM as new member agency

Tags: VA ORD, ICCVAM

In October 2020, the U.S. Department of Veterans Affairs Office of Research and Development (VA ORD) joined ICCVAM to become the 17th ICCVAM member agency. Joining ICCVAM will help VA ORD to coordinate efforts with other federal agencies in replacing, reducing, and refining animal use. It is part of an overall agency plan to reduce animal use while improving predictions of human health hazard.

Activities of the In Silico Carcinogenicity Protocol Workgroup

Tags: FDA, NIEHS, carcinogenicity

Scientists within the FDA Center for Devices and Radiological Health, FDA CDER, and NIEHS participate in an international interdisciplinary consortium to develop publicly available in silico protocols to support the hazard assessment of major toxicological endpoints. Within that consortium, the In Silico Carcinogenicity Protocol Workgroup has focused on developing an in silico protocol for the hazard assessment of carcinogens based on the 10 key characteristics of carcinogens (Smith et al. 2016). The workgroup has published an assessment (Tice et al. 2021) of how current in silico methods address each of the key characteristics and where additional methods or data resources need to be developed. The workgroup is currently developing case studies on four chemicals to determine whether data available for current in silico approaches would be sufficient to generate predictions of in vivo bioassay outcomes. In a related activity, members of the workgroup are compiling a review of receptor-mediated carcinogenesis that will examine the degree to which interaction with nuclear receptors is a key driver of chemically induced carcinogenesis, as opposed to a downstream effect.

Activities of the Carcinogenesis Health Effects Innovation Program

Tags: DoD, DOE, NIEHS, carcinogenicity

Approximately one in three people will be diagnosed with cancer at some point in their lifetime. Despite tremendous progress in screening and treatment methods, cancer is still the second most common cause of death in the United States and the leading health concern of the American public.

The Carcinogenicity Health Effects Innovation Program (CarciHEI) is a program within DNTP. The CarciHEI is developing innovative approaches to quickly and efficiently assess the cancer risk posed by environmental exposures to help identify and mitigate the factors contributing to these increases. The program's goal is to identify early molecular biomarkers of preneoplasia and neoplasia and develop in vitro screening assays that can predict a cancer hazard.

The CarciHEI is collaborating with the Murtha Cancer Center at Walter Reed National Military Medical Center and the DoD Serum Repository to identify cohorts of service members diagnosed with cancer believed to be related to environmental exposures. Through an interagency agreement with the DOE Pacific Northwest National Laboratory, the CarciHEI will conduct proteomic analysis of serum samples collected every 2 years from these service members with the hope of identifying biomarkers of preneoplastic and early neoplastic events that contribute to the subsequent development of cancer.

About ICCVAM and NICEATM

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

ICCVAM Establishment and Purpose

Tags: ICCVAM, NIEHS

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

Many currently accepted test methods use laboratory animals. Alternative test methods are methods that replace animal use with non-animal 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” has been adopted as a broadly descriptive reference to any technology, methodology, approach, or combination of these that can be used to provide information on chemical hazard and risk assessment and that support the 3Rs.

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

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

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

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

- Increase the efficiency and effectiveness of federal agency test method review.
- Eliminate unnecessary duplicative efforts and share experiences between federal regulatory agencies.
- Optimize utilization of scientific expertise outside the federal government.
- Ensure that new and revised test methods are validated to meet the needs of federal agencies.
- Reduce, refine, and replace the use of animals in testing, where feasible.

ICCVAM is a permanent interagency committee of NIEHS under the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). In October 2020, VA ORD joined ICCVAM to become the 17th ICCVAM member agency.

ICCVAM Member Agencies

Tags: ICCVAM

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

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

ICCVAM Duties and Activities

Tags: ICCVAM

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

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

ICCVAM Workgroups

Tags: ICCVAM, acute toxicity, ecotoxicity, IVIVE, metrics, nanomaterials

ICCVAM establishes ad hoc workgroups to perform specific tasks important for the development or validation of alternatives to animal testing. The workgroups are composed of

representatives from agencies that use or require data from the topic of interest. Representatives of partner organizations in the International Cooperation on Alternative Test Methods may also be invited to participate in a workgroup.

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

During 2020-2021, ICCVAM had active workgroups focused on the following areas:

- Acute toxicity: co-chairs Donald Cronce, Ph.D. (DoD) and Grace Patlewicz, Ph.D. (EPA)
- Consideration of alternative methods: co-chairs Jessie Carder (USDA) and Matthew Johnson, D.V.M., DACLAM (DoD)
- Ecotoxicology: co-chairs William Eckel, Ph.D. (EPA) and Natalia Garcia-Reyero Vinas, Ph.D. (DoD)
- In vitro to in vivo extrapolation: co-chairs Moiz Mumtaz, Ph.D. (ATSDR) and Cecilia Tan, Ph.D. (EPA)
- Metrics: transitioned to expert group status in April 2021; co-chairs Suzanne Fitzpatrick, Ph.D., D.A.B.T. (FDA) and Matthew Johnson, D.V.M., DACLAM (DoD)
- Nanomaterials: transitioned to expert group status in December 2021; chair Elijah Petersen, Ph.D. (NIST)
- Read across: chair Grace Patlewicz, Ph.D. (EPA)
- Validation: co-chairs Suzanne Fitzpatrick, Ph.D., D.A.B.T. (FDA), John Gordon, Ph.D. (CPSC), and Elijah Petersen, Ph.D. (NIST)

How NICEATM Supports ICCVAM

Tags: ICCVAM, NIEHS

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

In addition to providing support for ICCVAM, NICEATM:

- Supports NTP activities, especially those contributing to the U.S. government's interagency Tox21 initiative.
- Conducts analyses and evaluations, and coordinates independent validation studies on novel and high-priority alternative testing approaches.
- Provides information to test method developers, regulators, and regulated industry through its website and workshops on topics of interest.

NICEATM staff

NIEHS

Nicole Kleinstreuer, Ph.D., Acting Director
Helena Hogberg, Ph.D., Toxicologist
Kamel Mansouri, Ph.D., Computational Chemist
Matthew Stout, Ph.D., Toxicologist, Project Officer
Pei-Li Yao, Ph.D., Toxicologist

NICEATM Contract Staff (Integrated Laboratory Systems LLC, an Inotiv Company)

David Allen, Ph.D., Principal Investigator
Steven Morefield, M.D., Project Manager
Jaleh Abedini, M.S.
Todd Auman, Ph.D.
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Patricia Ceger, M.S., D.A.B.T.
Xiaoqing Chang, Ph.D., D.A.B.T.
Neepa Choksi, Ph.D.
Bethany Cook, M.S.
Amber Daniel, M.S.
Jon Hamm, Ph.D.
David Hines, Ph.D.

Agnes Karmaus, Ph.D.

Eric McAfee (subcontractor, Sciome LLC)

Oluwakemi Oyetade, M.S.

Jason Phillips (subcontractor, Sciome LLC)

John Rooney, Ph.D.

Catherine Sprankle, M.S.

Judy Strickland, Ph.D., D.A.B.T.

Kimberly To, Ph.D.

James Truax, M.A.

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 2020 and September 2021.

Roster of SACATM Members 2020-2021

Name	**Title**	**Company**	**Appointment End Year**
Antonio Baines, Ph.D. (ad hoc 2021)	Associate Professor, Department of Biological and Biomedical Sciences	North Carolina Central University, Durham, NC	N/A
Szczepan W. Baran, V.M.D., M.S.	Head of Emerging Technologies	Novartis Institute for Biomedical Research, Cambridge, MA	2024
Ellen Berg, Ph.D. (ad hoc 2021)	Chief Scientific Officer, Translational Biology	Eurofins Discovery, Burlingame, CA	N/A
Michael B. Bolger, Ph.D.	Chief Scientist	Simulations Plus, Inc., Lancaster, CA	2020

Joseph L. Charest, Ph.D.	Biomedical Solutions Program Manager	The Charles Stark Draper Laboratory, Inc., Cambridge, MA	2022
Amy Clippinger, Ph.D.	Director	PETA Science Consortium International e.V., Washington, DC	2022
Kelly P. Coleman, Ph.D., D.A.B.T., RAC	Distinguished Scientist and Technical Fellow	Medtronic PLC, Minneapolis, MN	2020
K. Nadira De Abrew, Ph.D. (Chair)	Senior Scientist (Toxicologist)	The Procter & Gamble Company, Cincinnati, OH	2022
Denis Fourches, Ph.D.	Computational Chemist	Oerth Bio, Raleigh, NC	2023
Sean C. Gehen, Ph.D., D.A.B.T.	Regulatory Sciences Team Leader	Corteva Agriscience, Indianapolis, IN	2022
Sue Leary, M.S.	President	Alternatives Research and Development Foundation, Jenkintown, PA	2024
Adrian Nañez, Ph.D. (ad hoc 2021)	Senior Medical Science Liaison – Oncology (Heme)	Takeda, San Antonio, TX	N/A
Priyanka Sura, D.V.M., M.S., D.A.B.T.	Director Regulatory and Product Stewardship, Trade Compliance Manager	ANGUS Chemical Company, Buffalo Grove, IL	2024
Tamara Tal, Ph.D.	Senior Scientist, Group Leader	Helmholtz Center for Environmental Research – UFZ, Leipzig, Germany	2023
Misti Ushio, Ph.D.	Chief Executive Officer	TARA Biosystems, Inc., New York, NY	2024

ClarLynda Williams-Devane, Ph.D.	Director, State Center for Health Statistics	North Carolina Department of Health and Human Services, Raleigh, NC	2020

Reference Pages

Agency Representatives in 2020 and 2021

Tags: ICCVAM

The individuals listed on this page served as representatives from ICCVAM member agencies in 2020 and 2021. ICCVAM includes three types of representatives:

- The **Principal Agency Representative** is the primary source of communication from his or her agency to the committee and NICEATM. He or she regularly attends all ICCVAM meetings and teleconferences, coordinates participation of other representatives from the agency, and casts the agency's vote on occasions when formal voting is required.
- **Alternate Agency Representatives** participate in ICCVAM committee activities in place of the Principal when necessary.
- An agency may designate one or more **Other Agency Representatives** to participate in ICCVAM to provide broader representation or expertise.

Agency (Office)	Representative	Representative Type (as of December 2021)	Service Continuing into 2022
Agency for Toxic Substances and Disease Registry	Moiz Mumtaz, M.Sc., Ph.D.	Principal	Yes
Agency for Toxic Substances and Disease Registry	Patricia Ruiz, Ph.D..	Alternate	Yes
National Cancer Institute	Brian Cholewa, Ph.D.	Principal	Yes
National Cancer Institute	Mark Miller, Ph.D., FAC-COR III	Other	-
National Cancer Institute	Ron Johnson, Ph.D.	Alternate	Yes
National Institute for Occupational Safety and Health	Richard Probst, D.V.M., M.P.H., DACLAM, DACPVM	Principal	Yes
National Institute of Environmental Health Sciences	Brian Berridge, D.V.M., Ph.D., D.A.C.V.P.	Principal	Yes

Agency (Office)	**Representative**	**Representative Type (as of December 2021)**	**Service Continuing into 2022**
National Institute of Environmental Health Sciences	Mamta Behl, Ph.D., D.A.B.T.	Other	-
National Institute of Environmental Health Sciences	Stephen Ferguson, Ph.D.	Alternate	Yes
National Institute of Environmental Health Sciences	Warren Casey, Ph.D., D.A.B.T.	Other	Yes
National Institute of Environmental Health Sciences	Nicole Kleinstreuer, Ph.D.	Other	Yes
National Institute of Environmental Health Sciences	Elizabeth Maull, Ph.D.	Other	-
National Institute of Standards and Technology	John Elliott, Ph.D.	Principal	Yes
National Institute of Standards and Technology	Elijah Petersen, Ph.D.	Alternate	Yes
National Institutes of Health	Harold Watson, Ph.D.	Other	-
National Library of Medicine	Pertti (Bert) Hakkinen, Ph.D.	Other	-
National Library of Medicine	Jeanne Goshorn, M.S.	Principal	Yes
Occupational Safety and Health Administration	Surender Ahir, Ph.D.	Other	-
Occupational Safety and Health Administration	Janet Carter	Alternate	Yes
Occupational Safety and Health Administration	Deana Holmes, M.T.	Principal	Yes

Agency (Office)	**Representative**	**Representative Type (as of December 2021)**	**Service Continuing into 2022**
U.S. Consumer Product Safety Commission	John Gordon, Ph.D.	Principal	Yes
U.S. Consumer Product Safety Commission	Kristina Hatlelid, Ph.D.	Alternate	Yes
U.S. Consumer Product Safety Commission	Eric Hooker, M.S.	Alternate	Yes
U.S. Consumer Product Safety Commission	Joanna Matheson, Ph.D.	Alternate	Yes
U.S. Department of Agriculture	Carol Clarke, D.V.M., DACLAM	Principal	Yes
U.S. Department of Agriculture	Kristina Adams, M.S.	Alternate	Yes
U.S. Department of Agriculture	Erika Edwards	Other	Yes
U.S. Department of Agriculture	Aisha Ellis, D.V.M.	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 Agriculture	Patrice Klein, M.S., V.M.D., DACPV, DACVPM	Other	Yes
U.S. Department of Agriculture	Jessie Kull	Other	Yes
U.S. Department of Defense	Matthew Johnson, D.V.M., DACLAM	Alternate (acting Principal)	Yes
U.S. Department of Defense	Emily N. Reinke, Ph.D., D.A.B.T. (Co-chair)	Alternate	Yes
U.S. Department of Energy	Prem C. Srivastava, Ph.D.	Principal	-

Agency (Office)	**Representative**	**Representative Type (as of December 2021)**	**Service Continuing into 2022**
U.S. Department of the Interior	Barnett A. Rattner, Ph.D.	Principal	Yes
U.S. Department of the Interior	Jessica Leet, Ph.D.	Alternate	Yes
U.S. Department of the Interior	Tim Bargar, Ph.D.	Other	Yes
U.S. Department of the Interior	Adria Elskus, Ph.D.	Other	-
U.S. Department of the Interior	Paula F.P. Henry, Ph.D.	Other	Yes
U.S. Department of the Interior	Luke R. Iwanowicz, Ph.D.	Other	Yes
U.S. Department of Transportation	Steve Hwang, Ph.D.	Principal	Yes
U.S. Department of Transportation	Ryan Vierling, Ph.D.	Alternate	Yes
U.S. Department of Veterans Affairs Office of Research and Development	Christopher Bever, M.D., MBA	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	George Lathrop, D.V.M., M.S., DACLAM	Other	Yes
U.S. Department of Veterans Affairs Office of Research and Development	Mario Rinaudo, M.D.	Other	-
U.S. Environmental Protection Agency (Office of Pesticide Programs)	Anna Lowit, Ph.D. (Co-chair)	Principal	Yes

Agency (Office)	**Representative**	**Representative Type (as of December 2021)**	**Service Continuing into 2022**
U.S. Environmental Protection Agency (Office of Pollution Prevention and Toxics)	Louis (Gino) Scarano, Ph.D.	Other	Yes
U.S. Environmental Protection Agency (Office of Research and Development)	Pamela Noyes, Ph.D.	Alternate	Yes
U.S. Environmental Protection Agency (Office of Research and Development)	Stephanie Padilla, Ph.D.	Alternate	Yes
U.S. Food and Drug Administration (Center for Biologics Evaluation and Research)	Robin Levis, Ph.D.	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)	Simona Bancos, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Devices and Radiological Health)	Jennifer Goode	Alternate	Yes
U.S. Food and Drug Administration (Center for Devices and Radiological Health)	Rakhi Dalal-Panguluri, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Drug Evaluation and Research)	Paul C. Brown, Ph.D.	Other	Yes

Agency (Office)	**Representative**	**Representative Type (as of December 2021)**	**Service Continuing into 2022**
U.S. Food and Drug Administration (Center for Drug Evaluation and Research)	Jill Merrill, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Food Safety and Applied Nutrition)	Patrick Crittenden, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Center for Food Safety and Applied Nutrition)	Suzanne Fitzpatrick, Ph.D., D.A.B.T.	Principal	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	Yes
U.S. Food and Drug Administration (Center for Veterinary Medicine)	Li You, Ph.D.	Other	Yes
U.S. Food and Drug Administration (National Center for Toxicological Research)	Donna Mendrick, Ph.D.	Other	Yes
U.S. Food and Drug Administration (National Center for Toxicological Research)	Mugimane (Manju) Manjanatha, Ph.D.	Other	Yes
U.S. Food and Drug Administration (Office of the Chief Scientist)	Tracy Chen, Ph.D., D.A.B.T.	Other	Yes

Agency (Office)	Representative	Representative Type (as of December 2021)	Service Continuing into 2022
U.S. National Coordinator for OECD Test Guidelines Programme	Wanda Hall	Other	
U.S. National Coordinator for OECD Test Guidelines Programme	Charles Kovatch	Other	Yes

NICEATM and ICCVAM Publications, 2020-2021

This page lists publications issued in 2020 and 2021 that describe NICEATM and ICCVAM activities.

NICEATM and ICCVAM reports

Tags: ICCVAM, NIEHS, eye irritation, IATA, metrics, mixtures toxicity, skin sensitization

ICCVAM. 2020. Biennial Progress Report 2018-2019 [Internet]. Research Triangle Park (NC): National Institute of Environmental Health Sciences [cited 17 February 2022]. Available: https://ntp.niehs.nih.gov/iccvamreport/2019/index.html.

ICCVAM. 2021. Measuring U.S. Federal Agency Progress Toward Implementation of Alternative Methods in Toxicity Testing [Internet]. Research Triangle Park (NC): National Institute of Environmental Health Sciences [cited 17 February 2022]. Available: https://ntp.niehs.nih.gov/iccvam/docs/about_docs/iccvam-measuringprogress-feb2021-fd-508.pdf.

NIEHS. 2020. Application of Non-animal Test Methods and Defined Approaches to Skin Sensitization Assessment of Isothiazolinone Compounds [Internet]. Research Triangle Park (NC): National Institute of Environmental Health Sciences [cited 17 February 2022]. Available: https://ntp.niehs.nih.gov/iccvam/methods/immunotox/it/niceatm-it-report-june2020-final-wappx-508.pdf.

NIEHS. 2021. Prospective and Retrospective Evaluation of the Eye Irritation Potential of Agrochemical Formulations [Internet]. Research Triangle Park (NC): National Institute of Environmental Health Sciences [cited 17 February 2022]. Available: https://ntp.niehs.nih.gov/iccvam/docs/ocutox_docs/formulationreport_2021_fd-508.pdf.

Federal Register notices

Tags: ICCVAM, NIEHS, mixtures toxicity, developmental toxicity, neurotoxicity

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

NIEHS. 2020. ICCVAM notice of public webcast; request for public input. Federal Register. 85(74): 21256-21257.

NIEHS. 2020. ICCVAM Biennial Progress Report: 2018-2019; availability of report. Federal Register. 85(137): 43249-43240.

NIEHS. 2021. ICCVAM Communities of Practice Webinar on Non-animal Approaches for Mixtures Assessment. Federal Register. 86(6): 1981-1982.

NIEHS. 2021. ICCVAM notice of public webcast; request for public input. Federal Register. 86(67): 18547-18548.

NIEHS. 2021. Request for data and information on new approach methodologies for efficacy testing of ectoparasiticide products to meet regulatory data requirements. Federal Register. 86(242): 72251.

NIEHS. 2021. ICCVAM Communities of Practice Webinar on New Approach Methodologies to Assess (Developmental) Neurotoxicity. Federal Register. 86(243): 72609-72610.

Journal articles

A full list of NICEATM and ICCVAM publications is available on the NTP website.

Abedini JA, Cook B, Bell S, Chang X, Choksi N, Daniel AB, Hines D, Karmaus AL, Mansouri K, McAfee E, Phillips J, Rooney J, Sprankle CS, Allen D, Casey W, Kleinstreuer N. 2021. Application of new approach methodologies: ICE tools to support chemical evaluations. Comput Toxicol 20:100184. https://doi.org/10.1016/j.comtox.2021.100184.

Allen D, Rooney J, Kleinstreuer N, Lowit A, Perron M. 2021. Retrospective analysis of dermal absorption triple pack data. ALTEX 38(3):463-476. https://doi.org/10.14573/altex.2101121.

Alves VM, Auerbach SS, Kleinstreuer NC, Rooney JP, Muratov EN, Rusyn I, Tropsha A, Schmitt C. 2021. Curated data in — trustworthy in silico models out: the impact of data quality on the reliability of artificial intelligence models as alternatives to animal testing. ATLA 49(3):73-82. https://doi.org/10.1177/02611929211029635.

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Tags: ICCVAM, NIEHS, VA ORD, acute toxicity, antibodies, COVID-19, MPS, skin sensitization

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Glossary of Key Terms

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

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

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

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

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

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

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

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

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

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

Aneugen: a chemical agent that causes dividing cells to pass on an abnormal number of chromosomes to the descendant cells.

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

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

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

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

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

Cardiotoxicity: toxicity to the heart.

Chorion: in fish, the outermost membrane of an egg.

Clastogen: a chemical that can cause breaks in chromosomes, leading to sections of the chromosome being deleted, added, or rearranged.

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

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

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

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

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

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

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

Environmental fate properties: properties of a chemical, such as biodegradability and soil adsorption, that affect how it will behave if released into the environment.

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

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

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

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

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

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

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

Genotoxic: capable of damaging DNA.

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

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

Hepatic: referring to the liver.

Hepatocyte: the main functional cell of the liver.

Hepatotoxicity: toxicity to the liver.

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

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

Homeostasis: the maintenance of physical and chemical conditions by living systems to allow optimal functioning of the organism**.**

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

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

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

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

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

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

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

Keratinocyte: the major cell type of the skin.

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

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

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

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

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

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

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

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

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

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

Ontologies: standardized nomenclature systems**.**

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

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

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

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

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

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

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

Polymorphisms: in the context of genetics, differences in an organism's DNA sequence. While most polymorphisms have no effect on health or development, some of these genetic differences are related to functional effects such as variation in chemical metabolism.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Subacute: animal experiment designed to study effects produced by the test substance when administered either in repeated doses or continually in food, drinking water, or air over a period of between 24 h and 28 days.

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

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

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

Toxicant: a toxic or poisonous substance.

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

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

Uterotrophic assay: an assay conducted in female rodents that measures the estrogenic activity of a chemical by assessing the chemical's effect on the weight of the uterus.

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

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

Acronyms and Abbreviations

3Rs	principles of replacement, reduction, or refinement of animal use for scientific research or product safety testing
ADME	absorption, distribution, metabolism, and excretion
AOP	adverse outcome pathway
API	application programming interface
ATSDR	Agency for Toxic Substances and Disease Registry
AWIC	Animal Welfare Information Center (U.S. Department of Agriculture)
BraCVAM	Brazilian Center for the Validation of Alternative Methods
CaCVAM	Canadian Centre for the Validation of Alternative Methods
CarciHEI	Carcinogenicity Health Effects Innovation Program (National Institute of Environmental Health Sciences)
CATMoS	Collaborative Acute Toxicity Modeling Suite
CDER	Center for Drug Evaluation and Research (U.S. Food and Drug Administration)
CPSC	U.S. Consumer Product Safety Commission
DNT HEI	Developmental Neurotoxicity Health Effects Innovation Program (National Institute of Environmental Health Sciences)
DNTP	Division of the National Toxicology Program (National Institute of Environmental Health Sciences)
DoD	U.S. Department of Defense
DOE	U.S. Department of Energy
DOI	U.S. Department of the Interior

DOT	U.S. Department of Transportation
dTP	developmental toxicity potential
ELISA	enzyme-linked immunosorbent assay
ENM	engineered nanomaterials
EPA	U.S. Environmental Protection Agency
EURL ECVAM	European Union Reference Laboratory for Alternatives to Animal Testing
FAIR	findability, accessibility, interoperability, and reusability (of data)
FDA	U.S. Food and Drug Administration
FXR	farnesoid X receptor
GHS	United Nations Globally Harmonized System of Classification and Labelling of Chemicals
HTS	high-throughput screening
httk	High-throughput Toxicokinetics (software package, U.S. Environmental Protection Agency)
IATA	integrated approach to testing and assessment
ICATM	International Cooperation on Alternative Test Methods
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICE	Integrated Chemical Environment (National Toxicology Program)
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
iPSC	induced pluripotent stem cell
IVIVE	in vitro to in vivo extrapolation]
JaCVAM	Japanese Center for the Validation of Alternative Methods
KM	half-maximal concentration for metabolism
KoCVAM	Korean Center for the Validation of Alternative Methods
LC50	in traditional acute inhalation or aquatic toxicity tests, the concentration that produces lethality in 50% of the animals tested
LD50	in traditional acute dermal or oral systemic toxicity tests, the dose that produces lethality in 50% of the animals tested
LEL	lowest effect level

LOAEL	lowest observable adverse effect level
MAT	monocyte activation tests
MPS	microphysiological systems
MPSCoRe	MPS for COVID Research working group
NAM	new approach methodology
NASA	National Aeronautics and Space Administration
nAChRs	nicotinic acetylcholine receptors
NC3Rs	National Centre for the Replacement Refinement & Reduction of Animals in Research (United Kingdom)
NCATS	National Center for Advancing Translational Sciences (National Institutes of Health)
NCI	National Cancer Institute (National Institutes of Health)
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences (National Institutes of Health)
NIH	National Institutes of Health
NIOSH	National Institute for Occupational Safety and Health
NIST	National Institute of Standards and Technology
NLM	National Library of Medicine (National Institutes of Health)
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OPERA	Open (Quantitative) Structure–activity/property Relationship App
OSHA	Occupational Safety and Health Administration
PAH	polycyclic aromatic hydrocarbon
PBPK	physiologically based pharmacokinetic
PFAS	per- and polyfluoroalkyl substances
PHMSA	Pipeline and Hazardous Materials Safety Administration (U.S. Department of Transportation)
PK	pharmacokinetic
POD	point of departure

QSAR	quantitative structure–activity relationship
RHDV2	rabbit hemorrhagic disease virus serotype 2
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
SARA	Skin Allergy Risk Assessment model (Unilever)
SEAZIT	Systematic Evaluation of the Application of Zebrafish in Toxicology (National Toxicology Program)
SOT	Society of Toxicology
ToxCast	Toxicity Forecaster (U.S. Environmental Protection Agency)
ToxRefDB	Toxicity Reference Database (U.S. Environmental Protection Agency)
TSCA	Toxic Substances Control Act (U.S. Environmental Protection Agency)
USDA	U.S. Department of Agriculture
USGS	U.S. Geological Survey (U.S. Department of the Interior)
Vmax	maximal capacity for metabolism