Summary Minutes

Scientific Advisory Committee on Alternative Toxicological Methods Meeting

September 17-18, 2024

National Institutes of Health

Bethesda, MD

I. Contents

II. Location of Background Materials and Presentations

Background materials and presentations for the 2024 Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) meeting are available on the National Toxicology Program (NTP) Past SACATM Meetings page (https://ntp.niehs.nih.gov/events/past/index.html?type=SACATM).

III. Frequently Used Abbreviations

3Rs	replacement, reduction, or refinement of animal use in research or testing
ACD	Advisory Committee to the Director (National Institutes of Health)
AI	artificial intelligence
AOP	adverse outcome pathway
API	application programming interface
ARPA-H	Advanced Research Projects Agency for Health
BMC	benchmark concentrations
CAMERA	Collection of Alternative Methods for Regulatory Application
CEBS	Chemical Effects in Biological Systems
cMax	pharmacokinetic measure to determine drug dosing; peak concentration
CoMPAIT	Collaborative Modeling Project of Acute Inhalation Toxicity
Complement-ARIE	Complement Animal Research in Experimentation
CSS	steady-state plasma concentration of a drug
DNT	developmental neurotoxicity
DPCPSI	Division of Program Coordination, Planning, and Strategic Initiatives (National Institutes of Health)
EFSA	European Food Safety Authority
EPA	U.S. Environmental Protection Agency
FAIR	findability, accessibility, interoperability, and reusability
FDA	U.S. Food and Drug Administration
FNIH	Foundation for the National Institutes of Health
GHS	United Nations Globally Harmonized System of Classification and Labelling of Chemicals
GUI	graphical user interface
HSUS	Humane Society of the United States
IATA	integrated approach to testing and assessment

ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICE	Integrated Chemical Environment
IIVS	Institute for In Vitro Sciences
IVB	in vitro battery
IVIVE	in vitro to in vivo extrapolation
LC50	in traditional animal tests for acute systemic inhalation or aquatic toxicity, the concentration that causes death in 50 percent of the animals tested
MAD	Mutual Acceptance of Data (OECD Test Guidelines Programme)
MDF	Method Developers Forum (ICCVAM)
MPS	microphysiological systems
NAMs	new approach methodologies
NCATS	National Center for Advancing Translational Sciences
NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NTP	National Toxicology Program
OBO	Open Biological and Biomedical Ontology
OECD	Organisation for Economic Cooperation and Development
OPFR	organophosphorus flame retardants
OPERA	Open (Quantitative) Structure–activity/property Relationship App
PCRM	Physicians Committee for Responsible Medicine
PETA	People for the Ethical Treatment of Animals
QSAR	quantitative structure–activity relationship
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
SARA-ICE	Skin Allergy Risk Assessment-Integrated Chemical Environment
SOT	Society of Toxicology
TSAR	Tracking System on Alternative Methods Towards Regulatory Acceptance (European Commission)
VQN	validation and qualification network
VWG	Validation Workgroup (ICCVAM)

IV. Attendance

SACATM met in person at the National Institutes of Health (NIH) in Bethesda, MD, on September 17 and 18, 2024. The following individuals attended the meeting in person. In addition to the participants named below, 126 people viewed the meeting via webcast on September 17, with 105 viewing on September 18.

SACATM Members

Antonio Baines, PhD, North Carolina Central University

Szczepan Baran, VMD, MS, VeriSIM Life (remote)

Ellen Berg, PhD, Alto Predict LLC

Sue Leary, MS, Alternatives Research and Development Foundation

Sue Marty, PhD, MPH, DABT, The Dow Chemical Company

Kristini Miles, PhD, DABT, The HoneyPot Company (remote)

Adrian Nañez, PhD, Servier, Inc.

Kathryn Page, PhD, DABT, ERT, The Clorox Company (Chair)

Nathan Price, PhD, Thorne Health Tech

Patricia Silveyra, MS, PhD, Indiana University

Priyanka Sura, DVM, MS, DABT, Gilead Sciences, Inc.

Sally Thompson-Iritani, DVM, PhD, University of Washington

Misti Ushio, PhD, Digitalis Ventures

Ad Hoc SACATM Members

Kambez Benam, DPhil, University of Pittsburgh

Corie Ellison, PhD, Procter & Gamble (remote)

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Principal Representatives

Warren Casey, PhD, DABT, National Institute of Environmental Health Sciences (NIEHS)

Brian Cholewa, PhD, National Cancer Institute

John Gordon, PhD, U.S. Consumer Product Safety Commission

Steve Hwang, PhD, U.S. Department of Transportation

Barnett Rattner, PhD, U.S. Department of the Interior

Other ICCVAM Representatives

Jennifer Goode, U.S. Food and Drug Administration Center for Devices and Radiological Health

Nicole Kleinstreuer, PhD, NIEHS

Charles Kovatch, U.S. Environmental Protection Agency (EPA); U.S. National Coordinator, Test Guidelines Programme, Organisation for Economic Cooperation and Development

Monique Perron, ScD, EPA

Elijah Petersen, PhD, National Institute of Standards and Technology

Rebecca Rothhaas, PhD, U.S. Department of Transportation

Natalia Vinas, PhD, U.S. Department of Defense, ICCVAM Co-chair

Menghang Xia, PhD, National Center for Advancing Translational Sciences (remote)

National Institutes of Environmental Health Sciences (NIEHS) Staff

Milene Brownlow, PhD, Designated Federal Officer

Robbin Guy

Helena Hogberg, PhD

Kamel Mansouri, PhD

Christopher McPherson, PhD

Andrew Newell, PhD

Heather Patisaul, PhD

Mary Wolfe, PhD

Rick Woychik, PhD

NIEHS Support Contractors

Ella Darden (Inotiv, contractor supporting the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods [NICEATM])

Parris Milly (NTT DATA, contractor supporting the NIEHS Office of Communications and Public Liaison) (remote)

Nathan Mitchiner (NTT DATA, contractor supporting the NIEHS Office of Communications and Public Liaison) (remote)

Steven Morefield, MD (Inotiv, contractor supporting NICEATM)

Emily Reinke, PhD (Inotiv, contractor supporting NICEATM)

Chris Schnur (NTT DATA, contractor supporting the NIEHS Office of Communications and Public Liaison) (remote)

Catherine Sprankle, MS (Inotiv, contractor supporting NICEATM)

Public

Stacy Adam, PhD, Foundation for the National Institutes of Health (NIH)

Lauren Brown, PhD, RTI International

Amy Clippinger, PhD, PETA Science Consortium International

Megan Culbreth, PhD, U.S. Food and Drug Administration

Ellen Gadbois, PhD, NIH

Denise Johnson, MS, Battelle

Vicki Katrinak, Humane Society of the United States (remote)

Paul Locke, DrPH, Johns Hopkins University School of Public Health

Iris Mangas, PhD, European Food Safety Authority (remote)

Shaun McCullough, PhD, RTI International

Chantel Nicolas, PhD, Abt Global

Margaret Ochocinska, PhD, NIH

Timothy Shafer, PhD, EPA (remote)

Kristie Sullivan, MS, Institute for In Vitro Sciences (remote)

Loza Taye, MS, Johns Hopkins University School of Public Health

September 17, 2024

V. Welcome and Opening Remarks

Dr. Kathryn Page, The Clorox Company, Chair of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM), called the meeting to order at 10:05 a.m. on September 17. SACATM members and in-person attendees introduced themselves.

Dr. Natalia Vinas, U.S. Department of Defense and co-chair of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) welcomed attendees to the meeting. Dr. Nicole Kleinstreuer, National Institute of Environmental Health Sciences (NIEHS), Director of the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) thanked the committee members for their participation and noted the importance of the SACATM members' input to the ICCVAM committee.

Dr. Milene Brownlow, NIEHS, the SACATM Designated Federal Officer, read the conflict-of-interest statement and reviewed meeting logistics.

In welcoming remarks, Dr. Rick Woychik, Director of NIEHS, reviewed the purpose of SACATM and the goals of this meeting. He noted the importance of validation activities and operationalizing the concepts articulated in the report published earlier this year by the ICCVAM Validation Workgroup.[1] The Complement-ARIE program is going to be very important to that effort, which supports recent recommendations from the Advisory Committee to the Director of the National Institutes of Health (NIH). He also noted that the meeting would consider progress in replacing animal use for developmental neurotoxicity (DNT) and implementation of an in vitro battery for DNT. Finally, the meeting will provide updates on NICEATM computational resources that are used by

[1] "Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies," available at https://doi.org/10.22427/NICEATM-2.

ICCVAM stakeholders. He thanked the departing SACATM members for their service: Dr. Page; Dr. Szczepan Baran, VeriSIM Life; Ms. Sue Leary, Alternatives Research and Development Foundation; Dr. Priyanka Sura, Gilead Sciences, Inc.; and Dr. Misti Ushio, Digitalis Ventures. He also thanked international partners who engage with ICCVAM through the International Cooperation on Alternative Test Methods and the Test Guidelines Programme of the Organisation for Economic Cooperation and Development (OECD).

VI. ICCVAM Biennial Report

Dr. Kleinstreuer noted that the production of the ICCVAM Biennial Progress Report is mandated by the ICCVAM Authorization Act.[2] The Act requires reporting on the activities of ICCVAM, but the Biennial Report has evolved to encompass activities relevant to replacement, reduction, and refinement of animal use (3Rs) across the ICCVAM member agencies. Dr. Kleinstreuer reviewed the process of compiling the 2022-2023 Biennial Report,[3] which is led by NICEATM but involves contributions from many scientists from the ICCVAM agencies who provide high-level summaries of activities ongoing during the reporting period. She reviewed the structure of the report. The report includes sections on Technology, Confidence, and Utilization, which reflect the goals articulated in the Strategic Roadmap. Other sections include Leadership, which encompasses funding, international interactions, and collaborations, and sections providing background and reference information. The report includes 212 articles that can be filtered by using 29 different topic tags such as carcinogenicity, inhalation toxicity, and skin sensitization. Filter tags are also available to search for activities of the 17 agencies that were members of ICCVAM during the reporting period. Dr. Kleinstreuer reviewed the key accomplishments highlighted in the report, including publication of the ICCVAM Validation Workgroup (VWG) document, articles describing agencies' information needs and testing requirements, curation and publication of a human skin sensitization database, broadening applicability of defined approaches for skin sensitization, development of approaches to predict and characterize cardiotoxic potential, and web tools for chemical exploration and toxicity prediction. She reviewed the evolution of the Biennial Report and data on stakeholder engagement with the Biennial Report, which reflects interest when it is first published but also increased engagement during ICCVAM public events and other activities. An artificial intelligence analysis of the content of the report over the last 10 years showed an exponential increase of activities around computational toxicity as well as increasing activity around acute toxicity, and to a lesser extent in ocular toxicity and biologics and vaccine testing. Notably, recent reports also reflect a steady increase in efforts to validate and accept new methods, specifically in international harmonization, adoption of policies to accommodate rapid method development, and establishment of frameworks to streamline acceptance processes.

Clarifying questions and comments: Ms. Sprankle noted that at the time of the meeting the PDF version of the report was not available, and encouraged attendees to engage with the report via the website version as it is the easier and more user-friendly

[2] 42 U.S.C. 285l-3; available at https://ntp.niehs.nih.gov/iccvam/docs/about_docs/pl106545.pdf.
[3] Available at https://ntp.niehs.nih.gov/iccvamreport/2023.

means to access all the content.

Public Comments

Written public comments were submitted for this section from the Humane Society of the United States (HSUS) and the Humane Society Legislative Fund, from People for the Ethical Treatment of Animals (PETA), and from the Physicians Committee for Responsible Medicine (PCRM).[4]

Oral Public Comments

Ms. Kristie Sullivan, Institute for In Vitro Sciences (IIVS), presenting remotely, felt that this report features some incredible work by the ICCVAM agencies, especially in the area of assay application. She suggested that NICEATM consider presenting a webinar reviewing the highlights of the report. Publication of the validation document was a seminal ICCVAM accomplishment, representing its international leadership in this area. IIVS is looking forward to ICCVAM agencies implementing these concepts to incorporate new approach methodologies (NAMs) into their decision-making in a transparent way. In that vein, Ms. Sullivan encouraged agencies to provide case studies that describe how they have done this. Considering broadly the area of systemic toxicity, it would be of interest to have an agency needs review to provide focus for NAMs development activities, and case studies for this area would be especially of interest. She thanked NICEATM and its Inotiv contract staff for their work, especially around the development and improvement of the Integrated Chemical Environment (ICE) web resource and encouraged them to expand training opportunities for online tools.

Clarifying questions and comments: There were no clarifying questions.

Ms. Vicki Katrinak, HSUS, presenting remotely, expressed appreciation for the agencies' follow-up activities after publication of the VWG document, and hoped that the availability of this resource will lead to faster approval times for NAMs under certain contexts of use. She praised the creation of the ICCVAM Method Developers' Forum (MDF)[5] but wanted to make sure appropriate follow-up activities are performed to build on this, especially interactions between method developers and agencies. HSUS looks forward to future forums and encourages agencies to take steps needed to make sure these meetings ultimately lead to regulatory acceptance of NAMs. She also praised NIH activities to establish validation networks for regulatory implementation of NAMs.[6] Regulatory input is crucial to ensure development of methods that will accelerate drug evaluation while also replacing animal testing.

Clarifying questions and comments: There were no clarifying questions.

Comments from Designated SACATM Discussants

Discussants for "ICCVAM Biennial Report" were asked to consider the following questions:

- How can the advancements highlighted in the ICCVAM Biennial Report be

[4] Written public comments are available at https://ntp.niehs.nih.gov/events/past/index.html?type=SACATM (click the link "Meeting Materials" in the far-right table column).
[5] Information available at https://ntp.niehs.nih.gov/go/developers-forums.
[6] Discussed in more detail in Section VIII.

leveraged to shape future initiatives and focal areas, and what are the potential obstacles and opportunities in this process?

- What research areas or testing approaches are currently underrepresented across ICCVAM, given the present challenges and emerging opportunities in toxicological testing methods?
- What mechanisms or strategies can be applied to enhance stakeholder engagement, address their concerns, and incorporate their feedback more effectively into ICCVAM's future activities?
- What is your impression of the ICCVAM Biennial Report web platform, and how can it be improved?

Dr. Sally Thompson-Iritani, University of Washington, first discussant, commented on the comprehensiveness of the report and stated that she was impressed with the evolution of the report over time. She suggested that stakeholders might find it useful for the report to be updated continuously. The reference pages are a good resource, and she found the information about validation activities and advances in skin sensitization of particular interest. She agreed with Ms. Sullivan's suggestion that a webinar or series of webinars on the report content might be of interest. Dr. Kleinstreuer clarified that NICEATM doesn't have the resources to continually update the Biennial Report, and that might also be inconsistent with the congressional mandate for the report. She noted that NICEATM activities are continually updated on the NICEATM website.

Dr. Ellen Berg, Alto Predict LLC, second discussant, found the report to be comprehensive and showcased the good progress in evaluation of NAMs by agencies. She suggested as we move towards implementation and demonstrating impact, it would be helpful if the report were to provide some summary metrics such as numbers of validation studies initiated or completed and have this information on the home page. A different organization of the report, perhaps highlighting key successes, might better reflect the evolution and maturation of ICCVAM activities. The work on skin sensitization was particularly praised as it reflects the fact that when human data are available, NAMs can be shown to be superior to animal tests and shows the importance of incorporating human data. Also, that the ability to map NAMs to specific pathway mechanisms improves confidence. Combinatorial NAMs are a future trend and ICCVAM needs to consider how to validate those, especially consideration of their individual components. She would also like to see more information about the impact of human variation of application of NAMs. She proposed NICEATM consider ways to automate compilation of the report. Dr. Kleinstreuer asked about specific recommendations about incorporating human outcome data and population variability into NAMs development, and Dr. Berg responded that some approaches could include using cells from diverse donors and using genetic data as a basis for better understanding human biology. Dr. Woychik noted that a partnership with the NIH All of Us[7] program could support this. Leaders of that program are interested in integrating environmental exposure into their studies, and that could be an avenue to exploring that question.

[7] https://allofus.nih.gov/.

Additional SACATM Comments

Dr. Sue Marty, Dow Chemical Company, felt that it would be useful to think about putting NAMs into integrated approaches to testing and assessment (IATA) frameworks. Batteries of in vitro assays are likely to become more common as NAMs are used to evaluate more complex endpoints; thus, IATAs could inform on situations such as getting one positive result in a battery of many assays (e.g., 17 assays in DNT battery). An IATA framework that allows verification of results from in vitro assays in organoids or more complex assays would be useful. With respect to a research area that may be underrepresented, she expressed interest in more investigations into kidney toxicity as this is a common target organ for repeat-dose systemic toxicity. Dr. Marty also welcomed descriptions in the report of projects that are adding metabolism to in vitro assays to assess the potential bioactivity of metabolites. Future projects should also focus on standardizing approaches to analyzing and integrating in vitro data.

Dr. Nathan Price, Thorne Health Tech, noted Dr. Kleinstreuer's highlighting of progress in skin sensitization and asked for her thoughts on trends in this area. Dr. Kleinstreuer responded that sensitization is a great example of how, when robust human data and data from cell-based assays that map to an adverse outcome pathway are available, it's possible to demonstrate how a human-based testing strategy can improve on animal tests. Skin sensitization is unique in this regard because of the nature of the data available, but it can establish a framework for how to build confidence in NAMs for other endpoints. Current trends in skin sensitization are to move beyond hazard and potency characterization to using probabilistic models to do quantitative risk assessment over populations and identify and protect sensitive populations. The Skin Allergy Risk Assessment-Integrated Chemical Environment (SARA-ICE) model, under consideration by both U.S. Environmental Protection Agency (EPA) and OECD, can accomplish this.

Dr. Patricia Silveyra, Indiana University, asked if there are any projects ongoing to tailor NAMs to assess the effects of sex and disease state on toxicity. Dr. Kleinstreuer replied there are no models currently available that accomplish this but there are initiatives underway with that goal. Dr. Woychik noted that NIH recently funded an exposomics research center[8] to characterize the impact of collective exposures to human health, and that is mandated to consider sex as a variable. This is modeled on the Human Genome Project. The center will collect data around the world to address the fact that effects of exposures cannot be characterized by looking at single chemicals. A number of NIH agencies are collaborating on this effort, and collaborating rather than competing is going to facilitate success in this endeavor.

VII. Validation Updates

Updates to OECD Guidance Document 34 of Validation of New and Updated Test Methods

Mr. Charles Kovatch, EPA, U.S. representative to the Working Party of National Coordinators of the OECD Test Guidelines Programme, summarized OECD and partner

[8] Described in a Columbia University press release at https://www.publichealth.columbia.edu/news/nih-award-creates-columbia-led-exposomics-coordinating-center.

activities to update "Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment," informally known as "Guidance Document 34."[9] As an introduction, Mr. Kovatch summarized presentations made on this topic at last year's SACATM meeting. He reviewed the concept of Mutual Acceptance of Data (MAD), which is a legal mandate for countries participating in the Test Guidelines Programme. The underlying principles of MAD are test guidelines and Good Laboratory Practices. The OECD test guideline development process begins with a proposal called a Standard Project Submission Form. It then proceeds to prevalidation and validation activities, with a draft test guideline being developed from a validation report. Expert groups discuss the validation report and the draft test guideline, which is followed by one to three commenting rounds by the Working Party. Guidance Document 34 is 20 years old and needs to be updated, and technological advances both within and outside the field of toxicology need to be considered in the update. For example, assay development and optimization are not currently discussed in detail in the guidance document. Other factors being considered in the revision include the role of interlaboratory transferability studies and making the process more practical. Top priorities identified for the revision include the concept of technical validation, assessment of relevance beyond accuracy, and guidance for validation of new technologies. Mr. Kovatch also reviewed how Guidance Document 34 needs to be expanded to include appropriate consideration of defined approaches. In addition, OECD has made a specific effort to address needs around financial and operational aspects of validation. An announcement in January 2023 called for mobilization of resources to assist in accelerating the pace of NAMs development. This was followed by two webinars, a stakeholder survey, and compilation of 12 case studies of validation from the community. A December 2023 workshop yielded specific recommendations in this area. Reviewing the last three years, Mr. Kovatch noted that current activities are focused on actual revision of Guidance Document 34 with subgroups established to consider specific questions. In the current round of OECD activities, ICCVAM agencies are co-lead on 15 projects under consideration by the Working Party and have numerous other relevant activities ongoing. He asked SACATM to provide input on how ICCVAM can be best leveraged to quantify validation processes and steps in support of Guidance Document 34; how ICCVAM can be more strategically engaged to communicate interagency method development, research and validation activities; and how they can collaborate on communications and training opportunities.

Clarifying questions and comments: Dr. Ushio asked for a characterization of the stakeholders that responded to the 2023 OECD survey. Mr. Kovatch responded that the goal was to identify the developers and the funding sources. He estimated that there were between 100 and 150 respondents that fell into five or ten groups that varied in their interests and goals. Rather than yielding any kind of quantitative analysis, the survey results helped OECD to structure future discussions. OECD has identified some qualitative action items, such as how to better publicize resources available, and establishing forums to promote opportunities. Dr. Kleinstreuer added that the survey was targeted toward method developers and validation bodies. It identified clear recurring areas of difficulty, such as cell sources, awareness of availability of NAMs for

[9] Available at https://www.oecd.org/en/publications/guidance-document-on-the-validation-and-international-acceptance-of-new-or-updated-test-methods-for-hazard-assessment_e1f1244b-en.html.

different endpoints, acceptability of these methods to specific regulators, and funding for independent validation. OECD and ICCVAM are both aware of these difficulties and are identifying approaches to address them. The NIH presentations later in the agenda will address these issues.

Reviewing the Method Developers Forum: Follow-on Activities from the VWG Report

Dr. Emily Reinke, Inotiv (contractor supporting NICEATM) explained that the concept of the MDF came out of the publication of the VWG document in March 2024. It is a proactive effort to highlight and implement the recommendations of the document. ICCVAM is anticipating holding three MDFs per year, each focusing on a specific endpoint. There are six stages to the MDF process:

1) Presentations are recorded by federal and industry stakeholders summarizing their information needs and decision frameworks for the endpoint of interest.
2) Recordings are posted on the NICEATM website.
3) Announcements are sent out calling for method developer presentations.
4) Method developers draft presentations describing their methods, guided by questions that correspond to the key concepts in the VWG document.
5) The MDF Steering Committee reviews and selects submissions for the MDF main event.
6) The MDF main event (a webinar) features brief presentations from selected method developers that address NAMs for the endpoint or toxicity of interest and includes time for discussion.

The first MDF focused on carcinogenicity. Ten background videos were prepared by six ICCVAM member agencies and two nongovernment stakeholders. These are available on the MDF page on the NICEATM website.[10] A call for submissions from method developers was announced and guidance provided for what information the developers should provide in their presentations. Dr. Reinke reviewed the topics of the submissions received, which included in vitro and computational methods and reporting frameworks. Presenters included representatives of industry, academic, and government organizations. About 230 people viewed August 21-22 webinar, and the videos from the webinar are available on the NICEATM website. Dr. Reinke reviewed the lessons learned from the first MDF webinar. These included:

- Building appropriate timelines to get clearance for the agency videos.
- Increasing engagement from industry, particularly for preregulated phases.
- Providing more opportunities for follow-up questions and discussion during the webinar.
- Finding ways to prioritize methods that are ready for specific applications.
- Providing clear evaluation criteria for method developers to follow, which improves the quality of presentations and helps the steering committee come to

[10] Available at https://ntp.niehs.nih.gov/go/developers-forums.

a consensus on acceptance.

Positive feedback from diverse stakeholders included appreciation that ICCVAM is trying something new. The next MDF will focus on cardiovascular toxicity. Dr. Reinke reviewed other areas under consideration for future topics. She also noted that opportunities are being explored for expanding this approach into other venues, such as partnering with federal agencies or nongovernmental organizations or presenting similar programs at scientific meetings.

Clarifying questions and comments: Dr. Page asked for clarification of participation criteria. Dr. Reinke explained that NICEATM puts out a public call and provides specific criteria on how the Steering Committee evaluates the proposal. The developer's proposal is the presentation that the developer will actually deliver at the webinar. Dr. Marty noted that some of the proposals received for the carcinogenicity MDF only focused on specific aspects of that process such as genotoxicity. She asked if addressing multiple modes of the toxicity of interest was a criterion used by the Steering Committee for selecting presenters for the forum. Dr. Renke replied that was not considered for this forum but that would be a topic of discussion for the Steering Committee. One lesson learned from the August forum was that more time was needed for dialogue between presenters and regulators. Dr. Kleinstreuer also noted that organizers discovered that this event provided a good opportunity to facilitate collaborations among method developers. Some of the most insightful questions asked were from other method developers, and this started some conversations about potential collaborations. Dr. Adrian Nañez, Servier Inc., asked if there were plans to produce a summary report coming out that would describe the outcome of the forum. Dr. Kleinstreuer replied that there is nothing planned but acknowledged that was a good suggestion.

In Vitro Inhalation Toxicity: A Case Study in Building Confidence in New Methods

Dr. Amy Clippinger, PETA Science Consortium International (PETA-SCI), introduced PETA-SCI and described their mission and scope of activities. This presentation focused on their project to develop and gain confidence in new methods for inhalation toxicity. The focus of the study was an in vitro approach for assessing portal-of-entry effects of chemicals exposed as liquids to a reconstructed human respiratory epithelial tissue model. The study was motivated in part by recognition of the differences in the rat and human respiratory tracts and the shortcomings of the rat as a model for human inhalation toxicity. In vitro/ex vivo air-liquid interface systems for assessing inhalation toxicity vary in complexity. The most complex system is not always the best system; the ideal model will depend on the experimental question. PETA-SCI performed a literature review to identify chemicals that were tested in in vitro or ex vivo systems and had corresponding in vivo data. Study designs were extracted from selected articles with the goals of informing a list of reference chemicals and a consensus testing protocol. The platform used for the literature review was Sysrev. Dr. Clippinger reviewed the criteria for the literature search, which was performed on July 15, 2024. The search yielded about 1000 articles, and the Sysrev autolabeler along with a human review were used to eliminate papers that failed to meet specific inclusion criteria. This step reduced the yield to 132 papers that proceeded to manual full-text review. A decision to focus on

liquid test chemicals reduced the pool to 52 articles including results on 259 chemicals. Dr. Clippinger discussed the concept of reference chemicals and how they differ from proficiency chemicals, the chemicals that a naïve lab would use to show competency with a valid method. Proficiency chemicals might be a subset of reference chemicals, but reference chemicals need to encompass additional properties, including representing a range of responses that are reproducible and having high-quality data. They should also be easy to procure, store, and discard. The number of reference chemicals is going to vary depending on the method and can reflect the range of responses or the availability of data. PETA-SCI established a list of 259 chemicals based on the outcome of the literature review and is currently organizing an expert review of the list. The results of the literature review were also used to identify test methods and protocols of interest, focusing on those that evaluated liquid exposure in reconstructed human respiratory epithelium models. The starting point was a protocol developed under the international "INSPIRE initiative."[11] Twenty papers with relevant study designs were found in the literature review and used to draft a consensus protocol that is now under expert review. Dr. Clippinger then turned to the question of how to establish scientific confidence in this approach, for which relevant criteria have been articulated in a 2022 publication[12] and in the ICCVAM VWG document. PETA-SCI activities on this project in the coming year are going to focus on demonstrating that the requirements of these frameworks have been addressed.

Dr. Clippinger closed by briefly discussing three related projects.

- A paper in press (Sharma et al., Archives in Toxicology) articulates reporting standards for in vitro inhalation assays and proposes an approach for comparing data across laboratories.
- PETA-SCI is undertaking a project to characterize the metabolic capability of respiratory airway models via RNA sequencing; results will be presented at the 2025 Society of Toxicology (SOT) meeting.
- A final project is focusing on minimizing variability in in vitro assays. Two key factors identified are the use of animal-derived reagents and antibodies, and current work is focusing on recombinant replacements for these.

She emphasized the importance of collaboration to advance the shared goal of advancing human health through more efficient testing strategies.

Clarifying questions and comments: Dr. Kleinstreuer asked about the rationale for focusing on liquids, given that most chemicals of concern for inhalation toxicity are gases. Dr. Clippinger replied that this decision was made to limit the scope of the project to approaches that had plentiful data and a straightforward experimental approach. Dr. Page added that liquids represent a large segment of the chemical of concern for this endpoint. Dr. Marty asked whether the selection of reference chemicals considered sensitivity in specific areas of the respiratory tract, and Dr. Clippinger agreed that was worth further consideration. Dr. Baran asked how criteria were established for biological relevance, given the complexity of the respiratory system. Dr. Clippinger responded that the goal was a representation of human biology as good as or better than the current

[11] Described in Sharma et al. https://doi.org/10.1093%2Ftoxsci%2Fkfad074.
[12] Van der Zalm et al. https://doi.org/10.1007/s00204-022-03365-4.

regulatory model. She acknowledged that the reconstructed tissue models have limitations but could be sufficient depending on the experimental question.

Public Comments

Public comments on this topic were presented with those for the following topic.

VIII. NAMs Pipeline: Future Directions

Catalyzing the Development and Use of New Approach Methods (NAMs) to Advance Biomedical Research: Implementation of Recommendations from the NIH Advisory Committee to the Director Working Group

Dr. Ellen Gadbois, NIH, provided an overview of recommendations by the NIH Advisory Committee to the Director (ACD) on development and use of NAMs to advance biomedical research. This work was inspired by a growing recognition of the value and increasing sophistication and utility of NAMs for biomedical research and the potential for them to improve on animal models, especially with regards to throughput and human relevance. A working group to the ACD was convened that included broad membership from academia and industry, as well as ex officio members from relevant government agencies. The charge to the working group was to consider the development and use of NAMs and assess their strengths and weaknesses with regards to studying human biology with the goal of applying them to complement or potentially replace animal models. Public input was obtained through a request for information and two public workshops. The working group focused on three modes of NAMs: in chemico, in vitro, and in silico methods. In combination, these can be useful for conducting basic research, uncovering human physiological and pathophysiological mechanisms, and translating knowledge into practice.

An example of the vision of implementing NAMs was articulated via cancer metastasis. Current challenges for this endpoint include the difficulty of studying this process and the poor predictivity of current models. Application of patient-specific NAMs and integrated approaches could ultimately benefit research participants and eventually patients. The working group developed an overall vision of a NAMs ecosystem that integrates combinatorial NAMs with interoperable, reliable datasets and effective dissemination. These would be supported by training, multidisciplinary collaboration, technological and social responsibility, and coordinated infrastructure. Dr. Gadbois reviewed the seven recommendations to the NIH director and asked for SACATM input on specific aspects of the recommendations.

- Recommendation 1, “Prioritize the development and use of combinatorial NAMs”:
    - Establish benchmarks and standards.
    - Support research comparing and benchmarking relevant animal, NAMs and human models.
- Recommendation 2, “Establish resources/infrastructure/collaborations to promote interoperable/reliable and well-curated/high-quality datasets”:
    - Identify or establish a designated repository for NAM data sharing.

    - Create alliances and collaborations for collecting, managing, sharing, and publishing high-quality NAMs data.
- Recommendation 3, “Promote effective dissemination and interconnection of NAMs technology”:
    - Establish mechanisms to support testing, validation, qualification, and benchmarking.
    - Create accessible and reliable sources and repositories for disseminating validated NAMs.
- Recommendation 4, “Invest in comprehensive training to bolster continuous advances in NAMs development and use”:
    - Invest in training across the research to implementation pipeline.
    - Promote awareness and understanding of NAMs.
- Recommendation 5, “Facilitate multidisciplinary teams with expertise across technologies and the lifecycle of NAMs development and use”:
    - Develop funding opportunities to support multidisciplinary teams.
- Recommendation 6, “Promote social responsibility in both the creation and deployment of NAMs across the research lifecycle”:
    - Foster equitable development and use of NAMs.
    - Strengthen interagency partnerships.
- Recommendation 7, “Support and maintain coordinated infrastructure to catalyze effective and responsible NAM development and use”:
    - Create mechanisms for disseminating NAMs resources, technologies, and expertise.
    - Promote or establish consortia and venues for sharing best practices.
    - Identify opportunities to build on existing efforts.

The NIH Division of Program Coordination, Planning, and Strategic Initiatives (DPCPSI) is leading implementation of the recommendations.

Clarifying questions and comments: Dr. Kleinstreuer noted that NICEATM and ICCVAM have been engaged with DPCPSI throughout this process and are working to implement these recommendations. Dr. Gadbois agreed and added that activities to address these recommendations are ongoing across many NIH institutes and centers.

Complement Animal Research in Experimentation (Complement-ARIE) Program Overview

Dr. Margaret Ochocinska, NIH, provided an overview of the NIH Common Fund program. This program and DPCPSI were established in 2006 to fund trans-NIH initiatives to catalyze discovery across all areas of biomedical and behavioral research important to the mission of multiple NIH institutes and centers. These projects are collaborative. They require input from scientists from diverse disciplines, who participate

in interdisciplinary consortia to tackle shared goals, with leadership of the participating institutions working together to design funding opportunities and oversee projects. Complement-ARIE[13] was established to catalyze the development, standardization, validation, and use of human-based NAMs that will transform the way NIH does basic, translational, and clinical sciences. The working group to develop Complement-ARIE encompassed participation from 23 NIH institutes and centers. It was led by Dr. Joni Rutter, Director of the National Center for Advancing Translational Sciences (NCATS), and Dr. Woychik of NIEHS, with Dr. Kleinstreuer providing strategic vision. Dr. Ochocinska reviewed the Complement-ARIE development timeline, which ran concurrently with the work of the ACD Working Group on NAMs. There was exchange of information between the two efforts throughout. A landscape analysis was done to assess data infrastructure needs and the current use of NAMs. Public input was solicited through an August 2023 workshop, three listening sessions with major stakeholders, a federal interagency retreat in October 2023 and an ideation/design prize to engage the community and identify opportunities in NAMs development, validation, and adoption. The program will build on NAMs activities across NIH, including digital twin models, in silico models, complex in vitro systems, and in chemico screening. The Complement-ARIE Challenge Prize Competition[14] was launched in late 2023; 20 entrants were each awarded $50,000 to advance projects relevant to Complement-ARIE goals. Dr. Ochocinska highlighted three winners focused on high-throughput drug screening, developmental toxicity prediction, and multiscale engineered models of cardiovascular health and disease.

Current components of the Complement-ARIE program fall into three key pillars: technology development centers, a NAMs data hub and coordinating centers, and a validation and qualification network for NAMs. These will be supported by community engagement and training efforts that will encompass skill building and consideration of societal and ethical considerations. A graphic in the presentation illustrated how the three pillars will interact. A notice of intent to publish a funding opportunity for the technology development centers will be issued in October.[15] Public-private partnerships will be key to the success of Complement-ARIE, and these will be central to the validation and qualification network. The network will be implemented in three phases. Year 1 will focus on design. Years 2-5 will focus on Implementation Phase 1, during which NIH will fund at least eight use-case validation studies and develop long-term sustainability plans. Years 6-10 will focus on Implementation Phase 2, which will include activities to apply reporting standards to NAMs in the technology development centers and continue validation and qualification support. The three pillars of Complement-ARIE will be informed iteratively by implementation and stakeholder input.

Clarifying questions and comments: Responding to a question from Dr. Thompson-Iritani, Dr. Ochocinska noted that Complement-ARIE's goal is to develop integrated solutions to refine, reduce, and potentially replace animal models, which is consistent

[13] Detailed information at https://commonfund.nih.gov/complementarie.
[14] Details at https://commonfund.nih.gov/complementarie/highlights/nih-announces-winners-complement-arie-challenge-competition.
[15] Announcement for technology development centers available at https://grants.nih.gov/grants/guide/notice-files/NOT-RM-24-012.html; announcement for data hub and coordinating centers available at https://grants.nih.gov/grants/guide/notice-files/NOT-RM-24-015.html.

with the 3Rs. Dr. Ushio asked about NCATS participation, and Dr. Ochocinska responded that the concept was led jointly by NCATS and NIEHS but has broad participation across NIH. Technology developed by NCATS was an important underpinning of this work. Dr. Elliott asked how Complement-ARIE will add value to the existing NIH in vitro assay portfolio. Dr. Ochocinska replied that Complement-ARIE is looking for solutions that integrate those assays with in silico or in chemico models to more broadly model human biology. This was a key goal of the crowdsourcing exercise; she emphasized that anything that moves forward in the program will need to be combinatorial.

NIH Status Update: Public-Private Partnership for NAMs; Update on the Design Phase and Implementation of a Validation and Qualification Network (VQN) for NAMs Adoption and Implementation

Dr. Stacey Adam, Foundation for the NIH (FNIH), explained that FNIH links public and private sector expertise to advance NIH goals. FNIH partners with world-class organizations, including public sector organizations, biopharma, and NGOs, to tackle pressing health challenges. FNIH has raised over $1B in private funds, has 122 active partnerships, and spends 90% of funds raised on programs. Three key areas of activity are:

- Accelerating prevention efforts, new therapies, diagnostics, and potential cures.
- Advancing global health and equity in care.
- Training the next generation of scientists.

Three approaches taken to funding include programs funded exclusively by public organizations, funded by both public and private organizations, and funded exclusively by private organizations, and Dr. Adam provided examples of programs in all three types. Some programs that illustrate how FNIH might approach advancement of NAMs include:

- Accelerating Medicines Partnership program for target identification and validation; disease areas include Alzheimer's, metabolic diseases, autoimmune and immune-mediated diseases, and Parkinson’s disease. This program encompasses 12 projects, with over $9M invested over 10 years. Collaborators include 36 industry partners, 16 NIH institutes and programs, and 43 nonprofits.
- The Biomarkers Consortium bridges the gap between basic research and practical needs for advancing drug development and regulatory science. Drug development tools for a number of disease areas have been developed collaboratively with involvement from academic, government, and industry scientists. This program has been going on for over 20 years and has over 60 active partners and over 40 ongoing projects. Over $100M in private funds has been raised in support of the project.

FNIH’s strength is in filling gaps to tackle complex health problems; their areas of expertise include governance, policy management, program management, fundraising and relationship management, project management, and intellectual property management. FNIH activities around supporting public-private partnerships to progress

NAMs will focus on the Complement-ARIE validation and qualification networks, specifically to get key private sector partners involved. A steering committee has been established for this, and a kickoff meeting has been held with key stakeholders to solicit interest. The current focus is on setting up working groups to develop project proposals, which will then be progressed as appropriate to full project development. FNIH will participate in the Complement-ARIE design phase, one product of which will be a white paper that will invite participation of partners. The next steps would be to create an official research plan with participants and raise and administer private sector funding. Dr. Adam described how FNIH will support Complement-ARIE Phase 1 design, including determining success criteria, developing a governance structure, and issuing requests for proposals to solicit nominations of late-stage NAMs to address priority needs. She detailed design phase activities that might include workshops with interested partners, coordinating with ICCVAM workgroups, determining the scope of validation efforts, and determining stakeholders' precompetitive data-sharing capacity. The white paper will detail budgets, timelines, scoping parameters, milestones, and expected outputs. The design phase is envisioned to cost about $450,000, 2/3 of which will be from NIH with the remaining being raised from the private sector. One goal of the design phase is to determine the funding that will be needed for the implementation phase. The scope and focus of working groups are still to be determined, and FNIH is soliciting recommendations on how to organize these. For example, six to eight working groups could be structured around therapeutic areas or technology types. In addition to establishing working groups, next steps include re-engaging the full steering committee every other month to assess progress and working with Complement-ARIE on documentation for collaboration. The design phase will begin in earnest in early 2025.

Clarifying questions and comments: Dr. Chantel Nicolas, Abt Global, asked about the criteria for private sector participation, specifically whether participants needed to provide funding. Dr. Adam replied that it depends on the situation. Both monetary and in-kind support are welcome, and participation agreements are often tiered by organization size. For example, participation in the Biomarkers Consortium was organized by size of participants' research and development budgets. FNIH may also bring in subject matter experts whose only contribution is advice. The goal is to be flexible to include all interested parties to the extent possible. Dr. Kleinstreuer asked Dr. Adam to expand on what FNIH has learned from the Accelerating Medicines Partnership program and the Biomarkers Consortium that might apply to the Complement-ARIE validation and qualification networks. She also asked for Dr. Adam's thoughts on the private sector's willingness to share data and what FNIH can do to facilitate this. Dr. Adam responded that FNIH has some portals that exist to support sharing data, including patient-level de-identified data and commercial data. She cited several examples relevant to Alzheimer's disease, oncology, and metabolic disease where data-sharing agreements were successfully implemented. These examples illustrate how FNIH can facilitate cooperation to advance a problem that appears intractable. Dr. Thompson-Iritani asked Dr. Adam to elaborate on how she thought the working groups might be organized. Dr. Adam replied that will happen organically depending on the consensus that comes out of the initial discussions.

Public Comments

Written public comments were submitted for this section from the Humane Society of the

United States and the Humane Society Legislative Fund, PCRM, and PETA.

Oral Public Comments

Ms. Sullivan, IIVS, presenting remotely, felt that the MDF is an exciting development that will help connect developers and regulators. She agreed with some of the lessons learned from the first MDF that Dr. Reinke presented. Additional suggestions included charging someone from the ICCVAM member agencies to facilitate the discussion and providing a slide template with presentation criteria; she felt that some of the presentations were too focused on research and basic science. She also encouraged ICCVAM agencies to share feedback on follow-up activities from each forum. Referring to the OECD case studies forum as an example, she suggested NICEATM ask for specific feedback from agencies about the methods presented: whether they would use the method, and if not, why not. This would be useful information for the developers. Regarding other validation efforts and Complement-ARIE, Ms. Sullivan felt that ICCVAM and NICEATM deserve credit for driving international harmonization efforts. She suggested ICCVAM scientists engage with the OECD Working Party on Hazard Assessment, which would provide an additional forum to discuss NAMs application to chemical safety. NAMs validation is needed for both toxicology and biomedical research. In IIVS' experience, validation is a highly iterative process. Relevant funding and expertise are needed to make sure it goes well, and Complement-ARIE will be an important avenue to provide both. Ms. Sullivan encouraged consideration of activities such as tissue shipping studies and evaluation of equipment calibration within the scope of "validation" in addition to parallel assessments of accuracy and reproducibility. Planning and funding are also needed for capacity building. For example, implementation of the DNT battery by a laboratory will require an investment in equipment that would be difficult to justify without a high certainty of success. In addition to funding, expertise and guidance in method validation are needed for success. She encouraged establishment of a coaching process to allow more experienced institutions to share their expertise and build around good in vitro methods practices.

Clarifying questions and comments: There were no clarifying questions.

Dr. Megan LaFollette, 3Rs Collaborative, presenting remotely, explained that the 3Rs Collaborative's mission is to advance better science for both people and animals through facilitating collaborative 3Rs efforts. Focus areas include digital biomarkers, microphysiological systems (MPS), and artificial intelligence (AI). Their MPS Collaborative consists primarily of commercial developers and consists of 42 institutions. The 3Rs Collaborative tech hub connects end-users with relevant commercial providers.

The Collaborative has three recommendations for SACATM.

1) Continue to enhance collaboration between government and nongovernment partners to advance NAMs. The MDF and the NIH ACD have been important activities to advance this. She encouraged SACATM members to review past and join upcoming 3Rs Collaborative and IQ-MPS affiliate MPS workshops, available on the Collaborative's website.[16]
2) Continue to provide investments for independent characterization and validation

[16] Located at https://3rc.org/.

of NAMs, especially commercialized ones. As an example, she described a partnership between the 3Rs Collaborative and U.S. Food and Drug Administration (FDA) Center for Drug Evaluation and Research to conduct a cross-platform project on MPS for drug-induced liver injury.

3) Continue to endorse and invest in implementing scientifically supported NAMs. The Complement-AIRE program and NICEATM computational tools are supporting balanced, accurate messaging to promote use of NAMs.

Clarifying questions and comments: In response to a question from Dr. Sura, Dr. LaFollette explained that the Collaborative is preparing a review paper on use of AI in preclinical safety assessment, including current efforts and challenges. Once that is published, the Collaborative will be recruiting additional subject matter experts for follow-up activities.

Dr. Shagun Krishna, PCRM, noted that her comments are supplemented by written comments submitted by PCRM. PCRM is happy to see progress on updates to Guidance Document 34. Clarifying terms and readiness criteria and defining the technical validation process will help streamline NAMs adoption. She also praised the MDF, the ACD working group's recommendations, and the Complement-ARIE program. Public-private partnerships facilitated by FNIH will play a crucial role in acceleration of the validation and broader adoption of NAMs. She encouraged SACATM to support harmonization of regulatory acceptance criteria across agencies. She also called for all stakeholders to support activities to make human data available for validation of NAMs.

Clarifying questions and comments: There were no clarifying questions.

Comments from Designated SACATM Discussants: Session IIA

For this session, discussion questions were broken into subtopics and assigned to specific discussants. Discussants for the subtopic of "Validation Updates" were asked to consider the following questions:

- In what ways can OECD Guidance Document 34 be improved to support the validation of emerging toxicological methods, and what challenges and opportunities do we anticipate?
- What proactive steps can be taken to build upon the Method Developers Forum to develop follow-on activities and address current validation challenges?
- How can the learnings from the in vitro inhalation toxicity case study be applied to other areas of toxicology to enhance method validation, considering both challenges and opportunities?
- What future efforts are needed to ensure sustained progress in method validation?

Dr. Baran, first discussant, was impressed with the previous year's accomplishments, noting in particular publication of the VWG document. The challenge with updating Guidance Document 34 is going to be in addressing needs for validation of in silico NAMs. Validation guidance should not be prescriptive, particularly when NAMs are

measuring novel endpoints. He encouraged focusing on validation of the output rather than the technology, and cited the Biomarker Consortium as exemplifying how that might be done. Publication is an important aspect of validation but also presents challenges with protecting proprietary data or information. Transferring of AI technology between labs is a challenge, especially to ensure protection of intellectual property. Other challenges include clearly differentiating among specific use cases for a NAM and developing approaches to validating combinatorial NAMs. He agreed with the need for the addition of funding for validation. Data access is also important and challenging; this needs to be addressed collaboratively. It will be helpful to develop a framework for return on investment, including metrics; accomplishing this will require buy-in from regulators. He spoke to the need for collaboration across industries and with nongovernmental stakeholders. Formalization of collaborations with NIH will be helpful.

Ms. Leary, second discussant, reflected on the advancement of NAMs since she attended her first SACATM meeting in 2003. Publication of the VWG document is an important milestone that marks a new era of democratization of the validation process. Leadership of the working group is still needed, as they can serve to advise federal agencies how to act on the report's recommendations. It will continue to be important to monitor how some agencies are going to approach validation; in particular, initiation of new animal studies for side-by-side comparison with NAMs is not necessary and should be avoided. Human-based approaches should be encouraged and will ultimately be more protective of human health. Two recurring questions that have been raised by SACATM over the years are "What alternative methods are available?" and "Which ones will the agencies accept?" There are a lot of case studies that are building the body of knowledge, but to be relevant they need to address these questions. She encouraged agencies to continue to be involved in the application of NAMs and to clarify their acceptance criteria. Her organization is seeing an increase in grant applications over the past year, which is reflective of the increased interest around NAMs, and she spoke to the need for funding to capitalize on this interest. It will be important to have education around NAMs use that is not tied to commercial entities. She praised the Method Developers Forum and efforts to update Guidance Document 34, and cited the PETA-SCI inhalation project as an example of the kind of case studies that are needed.

Dr. Kambez Benam, University of Pittsburgh, third discussant, commented on how informative this meeting has been, reflecting engagement by diverse stakeholders. He emphasized the need to engage biologists, in particular academics who do not necessarily have expertise in toxicology, in developing approaches to NAMs validation. He also felt the need to articulate a clear value proposition for in silico NAMs within the context of their proposed applications, to clarify whether it solves the problem better than existing approaches. Method development platforms need to be developed with an eye toward supporting innovation. The PETA-SCI project illustrates an effective approach to focusing the scope of a project and developing a stepwise approach to addressing it. Any engagement with regulators will support the creation of a value proposition and can help develop the model in a productive direction. He welcomed the NIH initiatives; collaboration will be needed to support innovation, and collaboration needs to be supported by leadership.

Additional SACATM Comments

Dr. Page noted the importance of the Guidance Document 34 update and the inclusiveness of this process, and credited Mr. Kovatch for facilitating this. She pointed out that not all U.S. agencies have accepted NAMs to replace animal use for six-pack tests. She encouraged ICCVAM to continue work on this, especially in assessing toxicity of mixtures and inhalation toxicity. She emphasized the importance of cross-functional collaboration and cited the PETA-SCI project as a good example of this. Regulators should not need to be relied upon to drive these projects, although they need to be included in the discussion. Clear communication around NAMs' ability to protect human health will help acceptance of NAMs. She agreed with Ms. Leary about discouraging new animal testing for parallel validation. Dr. Kleinstreuer noted that a lot of work has been done in the past year to review ICCVAM agency representation on OECD workgroups, and a lot of refreshing of membership has been done as a result.

Dr. Berg supported the concept of technical characterization of individual methods. She felt that standards of performance characterization of NAMs components for variability and uncertainty were needed, and metrics for these, as a pre-validation activity. Establishing performance metrics on a small number of reference chemicals could be done as a prerequisite for formal validation testing. Many complex NAMs are not easily portable, and these prerequisites could help address that issue. She noted the benefits of collaboration, citing an example of how outside input revived a project that initially seemed infeasible.

Dr. Nañez noted how the MDF is facilitating connections needed between the developer and the end user. The NIH initiatives have the potential to impact both the regulatory and preregulatory spaces.

Dr. Thompson-Iritani also praised the MDF and expressed appreciation of what the PETA-SCI project accomplished in a very short time. Dr. Page agreed that projects should not wait for regulators to take the lead.

Dr. Ushio suggested that those planning the upcoming MDF around cardiovascular toxicity should engage people involved in Health and Environmental Sciences Institute projects in this area.

Comments from Designated SACATM Discussants: Session IIB

For this session, discussion questions were broken into subtopics and assigned to specific discussants. Discussants for the subtopic of "NAMs Pipeline: Future Directions" were asked to consider the following questions:

- Which recommendations from the NIH Advisory Committee to the Director Working Group on NAMs would you prioritize for ICCVAM to help implement?
- How can ICCVAM best engage with the Complement-ARIE Program, and how can its success be measured and expanded upon, considering the challenges and opportunities ahead?
- How can the VQN initiative be integrated into the broader NAMs validation pipeline to support continuous development, and what future efforts are needed to address existing gaps?

Dr. Price remarked on the overlap between activities in personalized medicine and toxicology. Digital twins and multi-omics analysis are two examples where there are analogous activities going on. There are a lot of other large computational efforts going on, ARPA-H[17] for example, that might provide opportunities for collaboration. He noted the complexity of validating combinatorial NAMs and speculated that AI might be productively leveraged. He agreed with the NIH working group recommendations, especially the need for high-quality interoperable data sets. Engagement is very important for driving efforts forward. He spoke to the need for sources of information on validated methods. Workshops are good avenues for engagement, especially if they are industry-focused to address the regulatory pathway. He agreed with other commenters on the need for good case studies, specifically citing the need for examples of how NAMs have or could improve on animal testing. He applauded Complement-ARIE, noting that it will be exciting to see how the centers develop. He suggested that large longitudinal human studies might be an important source of useful multi-omics data. Dr. Kleinstreuer responded by noting the example of skin sensitization as a NAMs success story. More broadly, skin sensitization, skin irritation, and eye irritation are endpoints where it has been conclusively demonstrated that human biology-based approaches improve on animal testing.

Dr. Silveyra spoke to the importance of training and dissemination of information on NAMs. Workshops and information repositories are important resources, and standardization will be important in building confidence. She encouraged activities to raise awareness of NAMs in academic arenas, for example through curriculum development. Appropriate statistical approaches are needed to ensure appropriate rigor. In addition to infrastructure and funding for collaboration, she spoke to the need to educate grant reviewers about the validity of NAMs and providing them with supportive metrics. She agreed with Dr. Price's comment about the potential value of longitudinal human studies.

Dr. Antonio Baines, North Carolina Central University, remarked on the recent progress that has been made in this area; he noted that he was pleased to have served on the ACD working group. Citing cancer biology as an example, he spoke to the importance of broad collaboration in moving progress forward. The working group recommendations will facilitate this. He emphasized the need for data infrastructure, as well as the need for interconnection of technologies and dissemination of information to all interested parties. He also noted the importance of incorporating population diversity into cell lines. Multidisciplinary teams will be needed to move NAMs forward, and these should include public health workers, chemists, biologists, etc. The Complement-ARIE program is a step in the right direction, but he encouraged ensuring that efforts are adequately funded and especially that smaller universities and companies are included in the funding opportunities. Training and communication will be important to this. Agencies need to be transparent about acceptance criteria for NAMs, and information about those criteria needs to be broadly disseminated. Information on NAMs should not be limited to the academic literature but made accessible to students and the public. He closed by encouraging a focus on the big picture of advancing biomedical research, recognizing that improving public health requires engagement of the public from the start.

[17] Advanced Research Projects Agency for Health: https://arpa-h.gov/.

Additional SACATM Comments

There were no additional comments.

Dr. Page thanked the day's presenters and discussants and adjourned the meeting for the day at 4:39 p.m.

September 18, 2024

Dr. Page called the second day of the meeting to order at 9:46 a.m. SACATM members and in-person attendees introduced themselves. Dr. Brownlow reviewed meeting logistics and read the conflict-of-interest statement.

IX. Developmental Neurotoxicity (DNT)

International Efforts Implementing NAMs for Assessing DNT in Chemical Risk Assessment

Dr. Iris Mangas, European Food Safety Authority (EFSA), presenting remotely, discussed OECD activities to advance the developmental neurotoxicity in vitro battery (IVB). Efforts to identify developmental neurotoxicants are complicated by poorly understood targets and pathways, as well as human outcomes that are not modeled well in rodents. The IVB is being applied to address this issue, and OECD Guidance Document 377 addresses evaluation of data from the IVB.[18] EFSA supported development of the IVB through literature searches to review available methods, co-sponsoring the proposal for development of Guidance Document 377, and developing relevant case studies. The main EFSA goal in applying the IVB is reducing and refining animal use to arrive at a more informative risk assessment, in particular for pesticide testing.

Dr. Mangas reviewed the key points of Guidance Document 377. Context of use is a key consideration for applying IVB data. Assays in the IVB measure processes critical for normal development of the nervous system. Reviewing the process of neurological development, Dr. Mangas noted the differences in critical time points between human and rodent models. The guidance document discusses in detail the elements of weight-of-evidence analysis. Issues to consider include interpretation of data both from individual assays and from the battery as a whole. EFSA has been considering data from the in vitro battery in dossiers for pesticide registration since 2019 and has published three case studies describing the use of the battery. The EFSA case studies first used a systematic literature review to assess data quality, then data from the battery were integrated into the adverse outcome pathway (AOP) framework. Data gap and uncertainty analyses were applied, and the conclusion was developed from the full range of information available. Regulatory implementation of the DNT battery will depend on a number of factors, including establishing lab-to-lab transferability, addressing biological uncertainties, development of methods for quantitative in vitro to in vivo extrapolation (IVIVE), and establishment of a reference chemical list. To address this last need, EFSA will soon be releasing a report recommending 164 chemicals to be used for this

[18] Guidance Document 377, "Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In-Vitro Testing Battery," available at https://one.oecd.org/document/ENV/CBC/MONO(2023)13/en/pdf.

purpose.[19] Another need is to standardize the analytical pipeline. There are a number of ongoing efforts to develop more case studies for pesticides; EFSA and partners are also working on guidance for specific contexts of use and developing AOPs for specific brain health endpoints. Reviewing lessons and learnings from the process, Dr. Mangas emphasized the need for international agreement on guidance and a standard workflow to facilitate use of the IVB in regulatory frameworks.

Clarifying questions and comments: In response to a question from Dr. Sura, Dr. Mangas noted that most of the assays in the IVB use human cells, although some use rodent cells.

Integrated Approaches to Testing and Assessment (IATA) Case Studies for DNT

Dr. Helena Hogberg, NIEHS, presented a DNT IATA case study developed by NIEHS; these case studies constitute an appendix to Guidance Document 377. A recent paper (Kreutz et al. 2024[20]) describes an NIEHS-developed case study on applying the DNT IVB to organophosphorus flame retardants (OPFRs). Dr. Hogberg reviewed the biological basis of the DNT battery in the context of human brain development. Some small model organisms in the battery enable evaluation of behavioral endpoints. Development of the NIEHS case study was a multistep process including gathering of existing data and generation of new screening data. Data sources for the case study included literature sources on both chemical effects and exposure, assay data from ICE, and new in vitro data. Chemicals examined included novel aromatic OPFRs and older brominated and other flame retardants that have been phased out of use but have more data available. Concerns surrounding the aromatic OPFRs include their similarity to pesticides known to have neurotoxic effects. ICE Curve Surfer was used to search for a broad range of assay data on these substances, and focused the analysis on assays that were more sensitive than the DNT IVB.

DNT battery results for the ten chemical examples indicated that they had a variety of activities. Overall, the aromatic OPFRs were highly active in the in vitro assays, with levels of activity similar to those of the halogenated flame retardants. Some substances were data-poor and no conclusions could be made; for example, tris (2-carboxyethyl) phosphine was only tested in one assay, but the fact that it was inactive in a behavioral assay may help prioritize it for further study. There was a lack of concordance among assays, and the significance of this needs to be further examined. There is also some uncertainty around the relevant mechanisms, which could be addressed by integrating additional data from ICE and literature. Zebrafish behavioral assays were found to be the most sensitive endpoint for several of the chemicals, and points-of-departures for some chemicals were lowered by integrating additional data from ICE and literature. Most of the sensitive endpoints were annotated to glial differentiation, immune process, and endocrine activity. Biomonitoring data helped contextualize the data in terms of human exposure. The most sensitive points-of-departure were found to overlap the range of human exposure, which might serve to prioritize specific chemicals for further study. In summary, novel OPFRs were found to have comparable in vitro activity to

[19] Mundy and Crofton, forthcoming.
[20] Available at https://doi.org/10.3390/toxics12060437.

older flame retardants, and the IATA suggests that some of these should be prioritized for future testing. Dr. Hogberg closed by discussing next steps to expand the regulatory applicability of the DNT IATA. EFSA and NICEATM are leading an OECD project to develop an IATA framework template specific for DNT, the goal of which is to streamline the approach to make it more easily applicable. A workshop in October will address specifics for this, with a framework expected to be finalized and approved by the OECD Working Party on Hazard Assessment by the end of 2025.

Clarifying questions and comments: Dr. Kleinstreuer asked about plans to expand data collection on exposure, and Dr. Hogberg responded that efforts are ongoing to obtain some data from Dr. Heather Stapleton at Duke University. Dr. Sura asked about a possible mechanistic basis for the observed lack of concordance among assays, specifically for oligodendrocyte differentiation and myelination. Dr. Hogberg responded that the battery does not assess myelination at this time but that there appear to be some concordance for oligodendrocyte differentiation and migration that could be related to endocrine activity for this class of compounds.

The DNT In Vitro Battery: Establishing Confidence in and Using Data from the Battery

Dr. Timothy Shafer, EPA, presenting remotely, discussed EPA's evaluation of how the DNT IVB aligns with their criteria for establishing confidence in NAMs. Reviewing the assays in the battery, he noted that while most of the assays provide structural information, the rat neural network formation assay provides information about the function of networks as they develop. He used the evaluation criteria articulated by van der Zalm et al.[21] to discuss an approach for building confidence in the assays. Confidence can be supported by examining why assays were included in the battery; in the case of the IVB, those criteria included readiness for use, availability of data from a common set of chemicals, analysis in the ToxCast pipeline, and detailed methodological descriptions. All the assays in the battery have been described in the peer-reviewed literature providing independent review. Bal-Price et al.[22] provided a ranking system for development and performance of the methods, and only assays that were highly ranked were included in the battery. A review of the battery, which was international and comprehensive, took place between 2017 and 2023, and included a 2020 review by an EPA scientific advisory panel. Dr. Shafer highlighted some comments from that review, including their recommendation for its use as a screening tool. Use of the battery within EPA initially focused on DNT reference positive and negative chemicals, chemicals with in vivo guideline studies, and chemicals of programmatic interest to EPA. Data from these studies are available via the EPA CompTox Dashboard. Dr. Shafer noted the lack of in vivo data for DNT and contrasted the limited availability and transparency of these data to in vitro battery data. ToxCast assays are all documented in alignment with OECD Guidance Document 211 on describing in vitro test methods.[23]

Turning to the human biological relevance of the assays, Dr. Shafer noted that most of the assays in the battery use human cells; only three use rodent cells. Key

[21] Available at https://link.springer.com/article/10.1007/s00204-022-03365-4.
[22] Available at https://doi.org/10.14573/altex.1712081.
[23] Available at https://www.oecd.org/en/publications/guidance-document-for-describing-non-guideline-in-vitro-test-methods_9789264274730-en.html.

developmental processes covered by the battery include proliferation, migration, apoptosis, differentiation, synaptogenesis, and gliogenesis/myelination. He reviewed the clinical conditions and the in vivo outcomes relevant to each of these processes and discussed the human relevance of the assays using rat cells. The network formation assay records electrical activity of neurons, the biological underpinnings of EEG recordings. Primary cultures of cortical neurons provide a good representation of the function of the frontal cortex, and activities of these cells is conserved across species. EPA has conducted two case studies using the DNT NAMs. The impact of use of the DNT NAMs is illustrated by the example of glufosinate; results of the DNT NAMs battery were used to justify a waiver for L-isomers, representing a cost savings of about $2M as well as substantial time savings. In summary, there is a consensus that the battery will continue to evolve and improve but there is utility to applying it now.

Clarifying questions and comments: There were no clarifying questions.

Integrating Screening-Level DNT Information of Chemicals in a NAMs Battery to Identify Chemicals for Future Study

Dr. Christopher McPherson, NIEHS, discussed how NIEHS is using DNT NAMs for prioritization. The NIEHS DNT Health Effects Innovation program was established in 2019 and has four primary objectives: (1) generate screening-level data for prioritization of additional studies; (2) conduct human-relevant mechanistic, behavioral, and brain network assessments to address neurodevelopmental issues; (3) contextualize in vitro and in vivo findings with human exposure using IVIVE and in silico approaches; (4) establish stakeholder networks to support global progress on DNT. The NIEHS DNT battery differs from the standard battery in that it incorporates a zebrafish behavioral assay. The main objectives of ongoing NIEHS screening efforts are to screen chemicals in a battery of assays that covers key neurodevelopmental events, evaluate these assays for redundancy, develop methods to rank chemicals for DNT potential, prioritize chemicals for further testing, and integrate data into a public resource, DNT-DIVER. Over 700 compounds were nominated for testing in this project; a number of criteria were applied to assign these chemicals to the three phases of the project. The data presented here focused on Phase 1 of the testing, which included 115 chemicals. Phase 2 will include an additional 108 chemicals; testing of these is about 70% complete and includes more chemicals considered to be “DNT negative.” The analysis pipeline for these data includes quality checks that are done before the data are loaded into the database. Benchmark concentrations (BMC) indicated a range of activity across assays; these assays covered a variety of endpoints including proliferation, apoptosis, neurite outgrowth migration, and behavior. A number of selectively active compounds were identified, meaning that they had neurotoxic activity at lower levels than general cytotoxicity. Breaking down activities by compound class shows some similarities in activity within classes. Calculation of the Toxicological Prioritization Index (ToxPi) showed that higher-ranking compounds included fungicides, drugs, and insecticides. The Pareto Frontier tool was also used to prioritize chemicals according to BMC, activity confidence scores from active endpoints, and fraction of active endpoints. Rankings of chemicals were similar for both ToxPi and Pareto Frontier rankings. An overall analysis of the results showed that the battery was more sensitive than the Tox21 data. In summary, the results of this study support use of the IVB in screening and prioritization. However, the IVB doesn’t fully replace animal studies for full mechanistic understanding,

and its limited chemical coverage is also a consideration for use.

Clarifying questions and comments: Dr. Kristini Miles, The HoneyPot Company, asked how botanicals and mixtures were incorporated into the first two phases of testing. Dr. McPherson replied that six botanicals with some evidence of neurotoxicity and heavy public use were included, and two or three different formulations were tested of each botanical. There were also some industrial formulations included, and for these, components and mixtures were both tested. Chemicals tested are listed on the website.[24] Dr. Monique Perron, EPA, asked about the analysis procedure for arriving at a BMC, in particular how this was done to avoid overfitting. Dr. McPherson replied that they examine individual curves, and they are confident about what they are calling a selective hit. Dr. Berg asked for clarification of activity vs. cytotoxicity. Dr. McPherson responded that most chemicals showed activity, fewer showed cytotoxicity. Dr. Sura asked about the activity of the statin tested in Phase 1 and whether its mechanism of action was considered. Dr. McPherson answered that the compound's mechanism of action for cholesterol modulation was not modeled by the assays in the battery, but the group is considering further testing of that compound in a myelinization assay.

Considerations and Challenges Associated with the Transfer and Implementation of Select DNT In Vitro Battery Assays in a New Laboratory

Dr. Megan Culbreth, U.S. Food and Drug Administration (FDA), discussed FDA Center for Food Safety and Applied Nutrition efforts to transfer the DNT IVB developed by the EPA into new laboratories. She reviewed the individual assays in the IVB, which were developed in three different laboratories. FDA was not able to directly transfer the IVB into their laboratories because most of the original cell models are not commercially available. The one assay for which the model was commercially available was the neurite outgrowth assay developed at EPA. FDA is making every effort to base assays on human induced pluripotent stem cells. Other differences in the FDA assays versus the EPA assays include use of a different imaging system and different image analysis algorithms. Concentration-response modeling was based on the EPA ToxCast Pipeline; quality control of raw and normalized data is assessed for all experiments. Data for cadmium chloride was shown as an example; Dr. Culbreth discussed criteria for response but noted that there is no consensus about how to compare data across laboratories. However, FDA's activity concentrations for cadmium chloride were similar to that derived by EPA. Evaluation of a set of performance compounds showed good agreement for some compounds between EPA and FDA outcomes but discordance for others. Importantly, there was concordance among expected inactive compounds. FDA had problems with the neuron count assay EPA used to assess viability and have been using a separate assay for this endpoint. Dr. Culbreth closed by reviewing the status of assay transfers to FDA. She noted that selection of performance compound or training sets has been a significant challenge as these often lack reference data. FDA's next steps will be to continue to develop their concentration–response modeling pipeline and metrics; evaluate performance compounds in transferred assays; and implement approaches used at other institutions such as multiplexed assays and organoid-based neural network formation assays.

[24] At https://www.niehs.nih.gov/research/atniehs/dtt/strategic-plan/health/developmental.

Clarifying questions and comments: Dr. Menghang Xia, National Center for Advancing Translational Sciences, asked about comparison of results between the cell viability with the neuron count endpoint of the EPA neurite outgrowth assay, which is used as the viability endpoints for this approach. Dr. Culbreth responded that FDA is collecting that data right now. They are using the Promega CellTiter-Glo assay for cell viability because they could not reliably detect effects in the neuron count endpoint even for expected cytotoxic substances. In a follow-up comment, Dr. Xia noted the variability in sensitivity of various cytotoxicity assays and the importance of picking one that is not overly sensitive. Dr. Sura asked whether the concentration-response models included human-relevant exposure concentrations. Dr. Culbreth responded performance compounds are selected not necessarily based on human-relevant DNT, but because the concentration-range evaluated has not been determined, they may or may not have implications for human health effects.

Public Comments

One written public comment was submitted for this section on behalf of HSUS.

Oral Public Comments

Randolph Ashton, Neurosetta, presented an update on their RosetteArray platform for assessment of human brain and spinal cord morphogenesis. A concern with the IVB is whether it adequately models the chemical's ability to cross the blood-brain barrier or the morphogenesis process of the nervous system. Neurosetta has addressed this issue by bioengineering neural organoid morphogenesis. In their system, neural stem cells create a rosette structure that can be implemented in a screening pipeline to assess effects on neural tube morphogenesis. Endpoints include cell viability proliferation, neural differentiation, and rosette formation. This is a scalable assay that can be used for screening applications for DMSO-soluble chemicals. Neurosetta has demonstrated high reproducibility and has integrated some human metabolism. They have published studies showing that we can model genetic causes of spina bifida and autism spectrum disorder. Dr. Ashton showed sample data comparing dose–responses of compounds with a variety of risk levels. The assay has high sensitivity and specificity, a caveat being that current data have a limited number of negative controls; Neurosetta is working to address this. This model has the potential to be applied to chemical screening, personalized disease model development, and precision medicine drug discovery.

Clarifying questions and comments: There were no clarifying questions.

Comments from Designated SACATM Discussants

Discussants for "Developmental Neurotoxicity (DNT)" were asked to consider the following questions:

- What future directions should NICEATM/ICCVAM consider to enhance the OECD and EFSA frameworks for developmental neurotoxicity testing?
- How can the insights gained from IATA case studies be used to develop more robust and predictive testing approaches for DNT, addressing both current uncertainties and future opportunities?

- What other criteria and methodologies may be needed to accelerate the use of data from the DNT IVB to ensure higher confidence in results while considering existing challenges?
- What strategies might be employed to facilitate the transfer and implementation of DNT IVBs across different laboratories, addressing potential challenges and leveraging opportunities?

Dr. Marty, first discussant, noted how challenging the DNT endpoint is due to its complexity. More challenging endpoints are going to require a battery approach for nonanimal testing, and therefore it is important to develop an approach to evaluating test batteries. The DNT battery still has some gaps in it, raising the potential to miss positive substances. Relating positive assay results to in vivo adverse effects is also still a challenge. She praised today's presenters for their commitment to data transparency (DNT IVB data are publicly available on ICE and the CompTox dashboard); she emphasized the importance of reaching a consensus about how to interpret data, establishing a set of reference chemicals for the battery, and defining performance criteria for the assays. In particular, it is important to understand how to interpret single positives within the battery (e.g., one positive assay of 17 assays in the battery). For example, for some multi-endpoint assays (e.g., microelectrode array, neurite outgrowth) compounds thought to be negative for DNT (e.g., L-ascorbic acid and saccharin in Dr. McPherson's presentation) may yield a positive hit in a subset of assay endpoints. A regulatory decision framework will be helpful. She encouraged using the case studies to explore development of additional AOPs. Training of all stakeholders in how to look at these data will be important, as is expansion of the chemical domain beyond its current predominance in pesticides. Accelerating use of data will be supported by development of fit-for-purpose validation of complementary or orthogonal assays. She spoke to a need for an intermediate step between the IVB and the in vivo mammalian assay; this might be addressed by use of organoid models or zebrafish, or other small model organisms, which have the advantage of having metabolic competence. A drawback of some small model organism assays is that they lack standardized methodologies, and she expressed a hope that ongoing NIEHS efforts in this area[25] will help with this. She also encouraged the development of IVIVE models that include placental transfer and the blood-brain barrier. She acknowledged the challenge of transferability, with issues including specialized equipment, differing high-content imaging platforms, and limited availably of cells. Communication from regulatory agencies of willingness to use data from the IVB with a specific timeline will incentivize contract research organizations to bring them in house. She reiterated the importance of standardized data management practices and reference compounds to support this, as well as funding.

Dr. Sura, second discussant, concurred with comments about the complexity of the endpoint and the gaps in the battery. She emphasized the importance of considering exposure in developing risk assessments; assessments of this will be supported by epidemiology and clinical data, as well as available animal data to help understand mechanisms. The IVB needs to capture all key events. Mechanistic information is very

[25] See https://ntp.niehs.nih.gov/whatwestudy/niceatm/test-method-evaluations/dev-tox/seazit.

important; the etiology of developmental disorders is not well understood. She encouraged development of biomarkers for receptor binding effects in signaling pathways or pathways such as cholesterol synthesis. She reiterated the importance of validation and consideration of development of a tiered-testing strategy.

Dr. Miles, third discussant, noted that it is going to take a worldwide effort to support acceptance of these models. The broad commitment to the overall goal to reducing animal testing is evident, and she was optimistic about the possibility for global regulatory acceptance of the IVB and its potential to improve on in vivo models. The IVB has an important role to play in prioritizing testing. She expressed an interest in seeing the IVB applied to complex mixtures, and agreed with Dr. Marty's call for need to broaden its use beyond pesticides. Dr. Miles encouraged consideration of nonchemical factors such as stress that can impact development and suggested that zebrafish could be used to examine the effect of conditions such as temperature and malnutrition. She also encouraged consideration of approaches to identifying neuroprotective substances.

Additional SACATM Comments

Dr. Page asked how amenable the battery is to handing complex mixtures. Dr. Hogberg replied that investigation of that is in progress. NIEHS is looking at mixtures with different modes of action in Phases 2 and 3 of its project, specifically mixtures from Superfund sites; NCATS is also doing some Superfund site mixtures testing. Dr. Shafer added EPA will also include these mixtures in Phases 2 and 3 of their project. These assays are all 96-well plate assays and they are amenable to testing defined mixtures. There is potential to use an array design to assess whether effects are additive. Dr. Culbreth added that FDA has assessed heavy metal mixtures and are comparing the data to single-compound data.

Dr. Berg commented that the battery assays appeared to have been selected from a standpoint of modeling neurodevelopmental processes, and wondered how those fit in with key characteristics of neurotoxicants. She noted the importance of avoiding redundancy in screening models. Dr. Shafer responded that a paper in preparation describes key characteristics of DNT chemicals, and it indicates that there is a lot of overlap between key characteristics and neurodevelopmental processes.

Dr. Benam commented on how several of the presentations today relied on retrospective analysis and suggested that prospective testing done in collaboration with method developers might better capture unmet needs. He also asked for some clarification on the definition of NAMs, specifically whether these include emerging technologies such as organs-on-chips or deep learning; we need to make sure we leverage emerging technologies. He also wondered whether focusing on key endpoints might be an approach to reducing the number of assays in the battery. Dr. Kleinstreuer responded that ICCVAM considers NAMs to encompass any technology that advances 3Rs, and definitely includes emerging technologies. Dr. John Gordon, U.S. Consumer Product Safety Commission, agreed and noted that NAMs also encompass assays that have been used for many years and have a good track record. Ms. Jen Goode, FDA Center for Devices and Radiological Health, noted that although many agencies are looking for human-relevant assays, some agencies need alternatives for animal testing for animal endpoints.

X. Computational Resources

NICEATM's Integrated Chemical Environment (ICE): Updates, Enhancements and Advances

Dr. Kleinstreuer, presenting on behalf of Dr. Brad Reisfeld (Inotiv), provided a summary of recent updates of NICEATM's Integrated Chemical Environment (ICE).[26] She noted that recent improvements in ICE have been inspired by feedback from SACATM, and this is a standing agenda item for this meeting. ICE contains high-quality curated in vivo and in vitro test data, in silico toxicity prediction and chemical property data, reference chemical lists and chemical lists of interest to stakeholders. Tools for chemical characterization and predicting toxicity democratize access to these resources to stakeholders that are not computational toxicologists. From July 2023 to August 2024, over 19,000 user sessions were initiated in ICE from 27 countries, and Dr. Kleinstreuer highlighted a few references describing various uses of ICE data. ICE includes over 5 million experimental data points that are grouped into data sets relevant to a variety of acute and long-term toxicity endpoints, as well as groupings such as "Functional Use" and "ADME Parameters" for absorption, distribution, metabolism, and excretion. An additional 50 million data points are available as property predictions, mostly from the Open (Quantitative) Structure–activity/property Relationship App (OPERA). ICE data are highly annotated, and version 4.1 added Open Biological and Biomedical Ontology (OBO) Foundry data annotations. NICEATM has also added versioning metadata to data sets, and ICE is interoperable with the NTP Chemical Effects in Biological Systems (CEBS) database and the EPA CompTox dashboard. Dr. Kleinstreuer reviewed the models used in ICE for property, toxicity, and exposure prediction; ICE version 4.1 added applicability domain data for OPERA predictions, available via both the graphical user interface (GUI) and the application programming interface (API). Another feature of ICE is its Chemical Quick Lists, which include Reference Chemical Lists of chemicals that cause a specified well-characterized biological effect and therefore can be used to assess the performance of an assay designed to measure that effect. Version 4.1 added quick lists for per- and polyfluoroalkylated substances and updated NTP Report on Carcinogens classifications.

Dr. Kleinstreuer highlighted updates to ICE tools:

- Search: this tool is used to interact with the ICE database, and users can utilize multiple types of chemical identifiers to query the database. Results are presented as an interactive table that can be filtered. Links allow users to explore properties of substances of interest and information on a substance in CEBS and CompTox.
- Chemical Quest: supports finding information on similar chemicals. Query chemicals can be uploaded, and there is also a drawing tool that can be used to build queries.
- Chemical Characterization: allows users to explore properties of a list of chemicals, compare two chemical lists, and obtain information on use categories or presence of specific chemicals in consumer projects.

[26] Available at https://ice.ntp.niehs.nih.gov/.

- Curve Surfer: enables interaction with high-throughput screening data. This tool's filter chain allows narrowing a data set to specific chemical types, activity ranges, etc. Version 4.1 added information on data flags to results cards to highlight features such as assay interference.
- PBPK: forward-dosimetry modeling tool to explore chemical dosimetry using models from EPA's httk package. Results show the concentration of the chemical over time in plasma and tissue compartments from a defined exposure, as well as the distribution of peak concentration (cMax) and steady-state plasma concentration of a drug (CSS) across all chemicals in a query.
- IVIVE: reverse-dosimetry modeling tool that extrapolates back from in vitro activity concentrations to predicted in vivo effective doses. Model predictions can be compared to legacy data from animal studies or human exposure predictions.

In addition to features noted above, ICE version 4.1 implemented a comprehensive update of user guides and help videos. Recent strategic activities to improve ICE included an external review of the user interface for usability and an audit of the back-end database schema and software stack. This yielded robust and actionable feedback related to ICE improvement, growth, and sustainability for future discussions and prioritization. Based on this feedback and feedback from other stakeholders, goals for ICE 4.2 include:

- Aligning ICE with invitrodb v4.1.
- Updating the Tox21 Chemical Quick List.
- Integrating Chemical Quick Lists into the data pipeline.
- Allowing the PBPK and IVIVE tools to analyze user-provided data.
- Updating principal component analysis plots in the Chemical Characterization tool.

Clarifying questions and comments: Dr. Silveyra asked if the PBPK tool has an option to select animal sex as an input. Dr. Kleinstreuer acknowledged that that would be useful but is not a current option. ICE does have a population simulator incorporated into the back end of models that integrates gender and ethnicity, but it is generic, and its main objective is to protect sensitive subpopulations. Dr. Nañez asked Dr. Kleinstreuer to characterize the risk assessors and other stakeholders that NICEATM consider to be the target audience for ICE. Dr. Kleinstreuer responded that these are primarily U.S. federal regulatory agencies, but training sessions NICEATM has presented at scientific meetings indicate that there is interest in ICE within a much broader audience, particularly in industry. Dr. Nañez then asked about the source of ICE data. Dr. Kleinstreuer responded that all data in ICE are drawn from public data. NICEATM has a wish list of data to add to ICE and is also querying stakeholders about data they would find useful.

Collaborative Modeling Project of Acute Inhalation Toxicity (CoMPAIT)

Dr. Kamel Mansouri, NIEHS, provided an update on an ongoing project to develop a model for predicting inhalation toxicity. This is the latest in a series of modeling projects

that started with EPA projects to predict endocrine disruptors.[27] This proceeded to a project to model oral toxicity,[28] which has been well received and applied most notably to predicting pesticide toxicity.[29] These prediction models were developed via crowdsourcing projects that combined multiple models in an ensemble approach to leverage the strength of each model. Predictions for chemical properties and toxicity generated by the models are available via OPERA. A 2016 workshop identified a need for a database of inhalation toxicity data as the basis of modeling projects. Collection and curation of these data has taken a number of years but is now ready for modeling. Dr. Mansouri reviewed the sources and data collected for the inhalation database, and briefly described the curation process involved. The final data set included 2109 entries for 1025 chemicals; these are mostly LC50 data, but some data represent limit tests or ranges. He also reviewed the 23 functional use categories represented in the data set. The modeling strategy identified endpoints of interest: LC50 for a four-hour exposure plus predictions of EPA Office of Pollution Prevention and Toxics, EPA Office of Pesticide Programs, United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS), and U.S. Consumer Product Safety Commission hazard classifications. Classification modeling was complicated by the fact that inhalation exposure may consider gas, vapor, and/or aerosol phases depending on the approach used by hazard classification. Unfortunately, many of the data did not have phase reported so a phase prediction approach was implemented. Ultimately, a decision was made to limit modeling to LC50 point estimates in mg/L and ppm. Training and evaluation sets of 612 and 153 chemicals were provided to the modelers; models were then used to predict LC50s for the set of nearly 50,000 quantitative structure–activity relationship (QSAR)-ready structures used for the acute toxicity modeling project. The inhalation modeling strategy consists of four steps: creation of models, combining single models into a weight-of-evidence consensus model, assigning physical states according to physicochemical properties, and applying the corresponding thresholds for the different regulatory classification systems. Evaluation will be according to the five OECD principles for QSAR validation, which considers endpoint definitions; transparency of algorithm; definition of applicability domain; appropriate measures of goodness-of-fit, robustness and predictivity; and mechanistic interpretation (if possible). Modelers were provided with training and prediction sets on July 1; models are due on October 1. The organizing committee will evaluate the individual models during November, with development and evaluation of the consensus model expected to be completed by the end of January 2025. He acknowledged the approximately 20 groups participating in the project, and the goal is to make the model available in 2025.

Clarifying questions and comments: Dr. Page asked for details about the substances nominated by EPA for the project, and Dr. Mansouri replied they are from a list of marketed chemicals compiled by EPA, including substances listed under the Toxic Substances Control Act chemicals. Dr. Benam asked for clarification of why the LC50 was settled on as the endpoint of interest rather than a less severe endpoint. Dr. Kleinstreuer responded that the LC50 is of interest because it is the regulatory guideline

[27] Mansouri et al. 2016, https://doi.org/10.1289/ehp.1510267; Mansouri et al. 2020, https://ehp.niehs.nih.gov/doi/10.1289/EHP5580.
[28] Mansouri et al. 2024, https://doi.org/10.1289/EHP8495.
[29] Bishop et al. 2024, https://doi.org/10.1016/j.yrtph.2024.105614.

test. Dr. Benam then asked if negative controls were included in the training set. Dr. Reinke replied that they were and elaborated that the chemical set is skewed toward moderate- to less-toxic compounds, and there were no highly lethal substances included. Dr. Miles asked how substances were categorized as "fragrances," and Dr. Reinke clarified that these were fragrance ingredients, not mixtures. In response to a question from Dr. Corie Ellison, Procter & Gamble, Dr. Reinke noted that the modeling data just included rat data, as that is the regulatory endpoint, and everything was standardized to a four-hour timepoint.

Collection of Alternative Methods for Regulatory Application (CAMERA)

Dr. Kleinstreuer provided an overview of development of the CAMERA resource. This was requested by ICCVAM's federal agency partners and also suggested by a recommendation of the ACD NAMs working group. After exploring whether this need could be addressed by existing resources, including the European Commission's Tracking System for Alternative Methods Towards Regulatory Acceptance (TSAR), NICEATM found that a new resource needs to be developed. NIEHS is funding the initial version of this resource, which will include a front-end user interface and a back-end database. A project manager has been recruited to develop CAMERA, and it will be maintained by NICEATM via the support contract. The resource will be interoperable with ICE, the Complement-ARIE data hub, CEBS, and other relevant resources. CAMERA is envisioned to be the landing place for NAMs that come out of the Complement-ARIE program.

Dr. Kleinstreuer posed questions to the committee about how CAMERA should be defined.

- What should be included in the database? This could include types of NAMs, validation stage, validation study data, acceptance or guidance, use-case examples, protocols, applicability domain information, context of use.
- How should it be developed to avoid redundancy with similar resources (such as TSAR)?
- How should users be able to interact with CAMERA? Options could include ability to search by multiple dimensions, leveraging NICEATM cheminformatics workflows to query applicability domains of methods or other dimensions.
- What should the interface provide? Intuitive, user-friendly; data presentation vs. graphics, downloadable reports?
- How do we select or prioritize NAMs to be added to CAMERA? Considerations could include technical characterization, validation status, or alignment with concepts articulated in the VWG document. The starting point will be the methods listed on the NICEATM website, many of which are OECD test guidelines. Other sources might be ASTM, USP, etc. Should user submissions be accepted, and from whom?
- Who will provide oversight of the resource? ICCVAM is establishing a standing steering committee to oversee the resource and is also considering holding a workshop to solicit input from stakeholders.

- How often should NICEATM and ICCVAM conduct a landscape assessment of new NAMs?

Clarifying questions and comments: Dr. Page asked whether CAMERA is envisioned to replace or supplement production of specific guidance from regulatory agencies, and Dr. Kleinstreuer replied that would be up to the agency. Dr. Perron added that it would also depend on the program, but that EPA is open to building off existing resources. Direct communication with stakeholders is going to continue to be important, and she encouraged stakeholders to continue to engage early with regulators. Dr. Page then asked what the CAMERA data submission process might look like. Dr. Kleinstreuer replied that is still an open question. Responding to a concern expressed by Dr. Woychik about duplication of resources, Dr. Kleinstreuer responded that NICEATM and ICCVAM envision that CAMERA will provide functionality that is not provided by TSAR but will be harmonized and coordinated. Dr. Thompson-Iritani asked about NICEATM's existing list of alternative methods. Dr. Kleinstreuer clarified that this includes refinement and reduction alternatives and reiterated that a central question for CAMERA will be whether replacement NAMs will be emphasized. Dr. Heather Patisaul, NIEHS, asked how CAMERA would accommodate evolution or improvement of methods. Dr. Kleinstreuer replied that it might be best for each method described in CAMERA to include a link out to the method developer's site. They would then be responsible for providing the most updated version of the protocol. Dr. Baran commented that the willingness of developers and other contributors to share data might end up being a major factor in what can be included in CAMERA.

Public Comments

There was one written comment submitted for this section from HSUS.

Oral Public Comments

There were no requests to present oral comments for this section.

Comments from Designated SACATM Discussants

Discussants for "Computational Resources" were asked to consider the following questions:

- What functionalities and data sets should be added to ICE to address stakeholder needs and drive future innovations in toxicological assessments?
- What potential collaborative/outreach projects can be initiated to enhance the impact of CoMPAIT, and who should be engaged in these efforts?
- What are the key types of information that should be included in CAMERA?
- How can the CAMERA database and user interface be best designed and leveraged to maximize its contributions?

Dr. Nañez, first discussant, encouraged thinking broadly about who in addition to regulators might be considered to be risk assessors. He emphasized the importance of providing training for ICE, and encouraged NICEATM to consider leveraging AI resources for interacting with it and helping users build queries. He encouraged

NICEATM to seek out companies that might be able to contribute different types of data to ICE. He felt that it will be important for CAMERA users to be able to search on acceptance by regulatory agency. He also encouraged structuring CAMERA in a way that would support decision-making by users and noted that a method that is not fully validated could still be useful for decision-making. He reiterated the potential usefulness of building AI resources into the user interface. Considering what the 3Rs focus of CAMERA should be, he noted that while replacement is the ultimate goal, reduction might be the road to get there. He encouraged consideration of how CAMERA might support a community-based approach to adoption of NAMs, as opposed to NAMs acceptance being driven by what methods are accepted by regulators. He supported the idea of holding a workshop to solicit stakeholder input, and closed by suggesting that a well-curated resource like CAMERA could support regulatory adoption of NAMs.

Dr. Ushio, second discussant, appreciated the data presented on use of ICE and the thought that has been put into the curation of the data for CoMPAIT. She encouraged consideration of both regulatory and preregulatory testing when considering the needs of the CAMERA end-user. She also noted the importance of encompassing a broad range of chemical types, specifically mentioning inclusion of both EPA- and FDA-regulated substances. Commercial availability of a method should be a consideration for inclusion in CAMERA. She spoke to the usefulness of having available models of different complexity to serve a variety of testing needs. For pharmaceuticals, she also noted the usefulness of models that represent specific disease states, as well encompassing both models focused on toxicity and models that serve a discovery/efficacy purpose. Other questions include whether CAMERA would be organized by chemistry or biology, and how it will include methods for biologicals testing. Channels for input will include 3Rs organizations and MPS working groups. She noted the importance of defining upfront what success will look like; user uptake will be an important measure of this. She closed by noting her appreciation of the evolution of focus toward human-relevant data in her time on the committee.

Dr. Ellison, third discussant, noted that this was his first SACATM meeting and was glad to hear that this is a standing agenda item. Areas for improvement for ICE could include looking at the robustness of the fingerprints being used for chemical similarity. He noted the limitations of Tanimoto scores, and that better results might be obtained with approaches that consider factors such as metabolism, reactivity, and physicochemical properties. He suggested integrating expert knowledge and decision trees into ICE alerts, as well as incorporating saturable metabolism into ICE models and automating connectivity between modules. CoMPAIT looks like a promising project; he suggested that its success will depend on the comfort of the regulatory community with the in silico predictions and the clarity of the mechanistic interpretation of the output. He would like to see these efforts applied to support development of safer chemicals. He encouraged looking at ways to bring academics into these modeling efforts, specifically giving students opportunities to see real-world applications for their skills. He agreed with other comments made about ensuring that CAMERA is not redundant with other resources. There seems to be a natural connection between CAMERA and the MDF. He encouraged starting small and adapt progress to response. More broadly, he encouraged consideration of how both expert knowledge and natural language processing can be leveraged to drive hypothesis generation and model development.

Additional SACATM Comments

Dr. Kleinstreuer emphasized that the NICEATM and ICCVAM portfolio is much broader than what has been covered in this meeting. In particular, she noted that NICEATM is looking at ways to improve the ICE Chemical Quest tool. NICEATM is developing a Modeling and Visualization Pipeline, a chemical similarity tool that expands beyond a structure-based approach, and NICEATM is looking at how to bring that functionality into Chemical Quest. NICEATM is also addressing the question of how to bring expert knowledge and natural language processing into knowledge building via a collaboration with BioBricks to develop knowledge graph networks. She expressed a hope to be able to present updates on both projects next year.

Dr. Page appreciated the EPA case study presentation that highlighted the cost benefit of applying the DNT IVB. She welcomed the updates to the ICE IVIVE tool and that ICE allows both searching and modeling of data. She noted how user-friendly ICE is but expressed an interest in more support for exploring mixtures and would like to see on-demand training resources. Dr. Kleinstreuer noted that there are several hundred defined mixtures in the database. She suggested that it might be useful to incorporate the GHS mixtures equation into ICE tool, and Dr. Page agreed that might enable users to explore their own defined mixtures. For CoMPAIT, Dr. Page noted the challenge involved in incorporating phase data for the chemicals and the approach that was taken to address that problem, and she expressed appreciation that manuscript development is part of the project. The CAMERA project has tremendous potential, and she expressed interest in participating in any future workshop.

Dr. Thompson-Iritani asked how industry regulatory affair groups are engaged in driving application of alternatives, particularly with respect to global markets. Dr. Kleinstreuer noted that NICEATM is engaged with the user community. Dr. Gordon added that regulators are responsible for being clear about what will be accepted, because people will not use data if they do not understand how to use it. CAMERA is going to be a good way to connect information about methods with information about how regulators are going to use data from that method.

Dr. Price asked whether users can upload their own information into ICE. Dr. Kleinstreuer responded that users can query the API with a list of chemicals and return data from ICE, and NICEATM is also exploring how to automate calling ICE workflows via the API. In response to a follow-up question about ICE limits on size and frequency of queries, Drs. Kleinstreuer and Mansouri noted that they are exploring how to improve handling of large queries. ICE is being transitioned to a new server, which should help with that. Dr. Price then asked for details on ICE use, and Dr. Kleinstreuer responded that NICEATM is exploring ways to better characterize ICE use, both by tracking literature citations and by web analytics. She noted use of ICE by regulatory authorities in Europe and Asia, as well as research institution. The most frequently used ICE tools are the Search function to integrate different datasets, and the IVIVE/PBPK workflows to model dosimetry or predict effective doses. A recent collaboration with Brazilian scientists related in vivo dosimetry to in vitro predictions; projects like these can illustrate use of ICE for risk management.

Dr. Silveyra asked what could be done via training or outreach to accreditation programs to facilitate ICE being used more broadly globally. Dr. Kleinstreuer agreed that tools like

these could democratize access to computational tools to communities that are disproportionately exposed to environmental chemicals. She noted how she is expanding her own outreach to Spanish-speaking countries, which has been very well received. NICEATM also translated the Strategic Roadmap into five languages and is translating the VWG document into six.

Dr. Baines asked how to get this information and tools into undergraduate and graduate education programs. Dr. Kleinstreuer noted that NICEATM engages directly with several universities and also conducts training at SOT. They are open to engaging with more groups and welcome partnerships to facilitate that.

XI. Adjournment

Dr. Patisaul thanked everyone for an exciting and informative meeting, both the presenters for their information and the committee members for their feedback. She was pleased that the meeting landed on a note of emphasizing health protection, especially in the global community. These technologies have a great promise for enabling development of safer technologies. She thanked Dr. Page for her work as chair, and Dr. Kleinstreuer and NICEATM for organizing a great meeting.

Dr. Kleinstreuer thanked the committee for their thoughtful and actionable comments that challenge ICCVAM and NICEATM to improve their efforts. She also recognized the contributions of the NICEATM support team and the ICCVAM members.

Dr. Page adjourned the meeting at 4:24 p.m.

Kathryn Page, PhD

SACATM Chair

Date: December 13, 2024