

Summary Minutes

**Scientific Advisory Committee on
Alternative Toxicological Methods Meeting
September 21-22, 2023
National Institute of Environmental Health Sciences
Research Triangle Park, NC**

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II. Location of Background Materials and Presentations

Background materials and presentations for the 2023 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

ADME	absorption, distribution, metabolism, and excretion
AOP	adverse outcome pathway
API	application programming interface
CATMoS	Collaborative Acute Toxicity Modeling Suite
CEC	contaminant of emerging concern
CPSC	U.S. Consumer Product Safety Commission
DASS	defined approaches for skin sensitization
DNT	developmental neurotoxicity
EPA	U.S. Environmental Protection Agency
FAIR	findability, accessibility, interoperability, and reusability
FDA	U.S. Food and Drug Administration
GHS	United Nations Globally Harmonized System of Classification and Labelling of Chemicals
h-CLAT	human cell line activation test
IATA	integrated approach to testing and assessment
ICATM	International Cooperation on Alternative Test Methods
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICE	Integrated Chemical Environment
IVIVE	in vitro to in vivo extrapolation
LD50	in traditional animal tests for acute systemic oral or dermal toxicity, the dose that causes death in 50 percent of the animals tested
NAMs	new approach methodologies
NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development

OPERA	Open (Quantitative) Structure–activity/property Relationship App
OPP	U.S. Environmental Protection Agency Office of Pesticide Programs
OPPT	U.S. Environmental Protection Agency Office of Pollution Prevention and Toxics
PCRM	Physicians Committee for Responsible Medicine
PFAS	per- and polyfluoroalkylated substances
PECO	population, exposure, comparator, outcome
PETA	People for the Ethical Treatment of Animals
ppmv	parts per million per unit volume
QSAR	quantitative structure–activity relationship
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
SEEM3	Systematic Empirical Evaluation of Models (U.S. Environmental Protection Agency)
TSAR	Tracking System on Alternative Methods

IV. Attendance

SACATM met in person at the National Institute of Environmental Health Sciences (NIEHS) in Research Triangle Park, NC, on September 21 and 22, 2023. The following individuals attended the meeting in person. In addition to participants named below, 126 people viewed the meeting via webcast on September 21, with 105 viewing on September 22.

SACATM Members

Antonio Baines, PhD, North Carolina Central University

Szczepan Baran, VMD, MS, VeriSIM Life

Ellen Berg, PhD, Alto Predict LLC

Denis Fourches, PhD, Oerth Bio

Sue Leary, MS, Alternatives Research and Development Foundation

Adrian Nañez, PhD, Servier, Inc.

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

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

Tamara Tal, PhD, Helmholtz-Centre for Environmental Research UFZ

Misti Ushio, PhD, Digitalis Ventures

Ad Hoc SACATM Members

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

Kristini Miles, PhD, DABT, Nouryon Chemicals LLC (virtual)

Nathan Price, PhD, Thorne Health Tech

Patricia Silveyra, PhD, Indiana University (virtual)

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

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

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

John Gordon, PhD, U.S. Consumer Product Safety Commission, ICCVAM Co-chair

Anna Lowit, PhD, U.S. Environmental Protection Agency (EPA), ICCVAM Co-chair

Other ICCVAM Representatives

Nicole Kleinstreuer, PhD, NIEHS

Charles Kovatch, EPA; U.S. National Coordinator, Test Guidelines Programme, Organisation for Economic Co-operation and Development

David Reif, PhD, NIEHS

Natalia Vinas, PhD, U.S. Department of Defense

NIEHS Staff

David Balshaw, PhD

Milene Brownlow, PhD, Designated Federal Officer

Dori Germolec, PhD

Robbin Guy

Helena Hogberg-Durdock, PhD

Kamel Mansouri, PhD

Jose Teofilo Moreira Filho, PhD

Robert Sills, DVM, PhD, DACVP

Nigel Walker, PhD, DABT (remote)

Mary Wolfe, PhD

Rick Woychik, PhD (remote)

NIEHS Support Contractors

David Allen, PhD (Inotiv, contractor supporting the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods [NICEATM])

Michaela Blaylock (Inotiv, contractor supporting NICEATM)

Alexandre Borrel, PhD (Inotiv, contractor supporting NICEATM)

Ella Darden (Inotiv, contractor supporting NICEATM)

Bridgett Hill, MS (Inotiv, contractor supporting NICEATM)

Anna Kreutz, PhD (Inotiv, contractor supporting NICEATM)

Shagun Krishna, PhD, (NTP/NIEHS, contractor supporting NICEATM)

John Maruca (Image Associates, contractor supporting the NIEHS Office of Communications and Public Liaison)

Steve McCaw (Image Associates, contractor supporting the NIEHS Office of Communications and Public Liaison)

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

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)

Catherine Sprankle, MS (Inotiv, contractor supporting NICEATM)

Public

Agnes Karmaus, PhD, Syngenta Crop Protection

Alison Harrill, PhD, EPA

Amy Clippinger, PhD, PETA Science Consortium International

Monique Perron, ScD, EPA

Anne Gourmelon, Organisation for Economic Co-operation and Development

Patricia Ceger, MS, RTI International

Brian Oliver, PhD, National Institute of Diabetes and Digestive and Kidney Diseases

Reshan Fernando, PhD, RTI International

Gina Hilton, PhD, PETA Science Consortium International

Elizabeth Baker, JD, Physicians Committee for Responsible Medicine

Bridget Rogers, MS, PETA Science Consortium International

Hans Raabe, MS, Institute for In Vitro Sciences

Jeffrey Davis, PhD, Syngenta Crop Protection

Brianna Jackson, MS, Syngenta Crop Protection

Annie Jarabek, PhD, EPA

Shaun McCullough, PhD, RTI International

Valérie Zuang, PhD, European Commission, Joint Research Centre (remote)

Shannon Bell, PhD, RTI International

Matthew Linman, PhD, Verto Solutions

Carrie-Anne Malinczak, PhD, Helaina

Hyun Wook (Daniel) Lim, RadaHaim

Megan Culbreth, PhD, PETA Science Consortium International

Ron Baron, MS, Physicians Committee for Responsible Medicine

Saniya Rattan, PhD, U.S. Food and Drug Administration

Tiffany Zapata, MS, Syngenta Crop Protection

Weihshueh Chiu, PhD, Texas A&M University

September 21, 2023

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 21. SACATM members and key National Institute of Environmental Health Sciences (NIEHS) staff introduced themselves.

Dr. Anna Lowit, U.S. Environmental Protection Agency (EPA) and co-chair of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) thanked the SACATM members for the time they spent preparing for and attending the meeting. She noted the importance of the committee's advice in the context of ensuring that regulatory decisions are based on the best science. She also thanked the attending members of the public for their comments and engagement. Dr. John Gordon, U.S. Consumer Product Safety Commission (CPSC) and ICCVAM co-chair, echoed Dr. Lowit's comments. He noted the important opportunity this meeting and others such as the ICCVAM Public Forum provide to interact with the public.

Dr. Nicole Kleinstreuer, NIEHS, director of the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) welcomed the committee members' participation and comments, noting that SACATM members are selected for their specific areas of expertise.

In welcoming remarks, Dr. Woychik noted that SACATM is a group of nongovernmental scientists charged with providing advice on the development, evaluation, and implementation of new approach methodologies (NAMs). Reviewing the agenda, he emphasized that efforts for updating approaches to validation to better accommodate NAMs are taking place internationally and noted the robust response to the request for comments on a draft ICCVAM document on that topic. The meeting also considered using NAMs to improve environmental health protection, and Dr. Woychik described the prospect of using NAMs to address population variability and characterize emerging contaminants as a critical and exciting opportunity. He noted the planned Complement Animal Research in Experimentation Common Fund project and promoted upcoming listening sessions.¹ He thanked participants for their attendance in person and remotely,

¹ Information available at <https://www.niehs.nih.gov/news/events/complement-arie/index.cfm>.

noting in particular the international participants. He recognized departing SACATM members Dr. Denis Fourches, OerthBio, and Dr. Tamara Tal, Helmholtz-Centre for Environmental Research UFZ.

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

VI. Five Years into the ICCVAM Strategic Roadmap: Full Replacement of the Acute Toxicity Six-pack

Roadmap Implementation Plans: Update on Each of the Six-pack Endpoints, How Close Are We to Replacement?

Dr. David Allen, Inotiv (contractor supporting NICEATM), reminded the group of the existence and purpose of ICCVAM's 2018 Strategic Roadmap.² So far there are Strategic Roadmap implementation plans for the endpoints associated with the acute toxicity six-pack (i.e., skin sensitization, skin and eye irritation, and acute systemic toxicity via the oral, dermal and inhalation routes). Three salient ICCVAM reviews published in 2018 and 2019 described agency needs, requirements, and opportunities for application of NAMs to the six-pack.³ A NICEATM collaboration with EPA has demonstrated that acute oral toxicity data can be used to waive the requirement for acute dermal toxicity studies, and EPA issued guidance for such waivers for pesticide formulations and pesticide technical chemicals in 2016 and 2020, respectively.⁴ NICEATM and ICCVAM developed in silico models for acute oral toxicity that can predict LD50⁵ at least as well as the in vivo reference test. NICEATM is now compiling data to extend this effort to inhalation data. Prospective testing has been done on human-relevant in vitro ocular irritation methods; testing on agrochemicals using these methods has supported a defined approach that accurately identifies potentially hazards for determining personal protective equipment requirements. Dr. Allen reviewed the adverse outcome pathway (AOP) for skin sensitization and noted where test methods inform on key events in the pathway. Guideline 497 issued by the Organisation for Economic Co-operation and Development (OECD) describes defined approaches for skin sensitization (DASS) based on this AOP, and Dr. Allen presented a NICEATM evaluation of DASS applied to predicting skin sensitization hazard for chemicals of interest to ICCVAM member agencies. A NICEATM collaboration with Unilever is using a Bayesian approach to extend application of DASS to predicting skin sensitization potency. An item on the OECD work plan will integrate this into an update of Guideline 497. Other activities were reviewed supporting progress toward six-pack replacement. In conclusion, Dr. Allen noted that efforts to replace animal use for dermal lethality and skin sensitization can be considered complete; ongoing activity for other toxicity areas include the use of additivity equations for predicting toxicity of mixtures and continued

² Available at <https://ntp.niehs.nih.gov/go/natl-strategy>.

³ Choksi et al. <https://doi.org/10.1080/15569527.2018.1540494>; Strickland et al. <https://doi.org/10.1016/j.yrtph.2018.01.022>; Strickland et al. <https://doi.org/10.1007/s00204-018-2341-6>.

⁴ Available at <https://www.epa.gov/pesticide-registration/bridging-or-waiving-data-requirements>.

⁵ In traditional animal tests for acute systemic oral or dermal toxicity, the dose that causes death in 50 percent of the animals tested.

development of human biology-based defined approaches.

Clarifying questions and comments: Dr. Priyanka Sura, Gilead Sciences, Inc., asked for Dr. Allen's thoughts about the GARDskin, a gene expression-based skin sensitization test proposed as an alternative to the human cell line activation test (h-CLAT), which is currently used in several DASS. Dr. Allen replied that NICEATM's evaluation of the GARDskin suggests that it looks to be an acceptable alternative to h-CLAT and would provide the first internationally harmonized test based on genomics and machine learning algorithms to be used in this context. In response to a question from Dr. Fourches, Dr. Allen stated that NICEATM was not provided the quantitative composition and identity of all ingredients in the agrochemical formulations that were tested in the in vitro eye irritation methods.

Modernizing the Acute Toxicity Six-pack for U.S. EPA's Office of Chemical Safety and Pollution Protection

Dr. Monique Perron, EPA Office of Pesticide Programs (OPP), reviewed the activities of the EPA Office of Chemical Safety and Pollution Prevention, which includes OPP and the Office of Pollution Prevention and Toxics (OPPT). EPA efforts to reduce and replace animal testing are tied to a NAMs work plan released in 2020 and updated in 2021.⁶ Several guidance documents have been issued that focus on acquisition of data that will support risk assessment in a meaningful way rather than just checking boxes.⁷ She also noted EPA's goal of applying NAMs that provide "equivalent or better" information.

Dr. Perron described specific EPA activities to reduce testing for each of the six-pack endpoints.

- OPP has had dermal waiver guidance for several years for both formulations and active ingredients.
- The Collaborative Acute Toxicity Modeling Suite (CATMoS)⁸ has been developed for potential replacement of the acute oral toxicity test, especially for nontoxic compounds; analysis and conclusions from this work will be published soon. She also discussed use of the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) mixtures equation for predicting toxicity of mixtures.⁹ OPP uses predictions derived from the GHS mixtures equation in a weight-of-evidence approach for assessing acute oral toxicity.
- Dr. Perron noted the challenging nature of the inhalation toxicity endpoint. OPP issued waiver guidance for this endpoint based primarily on physicochemical properties, and efforts are ongoing to build predictive models. There is also a pilot project to apply the GHS mixtures equation to the acute inhalation endpoint, as well as ongoing investigations of in vitro assays and in-depth reviews of species differences to better anchor NAMs application to an understanding of biology.
- OPP has a framework in place for eye irritation assessment of antimicrobial

⁶ Available at <https://www.epa.gov/chemical-research/epa-new-approach-methods-work-plan-reducing-use-vertebrate-animals-chemical>.

⁷ For examples, see <https://www.epa.gov/pesticide-registration/registration-requirements-and-guidance>.

⁸ Information at <https://ntp.niehs.nih.gov/go/tox-models>.

⁹ Discussed in Hamm et al. <https://doi.org/10.1016/j.vrtph.2021.105007>.

cleaning products, which is applied on a case-by-case basis for other pesticide classes. A paper to be submitted soon describes defined approaches for agrochemicals leveraging test guideline assays already available. OPPT has a draft decision framework for using existing data for eye irritation classification that will be released for public comment.

- Similarly, EPA is looking at how to leverage available test guidelines for skin irritation testing. A manuscript in preparation describes testing strategies that utilize these guidelines for pesticides, and OPPT is developing a decision framework for this endpoint.
- For dermal sensitization, EPA's interim draft science policy accepting results from DASS is still in effect, and EPA also accepts results from the DASS described in OECD Guideline 497.

Dr. Perron noted that metrics on NAMs application for OPP are available on the EPA website. Data from NAMs still represents a small portion of submitted data for pesticides. EPA is developing approaches for tracking animal reduction efforts more comprehensively. This includes development and reporting of metrics for OPPT. Collaborations are contributing to success in this area and ensuring that the data meets regulatory needs. Dr. Perron then described some of the challenges to advancing adoption of NAMs. Developing policy guidance documents is labor-intensive and these documents can sometimes be out of date by the time they are released; EPA is looking at ways to expedite this activity. A new policy council has been established to improve consistency across OPP and OPPT. International harmonization is crucial to eliminating animal tests, as is ensuring coverage of relevant chemical spaces (e.g., pesticide vs. industrial).

In summary, Dr. Perron noted that OPP has flexibility in data requirements which has helped with implementation of NAMs data. OPPT has a statutory mandate to consider availability of NAMs. Both offices continue to assess progress and extent of adoption of NAMs and work with national and international stakeholders.

Clarifying questions and comments: Dr. Tal asked if EPA tracked either the success rate or the time to acceptance for submissions that do or do not use NAMs, and Dr. Perron responded that they do not. Dr. Sue Marty, The Dow Chemical Company, asked about utility of CATMoS for identifying highly toxic chemicals. Dr. Perron replied that EPA has not yet implemented CATMoS programmatically. Currently, the data are promising for nontoxic compounds and it may be possible to use it in a weight-of-evidence approach for more toxic chemicals, but further investigation into its ability to be used for highly toxic chemicals is needed. Dr. Ellen Berg, Alto Predict LLC, asked if EPA had any idea how broadly waivers are being utilized among EPA's stakeholders. Dr. Perron answered that this has not been specifically tracked but it would not be unexpected for waivers to be more broadly used by larger companies.

[Beyond the Six-pack: Strategic Roadmap Future Priorities?](#)

Dr. Kleinstreuer suggested that most of the scientific work has been done for replacement of the six-pack. However, development and validation of the methods are part of the process, and it is also critical for regulatory agencies to communicate their existence and acceptance. EPA has stated their commitment to replacing animal testing

for the six-pack. Similar guidance and policy documents are needed from all regulatory agencies to achieve universal elimination of animal tests in both the human health and ecological areas. Public-private partnerships are needed to communicate to regulated industries that agencies are both willing and eager to accept non-animal methods and may prefer them in some cases. The OECD integrated approach to testing and assessment (IATA) case studies program is an example of how to demonstrate ways NAMs can be used in regulatory decision-making, as a step toward development of codified test guidelines. There is also a need to raise awareness within the legal arena, which considers animal testing as a “safe space” and is not aware of the advantages of human-relevant NAMs. She noted how nongovernmental organizations are actively providing education about this topic, citing the example of the Physicians Committee for Responsible Medicine (PCRM) NAMs Use for Regulatory Application continuing education series, as well as resources developed by the American Chemistry Council.

Besides education and communication, another major challenge that needs to be addressed is extending application of NAMs to complex mixtures such as pesticide formulations and medical device extracts. Partnerships between government and industry are needed to share data to build confidence in using approaches like the GHS additivity approach for predicting mixtures toxicity. She cited publications that have described the utility of these approaches.¹⁰ The next step is to formalize the acceptability of this approach in guidance documents and test guidelines.

Other key future directions for NICEATM and ICCVAM are defining agency- and endpoint-specific contexts of use and using those to define appropriate validation frameworks. There is also a need to develop human-based testing approaches for more complex endpoints such as cardiotoxicity, carcinogenesis, and developmental neurotoxicity. Work is ongoing within the NIEHS Division of Translational Toxicology to address these endpoints by looking at large numbers of chemicals. NICEATM and ICCVAM are also considering how NAMs could be applied to the challenge of improving environmental health protection. They could be used to characterize population variability and susceptibility by applying probabilistic approaches to protect the most sensitive populations. Another area is incorporation of NAMs into rapid response plans for emerging contaminants. Dr. Kleinstreuer closed by noting NICEATM’s upcoming workshop on gastrointestinal models.¹¹

Clarifying questions and comments: There were no clarifying questions.

Public Comments

Written public comments were submitted for this section from the Humane Society of the United States and the Humane Society Legislative Fund, and from People for the Ethical Treatment of Animals (PETA).¹²

Oral Public Comments

Elizabeth Baker, representing PCRM, praised the progress made in this area but

¹⁰ Chushak et al. <https://doi.org/10.1021/acs.chemrestox.0c00256>; Hamm et al. <https://doi.org/10.1016/j.yrtph.2021.105007>.

¹¹ Information at <https://ntp.niehs.nih.gov/go/gut-models-2023>.

¹² 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).

expressed disappointment that there had been no presentation by the U.S. Food and Drug Administration (FDA) in this session. She encouraged them and other less active agencies to prioritize replacement of the six-pack tests. PCRM is happy to collaborate to support these efforts. She noted the importance of updating policies to get new methods into practice. She closed her statement praising Dr. Kleinstreuer's vision for achieving full replacement and for showing her passion and leadership.

Comments from Designated SACATM Discussants

Discussants for "Five Years into the ICCVAM Strategic Roadmap: Full Replacement of the Acute Toxicity Six-pack" were asked to consider the following questions:

- What are your thoughts on strategies to fulfill complete replacement for all six endpoints?
- What are your thoughts on areas that should be prioritized in the future?
- What's next (low-hanging fruit) for strategies?
- What can be done to ensure harmonization in regulatory guidance on the acute tox six-pack among ICCVAM agencies and international organizations?

Dr. Sura, first discussant, agreed with Dr. Kleinstreuer on the need for guidance from agencies. These need to not only provide waivers but to recommend appropriate NAMs. Agency delays slow the adoption of NAMs by industry. More guidance is also needed from agencies about how stakeholders should use in vitro and in silico approaches, especially if they are not to be used as a one-to-one replacement for an animal test. Dr. Sura would like to see these approaches applied to pharmaceuticals as well as pesticides. She also stressed the importance of sharing metrics and documenting successes as a way for agencies to demonstrate to their stakeholders, especially smaller companies, that they are open to accepting alternatives. Development of implementation plans by agencies will help with harmonization, and this in turn depends on open communication within and among agencies.

Ms. Leary, second discussant, described the adoption of the Strategic Roadmap as a game-changer representing a moment of consensus among agencies. It is important to maintain that level of engagement among all agencies. Specifically referring to the U.S. Department of Transportation, she noted that agencies that do not have the kinds of research components that EPA has appear to be slower to move beyond the animal tests. Communication was one of the priorities identified in the roadmap and continues to be important in achieving replacement of the six-pack. Models have really improved, and there have been huge efforts on an international level in generating some key data and building scientific confidence. The challenge is that these good methods are not being used. Ms. Leary characterized the ICCVAM Metrics Workgroup publication¹³ report as disappointing but acknowledged that some agencies are making good progress on reporting metrics. Reporting metrics provides the transparency needed to support stakeholder confidence in NAMs. She cited the decision-tree frameworks presented in the morning's presentations as good examples of the kind of

¹³ "Measuring U.S. Federal Agency Progress Toward Implementation of Alternative Methods in Toxicity Testing", February 2021, available at https://ntp.niehs.nih.gov/sites/default/files/iccvam/docs/about_docs/iccvam-measuringprogress-feb2021-fd-508.pdf.

communication needed, as well as regulatory guidance. A waiver is not a clear endorsement of alternatives; more specific guidance is needed. Funding for communications could be more robust, which would reduce the need to rely on private organizations for training. Ultimately, progress will be achieved by creating an atmosphere where companies have a “fear of missing out”. Simple reliance on champions within agencies and companies will not be sufficient to drive progress. OECD is a great venue to set priorities. She also suggested that agencies could set up helplines that could give stakeholders the opportunity to discuss testing options with someone other than their agency contact.

Additional SACATM Comments

Dr. Berg felt that more effort needs to be put into quantitative measurements of progress toward reduction of animal use and implementation of alternatives to figure out why the latter are not being more widely used.

Dr. Misti Ushio, Digitalis Ventures, noted that validation is not sufficient for a new test method to be broadly adopted. The question of commercial viability has not been sufficiently addressed. Agencies requiring specific NAMs to be used might help drive that, but it is possible simply educating users might be sufficient.

Dr. Marty commented that international coordination is important because NAMs may not be used if the animal studies will still be required in other geographies. Frameworks are important for transparent and consistent use of NAMs. She noted the importance of incorporating both consensus (global) and mechanistic models for predictive tools [e.g., addition of mechanistic tools to quantitative structure–activity relationship (QSAR) prediction tools such as CATMoS would allow identification of acute modes of action (MOAs) and allow for more targeted follow-up work as acute MOAs also can be relevant for repeat-dose toxicity].

Dr. Antonio Baines, North Carolina Central University, encouraged inclusion of academic partners, especially smaller institutes, and specifically noted the support this sector could provide in investigations of mixtures toxicity.

Dr. Nathan Price, Thorne Technologies, asked about the existence of case studies where use of NAMs might have expedited regulatory acceptance relative to use of an animal test. Dr. Kleinstreuer responded that it depends on demonstration that the NAM will better protect human health. This has been demonstrated with skin sensitization, and evidence is accumulating that this is true for other endpoints. Once the NAMs are established as the safer standard, it will be harder to justify not using them. Dr. Price suggested focusing on a narrow area that will provide a clear demonstration. Dr. Lowit responded that in the pesticide space, there is a common international set of data requirements; countries that are slower to adopt NAMs hold up global implementation of those NAMs because companies, especially smaller companies, do not want to pay to test in both the in vitro and in vivo assays. The pesticide sector does not have an international cooperative body like the International Council for Harmonisation in the pharma sector where there is international coordination on acceptable testing approach. There’s more opportunity for progress in the industrial space because there’s no specific requirement; companies are happy to run NAMs because they are faster and stakeholders are actively looking for ways to speed up their processes. Dr. Szczepan Baran, VeriSIM Life, suggested that rewarding use of NAMs could be considered as an

approach to encourage their use.

Dr. Adrian Nañez, Servier, Inc., felt that sharing experiences with early-stage testing could be a good opportunity to demonstrate validity of NAMs.

Responding to Dr. Ushio's comment about requirements to use NAMs, Dr. Kleinstreuer felt that agencies might be able to advise considering NAMs in an endpoint-specific way and identifying specific tests. Dr. Lowit added that, considering the legal implications of the word "require," it might be more practical for agencies to say they "prefer" a specific method. Regarding EPA requirements for pesticides, while there is a suite of data requirements, the relevant statutes specify no technology requirements. Guidance can discuss how NAMs represent better science. OPPT has no data requirements, and there's an opportunity for dialog between stakeholders and regulators as to how to speed up the registration process or get a more favorable review. Dr. Ushio commented that it might be helpful to encourage people to think about what is most current, beneficial, and most relevant.

VII. Five Years into the ICCVAM Strategic Roadmap: Evolution of Validation

ICCVAM Validation Workgroup Report

Dr. Gordon reviewed the roster of the ICCVAM Validation Workgroup and noted the robust contribution from a diversity of agencies to their efforts. The original ICCVAM document on validation was published in 1997 and very much needed updating. A major goal was to build on the Strategic Roadmap, which recognized that one approach to validation was not going to work for all testing contexts. Building confidence in new methods is key to promoting their use. A main theme in developing the new validation document is flexible, fit-for-purpose NAMs validation, which incorporates independent review of qualification of a method for a particular context of use.

The draft document "Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies" was released for comment in August 2023.¹⁴ Dr. Gordon reviewed the key topics considered in the document and the timeline of development of the document, which has been ongoing since the ICCVAM Validation Workgroup's establishment in March 2021. The public comment period on the document ended September 5. Ten public comments were received, of which Dr. Gordon summarized some key points:

- Terminology: how to define NAMs. Difference between "predictive" and "protective"; "validation" vs. "qualification"; flexible vs. prescriptive language; "as good or better" concept.
- Role of ICCVAM in the future: promoting communication among stakeholders, ensuring clear and timely communication from agencies; concept of the "5Cs" (confidence, collaboration, clarity, communication, commitment); add an executive summary to the document that clarifies the role of this guidance in supporting diverse needs among agencies and stakeholders communicating

¹⁴ Available at <https://ntp.niehs.nih.gov/go/ICCVAM-submit>.

directly with individual agencies.

- Context of use: how to expand an existing context of use, and what information and evaluation are needed to do so.
- Additional topics: when a transferability evaluation is needed; biological relevance; standards for animal reference data; need for standards or use of “good practices”.

Clarifying questions and comments: Dr. Ushio asked for elaboration on the question of protective vs. predictive. Dr. Gordon responded that both words are used in the document, and it may need to be reviewed specifically to make sure both are used consistently.

International Harmonization and Global Considerations: Organizational and Financial Aspects of Validation Studies

Ms. Anne Gourmelon described OECD’s efforts to collect information about operational and financial aspects of validation studies. In 2022, the Chemicals and Biotechnology Committee discussed the future of chemicals assessment, expressing support for proposals such as establishing guidance for the validation of NAMs, standardized reporting templates to facilitate regulatory use, development of new methods for exposure assessments, and considerations of technical readiness of NAMs for regulatory use and acceptance. Some validation principles seem to be universal, such as relevance and reliability/transferability. However, validation practices need to evolve to move away from predicting animal effects and address practical realities of requiring ring trials, which are expensive and logistically difficult. She noted that while a few national and (supranational) European validation organizations still coordinate validation of methods in some cases, the current trend seems to be a model of decentralization of validation with method developers managing the validation, including small companies that are asking for guidance on how to do this.

In January 2023, OECD called for increasing public funding for methods validation. A project has been started to update OECD Guidance Document 34, and OECD also conducted a survey of validation practitioners on practical and financial aspects of validation to inform a workshop to be held in December 2023. Objectives of the workshop include:

- Collecting feedback on recent and relevant experience with validation of new methods.
- Identifying and understanding drivers of validation and sources of funding.
- Identifying issues and challenges in operational aspects of validation.
- Proposing pragmatic, example-based good practices to illustrate the update of Guidance Document 34, focusing on operational and financial aspects.

Ms. Gourmelon provided an overview of questions asked in the survey, the goals of which were to develop an overview of the validation landscape, document experience, and identify what challenges and solutions may come from practitioners. The survey closed September 15. About 120 responses were received that are being compiled for presentation to the workshop steering group, which is preparing the agenda for the

workshop. A preparatory webinar is being planned for November in advance of the December workshop.

Ms. Gourmelon provided some high-level information about the survey responses, including what sectors the respondents self-identified as and what countries they represented. Selected responses from the survey indicated that:

- Validation is an integral part of assay development and should be funded by consortia of entities that have interest in the methods.
- Repositories are needed for both lists of reference chemicals and the chemicals themselves.
- Standardized formats are needed for standard operating procedures, as well as online training materials for a method entering validation.
- To enable faster validation, data generated using a method should be published promptly using FAIR (findable, accessible, interoperable, and reusable) data principles.
- Only methods with a high readiness and clear use case should be validated.
- The number of labs needed to demonstrate transferability should be limited.
- Main drivers of costs include technology transfer and training; demonstrating between-lab reproducibility; chemical procurement; and coding, blinding, and shipment.

Ms. Gourmelon pointed out important contradictions from the survey responses, such as lack of clear consensus on who should be responsible for funding and organization of validation studies, or on the question of standardization vs. flexibility of validation models. The key challenges for the workshop will be to synthesize the survey responses in a way that informs potential solutions; identify economic models that are fair and feasible; discuss operational and financial responsibility; balance public and private contributions and interests; and find support from stakeholders for shared responsibilities that lead to concrete actions. The overall goal is a minimum set of common NAMs trusted by all to generate chemical safety data.

Clarifying questions and comments: Dr. Kleinstreuer clarified that while ICCVAM does not coordinate validation studies, NICEATM does coordinate validation studies at the behest of ICCVAM for high-priority endpoints and methods. NICEATM just concluded a validation study on the electrophilic allergen screening assay for skin sensitization and are conducting one on a thyroid activity assay. Dr. Fourches asked for the source of the figures Ms. Gourmelon had quoted on the cost of a validation study, and she replied that those numbers came from survey responses. They are not verified but were meant to convey a general idea of the cost of a validation study.

[International Harmonization and Global Considerations: Update of Guidance Document 34 and ICATM Position](#)

Dr. Valérie Zuang, European Commission Joint Research Centre, provided an update on efforts to revise OECD Guidance Document 34 and summarized discussion on this topic at the recent coordination meeting of the International Cooperation on Alternative

Test Methods (ICATM). Update of Guidance Document 34 has been a topic of discussion by ICATM since an October 2018 workshop. In November 2022, the Joint Research Centre submitted a proposal to OECD to update Guidance Document 34; this was added to the OECD work plan in 2023, with the U.S., European Union, and Netherlands co-leading the project. In December 2022, the OECD Working Group of National Coordinators held a workshop on emerging technologies. The August 2023 ICATM coordination meeting built on action items from the 2018 meeting and activities since. The consensus from all these meetings is while the principles of validation articulated in Guidance Document 34 are valid, the process for validation and international acceptance of test methods articulated in the document no longer reflects the current state of the art.

Dr. Zuang summarized key points from the December 2022 workshop.¹⁵ As a follow-up to the workshop the concept of technical validation has been characterized with emphasis on three features of a test method: (1) the biological relevance of mechanistic methods that could be a part of a DA or an IATA, (2) method readiness, and (3) within-lab reproducibility/well-designed transferability to a second lab rather than success of ring trials. Turning then to the August 2023 ICATM meeting, she noted that validation perspectives discussed included cost and time, transferability, reference chemicals, acceptance criteria, and peer review. The discussion emphasized that some quality systems should be in place to ensure data integrity and transparency, recognizing that this is becoming a greater issue because of the trend toward validation studies being conducted by developers. The OECD Guidance Document on Good In Vitro Methods Practices¹⁶ represents a good standard. An adequate method description is critical, and requirements for this should be laid out in a revision of Guidance Document 34, as well as guidance for selection of reference chemicals. One suggestion made was to describe the chemical space that should be represented rather than a fixed list of chemicals. There was also a suggestion that it might be useful to establish a category of test method that would have less stringent criteria for adoption than a test guideline. This category would provide a test method description and data on within-lab reproducibility, potentially providing flexibility for communication of a method that might be suitable for incorporation into a defined approach. A discussion of the need for ring trials established that a demonstration of reproducibility is essential but acknowledged the time and expense required and that the results of such trials are often more reflective of a specific lab's quality and expertise than of the quality of the method. The applicability domain of a method does not need to be defined a priori and can evolve as the method is used. However, the Guidance Document 34 revision should clarify this concept and provide an opportunity for the method to push boundaries. Most participants at the ICATM meeting felt that a central repository of validated methods was needed. Ways to encourage developers to contribute to such a repository were discussed, such as making it a funding prerequisite. It was agreed that standalone guidance on technical validation is not needed, but rather this could be discussed within the revised Guidance Document 34. However, it was noted that the scope of the revision of this document has yet to be defined. Specific suggestions made considered that the edit should remove redundant terms and concepts and streamline the document overall. The revision should address

¹⁵ Report available at [https://one.oecd.org/document/ENV/CBC/MONO\(2023\)14/en/pdf](https://one.oecd.org/document/ENV/CBC/MONO(2023)14/en/pdf).

¹⁶

flexible approaches to validation and incorporate features of the QSAR assessment framework; and the scope of the revised document should focus on validation and not include discussion of international acceptance.

The ICATM participants also discussed the concept of validation of defined approaches. Reviewing the difference between defined approaches and IATAs, Dr. Zuang noted that defined approaches can be validated and fall under the OECD Mutual Acceptance of Data agreement while IATA may not. However, there is a need for a confidence-building framework for IATAs, and in particular some type of characterization of any areas of uncertainty. There was only limited discussion by ICATM on how to validate methods intended as part of a defined approach, but two suggestions made were either to (1) validate the component methods and the defined approach concurrently or (2) provide an approach that would expedite the validation of the component methods.

Clarifying questions and comments: There were no clarifying questions or comments.

Public Comments

One written public comment was submitted for this section, on behalf of People for the Ethical Treatment of Animals (PETA).¹⁷

Oral Public Comments

Dr. Amy Clippinger, representing PETA, noted the high degree of alignment among stakeholders on the challenges that remain and the direction in which we need to go. She encouraged efforts to bring more people into the conversation through open meetings and training efforts. Considering confidence frameworks, regulators and companies willing to make the effort to use something new need to take advantage of the opportunities to leverage leading-edge technologies that were not available to us before. A new confidence-building framework is needed that adheres to key principles but leverages cutting-edge tools that are available to us now.

Comments from Designated SACATM Discussants

For this session, discussion questions were broken into subtopics and assigned to specific discussants. Discussants for the subtopic of “Updated ICCVAM Validation Workgroup Report” were asked to consider the following questions:

- Are all the key areas included in the validation workgroup report or are there any gaps?
- From the common themes that emerged in public comments on the report, which should be considered in finalizing the report, and how?
- How could NICEATM and ICCVAM better engage with method developers to ensure that the key concepts in the report are taken into account?
- What are the best ways for NICEATM and ICCVAM to enhance communication

¹⁷ Available at https://ntp.niehs.nih.gov/sites/default/files/2023-09/PublicComment_SACATM2023_PETA_508_Redacted.pdf.

between methods developer and agency representatives?

Dr. Berg, first discussant, encouraged people considering this topic to take advantage of new data streams and leverage new data sources, particularly human data. The current state of knowledge is growing very quickly and moving beyond trying to identify one-to-one replacements for animal tests for six-pack endpoints. Leveraging human data is going to be especially important for addressing complex endpoints. To gain confidence in NAMs, a common standard is needed for characterizing them that will bridge the gap between toxicology and medical research. Characteristics of these would include reproducible identification of key positive controls, as well as characterization of variability and reproducibility. Standards for validation should be tied to context of use, but characterization standards could be more generic and would lead to an improved level of technological readiness. There are several weaknesses in evaluating specific chemical lists and validation metrics targets. It might be better to establish chemical lists and metrics targets that are more application-specific. NICEATM and ICCVAM are well positioned to compile and communicate these standards. Validation steps should evolve and incorporate periodic review and updates. One suggestion could be to use a small number of chemicals to develop the NAM and then expand the list to evaluate it. Consideration of between-laboratory validation raises the issue of how to validate methods that cannot logistically be easily transferred from one lab to another. One solution might be testing of blinded compounds overseen by an external entity.

Dr. Ushio, second discussant, felt that demonstration of relevance to human biology is very important, beginning with confidence in the cell or tissue model, which can support confidence in the resulting data. Discussions of implementation of methods should consider technical and business feasibility, and the needs of the end user. Validation should increase trust and credibility and commercial viability is needed to justify validation, which can be supported by building trust in the method by the end user.

Dr. Baran, third discussant, focused his comments on how NICEATM can engage with method developers. Workshops and webinars are good tools for promoting engagement, but he suggested those efforts be expanded to hosting focus groups and developing manuscripts in collaboration with method developers. Alignment of terminology is needed; he cited the example of the IQ Consortium working with FDA to agree on a definition of “microphysiological system”, which was then communicated to method developers and other stakeholders. Any approach to validation needs to be method-agnostic, applicable to *in silico*, *in vitro*, or other types of methods, which would make it easier for the technology developers and end users to interact. There is also a need in validation to differentiate between regulatory and non-regulatory applications and to clarify these differences to method developers. More communication is needed both between method developers and regulators and among stakeholders from different industries. More conversations and transparency are needed about how guidance impacts regulatory decisions, even to the point of sharing submission metrics. He referred to the earlier discussion about how it is more practical to suggest regulators express a preference for NAMs rather than saying any particular method is “required”. Method developers could also benefit from help with curating data and data review, for example with understanding FAIR concepts and defining what metadata are needed. They could also help technology providers reach users with training and educational materials; suggesting that nongovernmental organizations and industry consortia that

focus on a particular technology could help with this. He encouraged technology providers to expand beyond their industry to promote uptake. Funding for training, distribution, and validation studies would help. There is a particular need to identify situations where long-term funding is required, as well as to reach out to countries where NAM data are not widely accepted.

Additional SACATM Comments

Dr. Tal agreed that it would be useful to establish domain-specific chemical test sets, particularly for complex endpoints, and suggested that NICEATM could facilitate compiling chemical lists, providing chemicals, and then providing data through its Integrated Chemical Environment (ICE). There is also a need for guidance on how to combine methods into IATAs. She suggested the developmental neurotoxicity (DNT) endpoint could provide a good case study for such an exercise. The amount and variety of data and models available provides an opportunity to identify minimum chemical set needed to identify DNT effects.

Dr. Kristini Miles, Nouryon Chemicals LLC, suggested that NICEATM consider opportunities to create exchange programs for scientists and regulators from regions that historically lag in use of NAMs.

Dr. Marty supported the suggestions made about training method developers and broader education of stakeholders. She felt that orienting toward method predictivity, rather than protectivity, might be more useful for human relevance. She agreed that reference chemical sets should define positive and negative chemicals in the context of the battery being evaluated and its intended application. It is also important to consider how the error rates of methods within a battery can build on each other. She also noted that a lot can be learned from applying assays to an IATA.

Dr. Fourches agreed with Dr. Marty's preference for assay predictivity over protectivity; however, any approach should be able to detect "activity cliffs", small structural chemistry changes that make big differences in toxicity. The idea of replacing chemical lists with a "chemical space" is very ambitious because a chemical space can be defined in many different ways, and the chemical space in turn can define the applicability domain of a model. For in vitro assays, applicability domain will depend on the technical characteristics of the assay, for example whether a compound can enter the cell. Discussions of evaluation and validation need to consider whether a NAM considers new modalities. Approaches to development of agrochemical are changing and NAMs need to keep up.

Dr. Sally Thompson-Iritani, University of Washington, commented that predictivity should be evaluated in the context of the species of relevance, for example for environmental impacts.

Dr. Lowit responded to the comments on reference chemicals by noting that there is a lack of clarity around what is meant by this. For example, in the DNT space there are a small number of chemicals known to affect the developing brain, while other chemicals thought to have effects have some questionable data. If these are included on the list, they can confound the results of the validation study. Causal relationships need to be clearly defined for any chemical placed on a reference list.

Dr. Page encouraged consideration of selection of chemicals for methods geared

toward testing of mixtures. She noted that predictive results can be applied in a protective manner. She expressed appreciation of how the Validation Workgroup's report emphasized on fit-for-purpose validation, flexibility, and international harmonization.

Dr. Gordon thanked the participants for bringing new points for discussion by the Validation Workgroup. He asked for thoughts from the group on how often the new document should be revisited and updated, and what should prompt those efforts. Specifically, he asked for suggestions on what would be important enough to prompt an ad hoc update of the document, considering that an update of the document would take about two years. Dr. Berg suggested that the document should be revised every five years.

Responding to Dr. Lowit's comments, Dr. Tal agreed that as endpoints become more complex, it will be more difficult to identify reference chemicals as there will tend to be a lack of strong epidemiological or animal data. As more is learned about biology, there's a potential for additional tests to be developed but the necessity for these needs to be clearly identified in terms of improving the battery by filling mechanistic gaps.

Dr. Baran described the experience of the IQ Consortium generating chemical sets; it is important to define these sets to ensure reproducibility of the assay. Dr. Berg agreed but felt that the right group of stakeholders needs to be assembled to define these groups of chemicals and that the lists need to evolve as well. Dr. Page added that human relevance should also be considered, and Dr. Ushio agreed.

Dr. Kleinstreuer acknowledged the centrality of the question of identifying reference chemicals and the difficulty when available data have questionable human relevance. Context of use needs to be considered for the method and the reference chemicals in combination; there is a need to first demonstrate that a battery is human-relevant before examining how the chemical perturbs it. She agreed with the usefulness of establishing repositories of not only chemical lists but also chemicals. She noted that ICE has lists of chemicals with metadata, as does OECD, and asked for suggestions regarding whether more detail is needed on these. Dr. Berg suggested that they might include characterization of evidence for being a positive control. Also, the selectivity of the assay for the mechanism of interest is important for interpreting false positives.

Dr. Perron noted the importance of distinguishing between a positive control and a reference compound. There is also a need to make sure that the effects being induced by a chemical are reflections of its biological activity. She expressed concern about the possibility of reference compound lists becoming a hurdle to validation of NAMs. She added that context of use will affect a reference chemical list.

Dr. Marty noted that epidemiological data for complex endpoints such as DNT is limited to what is available and may be confounded by factors such as poorly characterized exposures. There is a potential for functional assays using small model organisms such as zebrafish to be informative here.

Comments from Designated SACATM Discussants

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

- What are your ideas on how to move on with validation studies based on updates presented?
- Are there aspects of the guidance that need to be modified or revisited to account for international harmonization (e.g., OECD GD34)?
- What can be done to ensure harmonization in regulatory guidance among international organizations?

Dr. Nañez, first discussant, felt that identifying a battery to replace an established assay like the two-year carcinogenicity assay is going to require some degree of standardization. It is also going to be necessary to consider how to incentivize adoption of new methods; anything that will streamline development will be an incentive. Considering technical validation, he felt that Good Laboratory Practice adherence will be important to support confidence. Reference chemicals need to be selected with an eye toward their utility for reproducibility. Transferability needs to be demonstrated. Efforts toward harmonization need to start with harmonization across U.S. agencies that includes dialog with stakeholders.

Dr. Marty, second discussant, noted that while working with OECD can be time consuming, it is the best forum for achieving harmonization. For transferability, clear standard operating procedures are important. Alternatives to ring trials should be considered where appropriate; perhaps this can be done in a stepwise fashion. For example, a less complex method that meets criteria for an interlaboratory transferability and reproducibility study among three labs might not need to be validated further. For international harmonization, communication and collaboration will be needed; early engagement is important for adoption, and OECD provides an opportunity for that because it engages various stakeholders with a vested interest. She noted work that OECD has done toward addressing the issue of methods that incorporate confidential business information, which is a topic that could possibly be discussed in this forum. Performance standards cannot be too strict (either the method will not be accepted or subsequent users will not meet all of the performance criteria), but they need to be developed to support harmonization.

Dr. Thompson-Iritani, third discussant, asked for clarification about existing repositories and thoughts on why they were not being used. Dr. Zuang noted the European Union's Tracking System on Alternative Methods (TSAR) system, which allows tracking of a method through validation and regulatory acceptance. Reasons that have been expressed for developers' reluctance to use TSAR include the need to describe the test method in a standardized way and the extra effort needed to characterize a method to a greater level than is typically provided in a scientific article.

Additional SACATM Comments

Dr. Page encouraged looking at NAMs as a new data stream that can improve human health protection rather than simply replacements for animals. With regards to the Validation Workgroup document, she cautioned against repeating ground that has been covered by similar documents; it would be good to develop it with an eye toward showing where efficiency can be improved. She appreciated the effort the document made toward defining responsibilities. Regarding harmonization, she noted that a good

place to start would be with agreeing on common definitions. For example, even within the U.S., CPSC and EPA differ in their definition of eye irritation.

Dr. Kleinstreuer agreed that lack of harmonization of endpoint definition is a challenge. Regarding the question of performance standards and working with OECD, she noted that the IATA case studies are useful to help figure out whether a weight-of-evidence approach can be standardized as a defined approach, which is more objective. It is also necessary to evolve away from date-stamped test guidelines to methods that are described as living documents. Updating these is currently an administrative burden; it would be easier to have a website reference maintained by the developer. This approach could also work for performance standards, which could be designed to evolve as knowledge increases.

Ms. Gourmelon responded that at OECD the IATA case studies are seen as an opportunity to put NAMs in a specific context of use and build confidence around that context of use among OECD countries. One aspect of discussions around evolving validation has been consideration of how to decouple the technical validation of a method with the regulatory application, which is a need the IATA case studies can address. She noted how resources such as TSAR can be used to regularly update a test method.

Dr. Page thanked the day's presenters and discussants and adjourned the meeting for the day at 4:43 p.m.

September 22, 2023

Dr. Page called the second day of the meeting to order at 10:01 a.m. SACATM members and key NIEHS staff introduced themselves. Dr. Brownlow reviewed meeting logistics and read the conflict-of-interest statement.

VIII. The Role of NAMs in Improving Environmental Health Protection: Population Variability and Susceptibility

Using NAMs to Address Variability and Susceptibility Across Populations: Report from the October 2022 Symposium/Workshop

Dr. Helena Hogberg-Durdock summarized the rationale and goals of a 2022 webinar series and symposium/workshop that focused on NAMs to address variability and susceptibility across populations¹⁸. Key points made by symposium speakers included:

- While several U.S. federal agencies have invested in research initiatives that aim to address health disparities and inequities, there is a need to integrate efforts to identify, characterize, and solve environmental problems with communities at risk.
- Effects of chemical exposures are exacerbated by non-chemical stressors and other risk factors.
- Vulnerable and sensitive populations are falling through the cracks.

¹⁸ Information at <https://ntp.niehs.nih.gov/go/popvar>.

- The burden to deal with exposures should not fall on the communities or individuals affected; exposures should be addressed and mitigated upstream.

Dr. Hogberg-Durdock reviewed the questions considered by a panel convened during the symposium and questions discussed by workshop breakout groups. NAMs that can be applied to address these issues include in vitro assays, microphysiological systems, stem cell-based models, small model organisms, and integrating existing data with NAMs.

Take-home messages from the panel discussions included:

- Technical and logistical challenges in this area include getting large enough sample sizes to accurately represent variability, obtaining data that reflect real population variability, evaluating models to ensure they are characterizing relevant variation, and understanding the difference between variability and (lack of) reproducibility.
- Research areas to prioritize include moving beyond genetic and epigenetic variation when considering sources of variability, considering impacts of social stressors, applying learnings to regulatory decision-making, and identifying relevant susceptibility biomarkers and integrating data from those with existing toxicity data.
- Priorities expressed by susceptible populations include engaging with communities, getting their input and guidance, considering and learning from historical failures, and developing new strategies to prevent and mitigate harmful exposures.

Dr. Hogberg-Durdock then presented questions that were considered by the breakout groups. These discussions identified scientific, regulatory, and community aspects needed to build confidence in application of NAMs to characterizing population variability and susceptibility. Suggestions for interaction with communities included education in environmental justice issues; involving social scientists and bioethicists; transparent communication about timelines, results, and capabilities; acknowledgement of community-level concerns; and clear communication of the common goal to understand and improve human health. Barriers and opportunities to applying NAMs include funding for larger, more interdisciplinary projects; inclusion of more probabilistic risk assessment practices; the need for more knowledge about susceptibility factors and co-/cumulative exposures; the ability for NAMs to provide for evaluation of subpopulations and improve on animal studies in this regard; and connection with existing data on health disparities.

In conclusion, the workshop identified a need to build confidence in NAMs and prioritize community engagement when planning and conducting research that aims to address environmental justice concerns. Long-term, results oriented collaborations could help focus research on high-priority issues. The development of additional workshops and working groups will provide opportunities for NAMs researchers to engage with community leaders and advocates. Dr. Hogberg-Durdock closed by encouraging interested people to submit relevant manuscripts to a special issue of Human Genomics focused on the topic of NAMs to address population variability and susceptibility.¹⁹

Clarifying questions and comments: Dr. Tal asked about the community groups who

¹⁹ Information at <https://www.biomedcentral.com/collections/NAMAPVS>.

participated. Dr. Hogberg-Durdock noted that Dr. Shirlee Tan, Public Health – Seattle and King County, was among the speakers, and representatives from community groups also participated in the breakout sessions. She acknowledged that efforts to engage relevant interested groups were not entirely successful and represents an area for improvement in the future.

Building Confidence in New Evidence Streams for Human Health Risk Assessment

Dr. Weihsueh Chiu, Texas A&M University, provided an overview of a recent National Academies report on this topic. The panel was convened at the request of EPA to address challenges in applying NAMs for human health risk assessment; there are few existing examples of applying NAMs for this purpose. Prior National Academies reports have highlighted decreasing reliance on apical endpoints and guideline studies, increasing use of in vitro and computational approaches, increasing role of systematic review-based evidence assessment methods, and increasing coverage of susceptible and vulnerable populations. The current report recommends building on these findings to improve toxicity testing and human health risk assessment. One specific issue the panel engaged with was the definition of NAMs; they found EPA’s working definition to be too narrow, and encouraged EPA to broaden their definition to encompass the full range of strategies and approaches that can be informative for human risk assessment.

One of the charge questions to the panel from EPA was to review the issue of variability in traditional laboratory mammalian studies. The panel felt that variability across assay results is not fundamentally a negative attribute. Some variability can inform the distribution of the toxic response and in turn the generalizability of a study’s results. Thus, the report recommended that EPA refrain from identifying a threshold of acceptable variability across all NAMs based on laboratory mammalian studies.

Another area of focus for the panel was to integrate and bridge two tracks identified among previous reports on building scientific confidence in NAMs: systematic review-based approaches and scientific confidence frameworks. Dr. Chiu discussed the applicability of PECO (population, exposure, comparator, outcome) statements. These are used to frame human health hazard-related questions in laboratory animal studies but are not currently routinely used for in silico, in vitro, and nonmammalian toxicity tests. The report recommended defining “target human” PECO for each NAM to better support evidence-based reviews. Such a practice would be expected to clarify the context of use of a test method for human health risk assessment. He provided examples of “parallel” PECO for several toxicity testing approaches such as the two-year cancer bioassay, high-throughput screening approaches, zebrafish early-life studies, and murine local lymph node assays studies. Incorporation of PECO can help synthesize the findings of systematic reviews and scientific confidence frameworks.

Scientific confidence frameworks typically include components such as purpose and context of use, internal validity, external validity, variability, and transparency. Dr. Chiu presented a domain-based approach for the structured evaluation of external validity. This might consider biological considerations around population and outcome, exposure, and concordance of the test methods to human outcomes; he outlined the qualitative and quantitative considerations relevant to each. Integrating systematic review-based approaches and scientific confidence frameworks can also be approached by integrating

evidence from multiple streams that can include consideration of susceptible populations and life stages. Similarly, for dose response assessment, external validity informs quantitative adjustments between the experimental systems and humans, while experimental and biological variability provides insight into uncertainty and variability in the overall human population.

The report recommends that EPA develop and utilize a framework for hazard identification and deriving toxicity values protective of public that does not require human epidemiologic or laboratory mammalian toxicity data. Key takeaways include:

- Decreasing reliance solely on apical endpoints and “guideline” studies.
- Increasing use of in vitro and computational approaches.
- Increasing role of systematic review-based evidence assessment methods.
- Increasing coverage of susceptible and vulnerable populations.
- Broadening the definition of NAMs.
- Bridging design and use of NAMs to support increased confidence.

The goals of the committee recommendations are to provide a path to build confidence in NAMs data and approaches, prepare for a future where NAMs may be the sole basis for risk management, and identify an opportunity for NAMs to address long-standing risk assessment challenges and thus better protect public health.

Clarifying questions and comments: Mr. Charles Kovatch, EPA, asked for clarification of the PECO concept; Dr. Chiu replied that PECO defines the evidence to be evaluated to come up with a hazard assessment. He cited an example of how it is now possible to achieve a classification of a substance as “possibly carcinogenic to humans” solely on mechanistic data. Dr. Lowit noted there are a lot of contexts in which NAMs could be used; in the case of hazard identification, it makes a lot of sense for EPA to improve its approach to systematic review. On the other hand, for decision points where you have a specific assay proposed for a specific context, there’s no basis for a systematic review. Dr. Chiu responded that because the panel’s charge was focused on the human health risk assessment application of NAMs, that case was not considered. He suggested one could do a parallel evaluation between the in vitro and in vivo system and make a judgment call as to regarding validity. Dr. Lowit observed that there may be confusion on that point by readers of this report, and perhaps there’s a need for the report to better characterize the concept of systematic review. She suggested some important contexts of use are not being addressed by this report.

Fruit Flies for Precision Toxicology

Dr. Brian Oliver, National Institute of Diabetes and Digestive and Kidney Diseases, discussed his work using fruit flies for modeling population variability. The similarities between the genomes of the human and the fruit fly have increased interest in fruit flies as a model for human health and biology. Relevance is usually characterized as a question of genotypic similarity, but consideration of gene expression is also important. He compared gene expression in similar organs in different species, where similar patterns of expression can be observed, which he contrasted to the divergence in gene expression patterns between different cell types in the same organism. This can be

applied to studying rare diseases by humanizing model organisms, which can be done rapidly. He cited an example of using zebrafish for modeling and developing treatment strategies for a rare lymphatic system disorder. Applying this to precision toxicology, different models are exposed to the same chemical to identify conserved pathways relevant to the toxic response. Current studies are focused on cell lethality, which exhibits a common expression pattern among multiple models. Initially, the endpoint of lethality was used to normalize across species, but some common behavioral phenotypes such as vomiting began to emerge. This led to studies in which video was used to study and score behavioral phenotypes, which are categorized according to established ontologies.

Clarifying questions and comments: Dr Kleinstreuer asked if there were any ongoing efforts to look at epigenetic changes in response to environmental exposures. Dr. Oliver responded no, explaining that their current focus is on evaluating population variability in panels of inbred flies. He acknowledged that some of those same techniques could be applied to look at epigenetic changes. Dr. Marty asked how exposure and internal dose were extrapolated from fly to human. Dr. Oliver responded that the only thing done toward that was to measure food intake and excretion.

Public Comments

One written public comment was submitted for this section on behalf of PETA.²⁰

Oral Public Comments

There were no oral comments presented.

Comments from Designated SACATM Discussants

Discussants for “Population Variability and Susceptibility” were asked to consider the following questions:

- What topics should NICEATM consider for follow-up workshops on population variability and susceptibility?
- Are there any other ideas on how to proceed with this topic?
- What NAMs that consider population variability and susceptibility should NICEATM and ICCVAM focus on?
- What are some ways to engage environmental justice communities?

Dr. Tal, first discussant, noted the importance of population variability and susceptibility and that this is not adequately addressed in current risk assessment practices. The background materials SACATM was given for this session illustrated both the importance and challenges. She emphasized the need to understand the mechanisms of variability in susceptibility and highlighted that more case studies are needed.

²⁰ Available at https://ntp.niehs.nih.gov/sites/default/files/2023-09/PublicComment_SACATM2023_PETA_508_Redacted.pdf.

Zebrafish, Drosophila and C. elegans can all provide good models for this goal. Generation of NAMs with human-relevant genetic variants is an underutilized strategy, and Dr. Oliver's talk illustrated well how this might work with the rare diseases examples provided. She envisioned similar studies for toxicology, for example focusing on Phase I /Phase II metabolism enzymes. It is likewise important to consider other sources of variability such as the microbiome or age and life stage. She noted the utility of stem cells for developing stage-specific susceptibility for neurotoxicants for example. While the main focus of discussions at this meeting has been on human-relevant NAMs, she encouraged consideration of how these approaches could be applied to ecotoxicology, for example protecting pollinators or amphibians. It is also important to consider the impact of multiple stressors: socioeconomic stress, urban stressors such as light and noise pollution, and so on. There's a lot to be learned from ecotoxicologists who routinely consider the effects of multiple stressors on chemical susceptibility and how it is impacted by factors such as exposure to mixtures, climate change, and nutrient stress. These can lead to adaptations that exact a fitness cost to the organism. She closed by acknowledging the environmental justice aspect of this area; it is important to ensure that communities realize real benefits from their participation, for example by receiving funding for remediation.

Dr. Thompson-Iritani felt that the areas discussed in this session represent good sectors for NAMs use because the lack of data provides an opportunity for them to become the gold standard. This concept might be considered as a topic for a future workshop. She agreed with Dr. Chiu's assertion that variability is not necessarily a bad thing as long as the testing systems being used incorporate appropriate rigor, reproducibility, and standardization. Understanding variability can help us understand its impact on the system. She wondered whether digital twins might be constructively applied to this problem and suggested that as another potential workshop topic. She agreed with previous comments noting the importance of engaging the environmental justice community. She observed that these communities are often overburdened and collaborators should be cautious about giving them tasks that they do not have bandwidth for. She closed by suggesting that, as we discuss in silico models and similar approaches, we should keep in mind the environmental impact of high-performance computing.

Additional SACATM Comments

Dr. Baran agreed with Dr. Thompson-Iritani that digital twins might be a useful approach. He suggested that data centralization and access should be considered as a topic for a future workshop. He mentioned a recent National Academies workshop on veterinary applications of microphysiological systems, suggesting that the outcomes might inform measuring effects on different species.

Dr. Page expressed interest in funding NAMs in the academic space to study population variability, and the need to avoid over-engineering NAMs to encompass variability that may or may not exist.

Dr. Baines encouraged early engagement of environmental justice stakeholders to allow them an opportunity to develop the game plan. He stressed the importance of having people that look like the community members involved, which can be facilitated by engaging institutions such as historically Black colleges and universities that already

have relationships with the participating community.

Dr. Kleinstreuer noted the importance of not conflating different kinds of variability. The National Academies panel was charged with looking at variability in data from regulatory guideline in vivo mammalian toxicity studies that use genetically inbred strains and characterize this variability in the context of providing an appropriate benchmark for NAMs validation. She contrasted that to the consideration of human variability to characterize variable susceptibility to toxicity. Acknowledging Dr. Baines' suggestions about engaging communities, she named some of the communities engaged in the 2022 workshop. She stressed the importance of bringing a listening approach to these conversations so that scientists can learn from these groups in a way that can effectively inform future work.

Dr. Woychik echoed Dr. Kleinstreuer's comments and the importance of acknowledging and supporting the goals of the collaborating organizations, which are focused on improving their communities. He asked the group, in the context of induced pluripotent stem cells for in vitro testing, to consider how big a population would need to be sampled to effectively characterize human variability, and mentioned that this is part of the goal of the All of Us project.²¹ Dr. Berg responded that industry has traditionally used a minimum of 30 donors to represent diversity. She suggested that NAMs could play a role in integrating data from genetically variable populations.

Dr. Fourches remarked that human lymphocyte antigen markers might be a fruitful area of study for understanding variability in human susceptibility to toxicity, for example in the context of liver toxicity and immune response.

Dr. Price commented that resources like the UK Biobank and All of Us will be useful for characterizing genetic sources of susceptibility; he cited the example of variability in lead toxicity association with amyotrophic lateral sclerosis susceptibility. Perhaps identifying the extremes can reduce the number of samples we need to characterize variability.

IX. The Role of NAMs in Improving Environmental Health Protection: Coordinated Responses to Contaminants of Emerging Concern

An Interagency Initiative to Address Contaminants of Emerging Concern

Dr. David Balshaw, NIEHS, described the National Emerging Contaminants Research Initiative. A contaminant of emerging concern (CEC) is a material for which there is no regulatory standard, that may have toxicity at lower levels of exposure than previously characterized, is difficult to address across jurisdictions, and for which it is difficult to share information with stakeholders in a timely manner. Many of these are of interest to multiple federal agencies, which has given rise to efforts to coordinate addressing them. In 2018, the White House Office of Science and Technology Policy established a task force that looked at CECs in drinking water, which identified research gaps and opportunities for collaboration. This gave rise to the Joint Subcommittee on Environment,

²¹ Information at <https://allofus.nih.gov/>.

Innovation, and Public Health. A subgroup of this committee focuses on CECs; others focus on per- and polyfluoroalkylated substances (PFAS), sustainable chemistry, and veterans' toxic exposure. The mission of the CEC team is to coordinate federal programs and activities to address CECs; 18 agencies are involved including NIEHS, EPA, and others. Also in 2020, the omnibus National Defense Authorization Act required establishment of the National Emerging Contaminants Research Initiative; a report of this effort summarizes current government activities and future needs.²² Specifically, the report articulated a vision to provide access to clean and plentiful drinking water for everyone in the U.S. and specified five goals to achieve this. Three coordination teams will focus on (1) non-targeted analysis and effects-based monitoring to discover and screen CECs; (2) characterizing risk by assessing the potential hazards and exposure; and (3) formulating joint solicitations across agencies. This last activity will involve making a plan for how to work together to craft solicitations and manage them. A draft implementation plan is under agency review, with a goal to be published this fall. The plan outlines a series of short- and long-term activities that are anchored to success metrics. NAMs are seen as a critical tool for potential hazard characterization.

Clarifying questions and comments: Dr. Miles asked what criteria are being used to identify and prioritize CECs. Dr. Balshaw responded prioritization of chemicals is not within the committee's remit, and they are currently developing the criteria. Dr. Marty asked if the committee was developing criteria that would be applicable for a broad range of chemicals, citing difficulties with characterizing toxicity of metals. Dr. Balshaw replied that this is part of the committee's rationale for supporting development of new tools.

ICCVAM PFAS Workgroup

Dr. Vinas described the establishment and current activities of the ICCVAM PFAS workgroup. The term PFAS refers to a large and diverse group of chemicals whose properties tend to make them very persistent in the environment. The Joint Subcommittee report to Congress²³ provided a high-level overview of research on PFAS as a chemical class, which identifies gaps and opportunities for the federal government. A key finding of the report was that there is no universally accepted definition of what PFAS are; some encompass hundreds of chemicals, others encompass thousands. Areas of research and development identified in the National Defense Authorization Act included removal and destruction of PFAS, development of safer alternative chemicals to replace PFAS, understanding sources of PFAS contamination, and understanding PFAS toxicity. Dr. Vinas showed a graph that illustrated the breadth of organ systems potentially affected by PFAS and the differences in these among subclasses of PFAS. As part of an overview of challenges and opportunities around toxicity, she noted that development of PFAS-specific high-throughput assays was identified as a key area for toxicity evaluation.²⁴

A presentation of the Joint Subcommittee report to the ICCVAM committee early in 2023

²² Available at <https://www.whitehouse.gov/wp-content/uploads/2022/08/08-2022-National-Emerging-Contaminants-Research-Initiative.pdf>.

²³ Available at <https://www.whitehouse.gov/wp-content/uploads/2023/03/OSTP-March-2023-PFAS-Report.pdf>.

²⁴ The topic of high-throughput screening of PFAS is discussed further in: Smeltz et al. 2023. *Toxicol Appl Pharmacol* 459:116355; Patlewicz et al. 2022. *Comput Toxicol* 24:100250; Carstens et al. 2023. *Chem Res Toxicol* 36:402-419.

provided a rationale for the recent establishment of the ICCVAM PFAS workgroup. The workgroup's charges include:

- Evaluate the state of the science of current PFAS definitions and groupings.
- Evaluate which NAMs are currently being applied to PFAS by regulatory agencies, and assess their potential to fulfill regulatory requirements and address risk assessment needs.
- Identify requirements of different agencies for the use of NAMs for PFAS testing and risk assessment.
- Identify PFAS toxicity endpoints of interest to federal agencies, and commonalities in these among agencies.
- Identify research challenges and data gaps for the use of NAMs for PFAS testing and risk assessment.

The workgroup's deliverables include:

- Publication of a summary white paper (6-9 months).
- Workshop or conference on NAMs for PFAS (1-1.5 years).
- Manuscript in scientific journal that discusses outcomes of the workshop as well as challenges and data gaps and ideas to overcome these challenges with specific case examples (2-3 years).

Clarifying questions and comments: Referring to a table in Dr. Vinas' presentation that summarized target organ effects, Dr. Fourches asked whether blank cells meant that there is no effect on that organ system, or if there's no data for PFAS effects on that organ system. Dr. Vinas responded that they probably represent both cases, but she would have to verify that. Dr. Thompson-Iritani asked whether at this point the workgroup was favoring a broader or more narrow definition of PFAS. Dr. Vinas said that would be informed by the regulatory agencies' input. Dr. Kamel Mansouri, NIEHS, asked for a rationale for the focus on PFAS specifically rather than looking at them in concert with other chemicals that might share, for example, properties or functional groups. Dr. Vinas responded that a lot of the effects and problems that the workgroup is interested in are unique to PFAS; for example, a lot of existing QSAR models do not work well for PFAS.

Public Comments

No public comments were submitted for this section.

Oral Public Comments

There were no oral comments presented.

Comments from Designated SACATM Discussants

Discussants for "Coordinated Responses to Contaminants of Emerging Concern" were asked to consider the following questions:

- How much confidence do we need in NAMs before deploying them for use in an environmental event/emergency event?

- Are there other resources currently not considered that can be used in response to contaminants of emerging concern?
- Are there NAMs that have shown to be useful in testing PFAS? What are research needs to enhance testing these classes of compounds?

Dr. Baines, first discussant, expressed appreciation for the current interest in CECs and PFAS by ICCVAM and the broader federal government, and especially the interagency aspects of these efforts. Regarding deployment of NAMs to address environmental emergencies, having NAMs to detect toxins in the environment either for ongoing surveillance or assessment after events would be very useful. However, they need to provide reproducible and consistent results to inspire confidence. At the same time, we need to be careful not to hold NAMs to a higher standard than traditional tests. Monitoring environmental species and wastewater might be good strategies for water quality monitoring. He stressed the importance of developing NAMs that could be used to test airborne contaminants as well as those in the water supply. He asked that students and trainees be included in these efforts; in addition to helping educate them about NAMs, it might generate new ideas about how to approach these problems. High-throughput screening will be a key approach to address characterization of PFAS and CECs. A better understanding of these substances will enable addressing any health effects they might induce.

Dr. Patricia Silveyra, Indiana University, second discussant, agreed with Dr. Baines' comment about the importance of involving trainees. It is also important to consider how these substances might induce toxicity at multiple levels, such as on the endocrine system. Integrating different testing methods will support a better characterization of toxicity. She encouraged both Dr. Balshaw and Dr. Vinas to consider community engagement as part of their groups' mandate. In particular, she noted the importance of communicating findings in a timely manner, which can build trust in science within communities.

Additional SACATM Comments

In response to Dr. Silveyra's comment about community engagement and Dr. Baines' comment about the certainty of measures, Dr. Balshaw emphasized that scientists must be careful not to use uncertainty as an excuse not to engage with the communities. It is important to be honest with the affected communities about both what is known and the relevant uncertainty.

Dr. Fourches noted that it is unlikely that every chemical identified as PFAS can be tested, simply because some of them will be hard to obtain. Perhaps efforts should focus on characterizing, for example, the ten that are most abundant in the environment, and formulate questions that will be broadly informative. He also encouraged consideration of the utility of QSAR models and seeking data from industry partners on properties such as degradation in soil.

Dr. Tal commented that in addition to the number of chemicals, it is also important to consider that many of these substances are in mixtures, as well the potential presence and effects of unknown precursor compounds. Researchers need to avoid the temptation to just repeatedly test the usual suspects. Dr. Vinas noted her agreement

with this comment.

X. Update on NICEATM Computational Resources

The Integrated Chemical Environment: Open-access Tools to Support Chemical Evaluations

Ms. Victoria Hull, Inotiv, provided a summary of recent updates of NICEATM's Integrated Chemical Environment (ICE)²⁵ Specific information about ICE updates can be found in release notes. She reviewed the types of data available through ICE and noted that a lot of work this year has focused on harmonizing and updating data sets. Large data exports from ICE can be facilitated by use of the ICE application programming interface (API). ICE also includes predictions of chemical properties from the Open (Quantitative) Structure–activity/property Relationship App (OPERA) as well as predicted data on exposure from EPA's Systematic Empirical Evaluation of Models (SEEM3). She reviewed the available ICE Chemical Quick Lists; additions to these for 2023 include "Mixtures and Formulations ICE" and "Toxcast Phase I, Phase II, and e1k". Proceeding through an example workflow, Ms. Hull described new features of the following ICE tools:

- Search: now supports use of chemical names and synonyms as input; provides access to new and improved data sets.
- Chemical Quest: allows identification of chemicals in ICE that are similar to a query chemical; no major updates this year.
- Chemical Characterization: provides an opportunity to explore chemical properties. The former "Consumer Use Categories" visualization within this tool was rebranded this year as "Curated Product Use Categories" to reflect a broadening of the information provided. The updated tool also provides heatmaps to visualize OECD and predicted functional use categories for a chemical. These functional use categories are derived from EPA's Chemicals and Products Database (CPDat).
- Curve Surfer: allows graphic exploration of high-throughput screening data in ICE; no major updates this year.
- Physiologically Based Pharmacokinetics: has a new gestational physiologically based toxicokinetic model; its inhalation model now accepts concentration in units of parts per million per unit volume (ppmv).
- In Vitro to In Vivo Extrapolation (IVIVE): has the new gestational model; inhalation model now accepts concentration in units of ppmv; EPA SEEM3 exposure predictions can be overlaid on graphic outputs of equivalent administered dose.

Work is in progress on providing endpoint-specific visualizations of ICE Search results and incorporating genetic variability in metabolism enzymes into physiologically based pharmacokinetic models.

Clarifying questions and comments: Dr. Price asked about the source of the

²⁵ Available at <https://ice.ntp.niehs.nih.gov/>.

exposure data, and Ms. Hull explained the SEEM3 model.

OPERA: Open-source QSAR Models for Regulatory Support

Dr. Mansouri, NIEHS, provided an update on OPERA. OPERA provides QSAR-based predictions for several chemical properties and endpoints. OPERA models support OECD principles for QSAR models for regulatory purposes. OPERA was built with open-source code, and its algorithms and performance are transparent, as are the applicability domains and limitations of the models. It can be run via a command-line interface or a graphical user interface and has a variety of input options, including structure identifiers and files. OPERA 2.9 updated a number of models for physicochemical properties and added models for absorption, distribution, metabolism, and excretion (ADME) properties; this update also added information on chemicals of regulatory interest. Version 3.0 is coming soon. Referring to earlier discussion of PFAS, Dr. Mansouri noted that OPERA has been shown to effectively predict properties of PFAS, even in earlier versions where PFAS were not included in the training data. OPERA includes data from three collaborative projects to predict estrogen activity, androgen activity, and rat acute oral toxicity. Many users focus on OPERA's predictive capability but it is important to keep in mind that OPERA also provides information on applicability domain, accuracy, nearest neighbors, confidence interval, molecular descriptors, and it uses standardized chemical structures. Reiterating a point made by Ms. Hull, Dr. Mansouri noted that OPERA predictions are available through ICE as well as via the OPERA tool and can be obtained using the ICE API. OPERA is also available via the PrecisionFDA²⁶ computational tools platform and as an extension to the OECD QSAR Toolbox.

Clarifying questions and comments: In response to a question from Dr. Casey, Dr. Mansouri reiterated that all predictions in ICE are in OPERA. Dr. Fourches asked if neighbors are shown in outputs, regardless of whether the k-nearest neighbors modeling approach was used. Dr. Mansouri responded that is correct; the descriptor selected for the specific model is used to identify the nearest neighbors. Referring to a tool available in ICE, Dr. Marty asked if users could upload ADME parameter data for IVIVE models. Dr. Mansouri replied no, and Dr. Kleinstreuer added that while that is not currently possible, users can upload their own in vivo and in vitro endpoint data. She indicated that NICEATM would take her suggestion as an action item for a future ICE update.

Web Application to Predict Skin Sensitization Using Defined Approaches

Dr. Kim To, Inotiv, gave an overview of the DASS App, a web application that uses accepted defined approaches to predict whether a chemical might be a skin sensitizer. Reviewing the AOP for skin sensitization, she identified test methods that are aligned with key events on the AOP. While these methods are not recommended for use on their own to identify skin sensitizers, three defined approaches incorporating these methods have been accepted by regulatory agencies for predicting whether a chemical might be a skin sensitizer. These methods are structured and do not require expert judgment but applying them can be difficult, especially for a large data set. NICEATM developed the DASS App to address this limitation. The DASS App provides a five-step protocol for selecting a defined approach, uploading and preparing data, and viewing results. In

²⁶ Available at <https://precision.fda.gov/>.

addition to context-specific instructions in the interface, the app has a downloadable user guide. Results can be downloaded as an Excel or tab-delimited file.

Clarifying questions and comments: There were no clarifying questions or comments.

ChemMaps.com v2.0 – Exploring the Environmental Chemical Universe

Dr. Alex Borrel, Inotiv, explained the concept of chemical space as the positioning of chemicals relative to each other according to relationships established by a particular set of molecular descriptors. The principles used by tools such as Google Maps for visualizing geographic space can also be used to visualize chemical space. NICEATM developed ChemMaps.com as a way for users to navigate chemical space efficiently and visualize relationships among chemicals, their properties and structures. The first version of ChemMaps, released in 2018, was limited to the 50,000 chemicals in the EPA's Toxic Substance Control Act inventory and the Canadian government's DrugBank tool²⁷. ChemMaps v2.0, released earlier this year, added data on about one million environmental chemicals, and data displayed include activity concentrations from Tox21 and ToxCast high-throughput assays. ChemMaps resides on a freely available website. Using the PFAS space as an example, Dr. Borrel showed how chemicals can be located and properties visualized interactively. Users can choose what to visualize from over two dozen structural, physicochemical, and bioactivity properties. Future plans for ChemMaps include a standalone version, training material, customizable map, linking to ICE, and incorporating bioactivity data from other sources.

Clarifying questions and comments: Dr. Tal asked how specific chemicals are located on ChemMaps and how chemical groupings can be visualized. Dr. Borrel replied that the chemicals are positioned relative to each other according to relationships established by a particular set of molecular descriptors. A user can begin a search by choosing a specific bioactivity property to explore or by entering the name of a particular chemical and exploring the space around it.

Public Comments

There were no written comments submitted for this section.

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 other tools would be useful or should be prioritized?
- Of the tools presented, are there modifications or additions that would enhance their utility?
- Do you have ideas on promoting or outreach to communicate availability of the

²⁷ Available at <https://go.drugbank.com/>.

tools?

Dr. Fourches, first discussant, noted that NIEHS is on trend with their application of artificial intelligence to toxicology. He congratulated the ICE team for implementation of the API and inclusion of exposure data, and in general for their responsiveness to implementing SACATM suggestions. He encouraged adding more formulation data for mixtures, data or links to information about binding sites, and more data about metabolism. Noting the figure of 6000 downloads cited for OPERA, he expressed interest in seeing metrics about how much ICE is being used and how many results are being downloaded. Regarding OPERA, he emphasized the importance of communicating the limitations of the data used to build the models, but added that OPERA was doing a good job of implementing best practices for structural curation and noted the significance of the launch of the OECD toolbox plugin. He felt that ChemMaps having direct links to ICE and EPA's CompTox Chemicals Dashboard will improve its utility. Over the next 10 years, the chemical space of, for example, agrochemicals will change; many of the tools that work well today will have to be retrained, and this needs to be anticipated during the development of new tools. It is also important to keep in mind what types of chemicals, for example cosmetics, might be missing from a chemical space. Responding to Dr. Fourches' comments, Dr. Kleinstreuer noted that in three months this spring users initiated between 600-900 ICE sessions per month. Dr. Mansouri noted that OPERA supports a QSAR modeling reporting file format that details all the technical information about the training set, as well as an applicability domain index.

Dr. Price, second discussant, focused his comments on how he envisioned these tools might be used. He encouraged the developers to broaden their concept of how users might interact with databases, for example providing the ability to understand text questions. Emerging tools for metabolomics are enabling generation of a lot of information on metabolites and allowing a better sense of the importance of variation in metabolism and effects of different metabolites. For example, it is becoming clear that the functionality of metabolic enzymes changes and degrades with aging. He encouraged consideration of leveraging genome-scale metabolic models of humans to explore how a chemical might affect a particular enzyme's function. Digital twins are going to be useful in toxicology in that they enable simulation of small effects over long time scales, for example the effect of low vitamin D on Alzheimer's risk, which simulations indicate would require a 10-year clinical trial to observe. The reverse is also true; these allow simulation a long-term exposure to a compound in a way that would not be practical in a laboratory experiment. It would also be useful to explore the effects of the microbiome, which has been demonstrated to have effects on drug efficacy. We need to consider the utility of computational models in evaluating effects of variability and be very clear about the difference between reproducibility and variability. Digital twins can also be useful in modeling populations, in that they enable assessment of the variability of known effects on toxicants and characterize the spread in a population. These demonstrate an understanding of how to make tools accessible, but he encouraged NICEATM to explore opportunities to provide training.

Additional SACATM Comments

Dr. Baran suggested implementing user experience software to assess usability of the

tools.

Dr. Berg congratulated developers on efforts to make these tools user-friendly and accessible to those who might not be sophisticated computationally. She encouraged NICEATM to continue to leverage technology to develop and improve these tools to make them approachable and accessible. She expressed appreciation for the fact that all tools are open access and noted that automating even simple tasks is very important. Dr. Page concurred with these remarks.

XI. Adjournment

Dr. Page invited SACATM members to share concluding remarks. Dr. Ushio noted the shift from technology development and toward implementation and emphasized the importance of communication of when data is better and more relevant and going beyond just reducing animal use. Dr. Fourches encouraged NICEATM and ICCVAM to continue to seek and act on stakeholder feedback. Reports are good but data and tools are more useful to many stakeholders. He also advised them to be aware of the development of new chemistries and how testing approaches might need to be adjusted or even replaced to accommodate them.

Dr. Sills encouraged research to continue to link efforts to human disease and maintain high-quality work. Dr. Gordon reiterated the value of the perspective of the SACATM members and their advice. He thanked the speakers for their participation, recognizing in particular Ms. Gourmelon for traveling from Europe to participate. Dr. Kleinstreuer concurred with these remarks and thanked the international viewers who are attending at late hours and Dr. Lowit, who is stepping down as ICCVAM co-chair at the end of the year.

Dr. Page adjourned the meeting at 3:57 p.m.

Kathryn Page, PhD

SACATM Chair

Date: xxx