U.S. EPA Conference on the State of Science on Development and Use of New Approach Methods (NAMs) for Chemical Safety Testing

Conference Summary

November 5–6, 2024

U.S. Environmental Protection Agency (EPA)

Table of Contents

Conference Overview 4
Day 1 Summary 4
Welcome 4
Maureen Gwinn (U.S. EPA): Welcome 4
NAMs Work Plan Implementation and Validation Update 4
Rusty Thomas (U.S. EPA): Day 1 Overview and NAMs Work Plan Progress Update 4
Nicole Kleinstreuer (NICEATM): ICCVAM Validation Report and NICEATM Activities 8
Alison Harrill (U.S. EPA): Building Confidence in NAMs via Validation Standard Setting in a Revised OECD GD 34 9
Exposure NAMs 12
Kristin Isaacs (U.S. EPA): Development and Application of Exposure New Approach Methodologies in EPA's ExpoCast Project 12
Kyle Messier (NIEHS): Integrating Geospatial Exposure Models with NAMs to Evaluate Health Risks from Environmental Chemicals 14
Sin Urban (Maryland Department of Health): Leveraging Non-Targeted High-Resolution Mass Spectrometry to Reveal the Complete PFAS Fingerprint in Maryland 15
Carsten Prasse (Johns Hopkins University): Human and Environmental Exposure Framework for Biosolids 16
Annette Guiseppi-Elie (U.S. EPA): Session Wrap-Up 18
Day 2 Summary 18
Welcome and Opening 18
Krystle Yozzo (U.S. EPA) and Monique Perron (U.S. EPA) 18
'Omics 19
Connie Mitchell (Health and Environmental Sciences Institute, HESI): Collaborative Vision for 'Omics-Based Chemical Testing 19
Logan Everett (U.S. EPA): Development and Application of Transcriptomics in EPA 20
Jessica LaRocca (Corteva Agriscience): Use of 'Omics to Inform Development and Safety of New Pesticide Active Ingredients 22
Chris Martyniuk (University of Florida): Knowledge Gaps and Opportunities for 'Omics Data in Environmental Assessments 24
Matthew Meier (Health Canada): Fostering Transparency and Reproducibility Using the OECD 'Omics Reporting Framework 26
Monique Perron (U.S. EPA): Session Wrap-up 28
In Vitro to In Vivo Extrapolation 28
John Wambaugh (U.S. EPA): Recent Developments in High-Throughput Toxicokinetics Including Confidence Assessment 28
Fabian Fischer (University of Rhode Island): Translation of In Vitro Effect Concentrations: Equilibrium or Kinetic Distribution Models? 30
Shane Hutson (Vanderbilt University): Impact of Chemical-PDMS Interactions on IVIVE from Microphysiological Systems 32
Jon Arnot (ARC Arnot Research and Consulting): Developing Internal Thresholds of Toxicological Concern 34
Alicia Paini (EFSA): Existing OECD Guidance on Toxicokinetics, In Vitro-to-In Vivo Extrapolation, and Exploring

Future Opportunities 35
Rusty Thomas (U.S. EPA): Session Wrap-Up 36
Meeting Closing 37
Rick Keigwin (U.S. EPA): Meeting Wrap-Up 37
Appendix A: Acronym List 38

Conference Overview

Conference Purpose

The purpose of the 2024 U.S. Environmental Protection Agency (U.S. EPA) State of Science on Development and Use of New Approach Methods (NAMs) for Chemical Safety Testing Conference was to engage a cross-sectional mix of stakeholders and scientific experts in a dialogue about using NAMs to evaluate chemicals for potential health effects. The conference consisted of presentations by scientific experts from inside and outside of the Agency to inform attendees on advances in the NAMs field. EPA presenters also provided an update on the deliverables outlined in the EPA NAMs Work Plan. Speakers presented on the state of the science in NAMs related to exposure NAMs, 'omics, and *in vitro* to *in vivo* extrapolation (IVIVE).

As outlined in the EPA NAMs Work Plan, the Agency hosts regular conferences to exchange information and solicit feedback, and this was the fourth such conference. This report summarizes the discussions and presentations that occurred.

Conference Dates, Location, and Materials

November 5 and 6, 2024
U.S. EPA Conference Center in Research Triangle Park, NC and online via Zoom Webinar
Link to NAMs conference website for agenda and presentations - https://www.epa.gov/chemical-research/epa-nams-conference

Participant Summary

There were 130 in-person participants and 818 virtual participants at the two-day conference. The participants were from various sectors, including academia, government, and industry.

Day 1 Summary

Welcome

Maureen Gwinn (U.S. EPA): Welcome

Dr. Maureen Gwinn opened the conference and welcomed participants. She indicated that the conference would highlight EPA's continued commitment to the development and implementation of NAMs and showcase how EPA continues to serve as a leader in developing these methods and technologies to reduce the need for the use of animals in research while using the best science to support Agency decisions. Dr. Gwinn emphasized EPA's comprehensive NAMs Work Plan, which was created to prioritize Agency efforts and resources toward activities aimed at reducing the use of vertebrate animal testing while continuing to protect human health and the environment. She also noted the ongoing efforts to maintain and update the list of EPA-recommended NAMs, per the Toxic Substances Control Act (TSCA), which includes an upcoming release of a draft proposal on the process for selecting which NAMs will be included in future versions of said list. Dr. Gwinn also discussed the importance of stakeholder engagement. She described EPA's ongoing stakeholder engagement efforts which include regular updates to EPA's NAMs website,[1] a NAMs training program, soliciting input from stakeholders on the development and updates to NAM methods and confidence evaluation, and the allocation of research grants to advance NAMs. She concluded by stating that the presentations and discussions at the conference aim to chart the path forward for using NAMs in chemical safety testing with a focus on aggressively reducing the use of animal testing.

NAMs Work Plan Implementation and Validation Update

Rusty Thomas (U.S. EPA): Day 1 Overview and NAMs Work Plan Progress Update

Dr. Rusty Thomas started the conference by reviewing the overarching goals of the NAMs Work Plan and discussed that one objective of these conferences was for EPA to share progress towards meeting the deliverables and timeline in the Work Plan. He reported on the status of Work Plan deliverables, including metrics on animal use at EPA, recent EPA Science to Achieve Results (STAR) grant awards, partnerships with external organizations related to

[1] https://www.epa.gov/nam

increasing confidence in the use of NAMs, development of relevant case studies, and the implementation of training programs.

Summary

- The Work Plan, originally released in 2019, aims to develop baselines and metrics, evaluate regulatory flexibility, establish scientific confidence, demonstrate applications, address information gaps, and engage with stakeholders with the aim of reducing animal testing and research while still adhering to the mission of the Agency to protect human health and the environment. An updated Work Plan was released in December 2021, which expanded the species covered to be consistent with TSCA to include all vertebrates and which also included updated deliverable timelines.
- Dr. Thomas presented a high-level overview of the status of specific Work Plan deliverables which included:
    - Releasing a report on a review of existing statutes, programmatic regulations, policies, and guidance that relate to vertebrate animal testing and the implementation and use of appropriate NAMs for regulatory purposes (originally projected to be complete in 2022 but finished in 2024).
    - Reporting annual progress and summary metrics on reducing vertebrate animal testing requests and use (annually since 2022).
    - Finishing a National Academies of Sciences, Engineering, and Medicine (NASEM) study that evaluated the variability and relevance of existing mammalian toxicity tests and reviewed frameworks for validation and establishing scientific confidence in testing methods (2023).
    - Developing a scientific confidence framework to evaluate the quality, reliability, and relevance of NAMs (end of 2024).
    - Developing an initial set of reporting templates that may be used by EPA and stakeholders to capture the range of specific NAMs used for Agency decisions (end of 2024).
    - Ongoing development of case studies for evaluating application to risk assessment and demonstrating protection of human health and the environment.
    - Releasing the EPA Strategic Research Action Plans (StRAPs) outlining research products to develop and apply NAMs.
    - Encouraging development of NAMs through mechanisms such as the STAR program and facilitating partnerships with organizations focused on establishing scientific confidence in alternative methods.
    - Publishing the EPA website to house information about NAM efforts and progress against the Work Plan, ongoing public webinars, peer reviews on deliverables from the Work Plan where appropriate, completing a NAMs pilot training program that will continue to expand, and providing regular scientific exchanges and progress updates through Agency-sponsored and partner-organized events.
- Dr. Thomas presented data showing the metrics on animal use for the Office of Research and Development (ORD) and Office of Pesticide Programs (OPP) from Fiscal Year (FY) 2019 to FY 2023. ORD had a significant reduction in use of mammals from 2019 to 2021 due to ORD reorganization, laboratory remodeling and the coronavirus pandemic, but in FY23 animal use is almost back to the baseline levels established as the average of FY 2016 – 2018. A method for more accurately counting amphibian and other nonmammalian vertebrates in addition to mammals has recently been developed and ORD will work to establish an updated baseline after data have been collected over multiple years. OPP had varying animal usage with no timeline trend, but the data reflect the submissions received. Beginning in 2023, OPP expanded its tracking of data waivers granted for the acute “6-pack”. The use of waivers and NAMs as part of data packages is welcome.
- Dr. Thomas summarized the results of the EPA report which reviewed statutes and regulations to identify the flexibility of incorporating NAMs and to identify barriers and opportunities for implementation. The report found that the relevant statutes are fairly broad and would allow the use of NAMs as long as EPA is using the best available science. Some EPA regulations currently require a minimum set of vertebrate animal testing for decision making.
- EPA has awarded several STAR grants to various institutions since 2019 to advance alternatives to vertebrate animal testing and to develop innovative approaches to test chemicals. EPA will announce new awards for Advancing Sustainable Chemistry soon.
- Partnerships with external organizations continue to be formed to help establish scientific confidence in NAMs. Examples of these partnerships include collaborating with four external organizations on an inter-laboratory pre-validation study of a human thyroid microtissue assay, partnering with five external organizations on the development and validation of 17 assays for developmental neurotoxicity (DNT), co-

leading an Organisation for Economic Cooperation and Development (OECD) activity to update Guidance Document (GD) 34, collaborating with National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) to catalog characteristics of OECD Test Guideline (TG) validation studies, participating in the National Institutes of Health (NIH) Complement Animal Research in Experimentation (Complement-ARIE) program, and co-authoring an Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) report in 2024.

- Dr. Thomas highlighted six case studies evaluating the application of NAMs to risk assessment and demonstrating protection of human health and the environment, five of which were case studies being performed through the inter-governmental Accelerating the Pace of Chemical Risk Assessment (APCRA) consortium. Case study topics include using bioactivity for screening level risk assessment, applying *in vitro* toxicokinetics (TK) to regulatory decisions, and developing quantitative structure use relationship models for predicting chemical functional use.
- Dr. Thomas described the NAMs training pilot program and trainings already completed to date (e.g., ECOTOX Knowledgebase, Generalized Read Across). He provided information about where to find the resources, including past video trainings,[2] and announced that EPA will host multiple additional trainings in the coming year.
- To conclude, Dr. Thomas reviewed the agenda for the rest of the day by first describing how the topics were chosen based on a survey from stakeholders and then describing the agenda at a high level and thanking the organizing team members.

Questions answered during the conference included:

- **(Verbal, in-person) Jon Arnot (ARC Arnot Research and Consulting):** Considering the unique biological and TK properties of avian species, is there potential within the vertebrate NAMs development space to include testing and developing NAMs specifically for birds?
    - **(Verbal, in-person) Rusty Thomas**: EPA is working on avian NAMs primarily in the Great Lakes Toxicology and Ecology Division in Duluth, Minnesota. EPA is mostly focused on the impact of per- and polyfluoroalkyl substances (PFAS) on avian species in field studies. However, efforts are more focused on fish and amphibians [and mammals] than avian species at this time.
- **(Online chat) Marc Williams (Defense Health Agency):** Did the COVID-19 pandemic contribute to the dampened use of animals reported for 2020 and 2021; i.e., disrupted laboratory studies and an apparent but not "real" reduction in animals in lab studies?
    - **(Online chat) Christina Baghdikian (U.S. EPA):** Hi Marc- More information on the metrics can be found here: https://www.epa.gov/chemical-research/new-approach-methods-nams-animal-use-metrics-research-and-development.
- **(Online chat) Kristie Sullivan (Institute for In Vitro Sciences):** Regarding the numbers of animals used by ORD, is there any granularity as to what those animals are used for? Can this information be shared publicly?
    - **(Online chat) Christina Baghdikian (U.S. EPA):** Hi Kristie- More information on the metrics can be found here: https://www.epa.gov/chemical-research/new-approach-methods-nams-animal-use-metrics-research-and-development. Note that page has ORD metrics and there's a link to the OCSPP metrics.
- **(Online chat) Zachary (unknown surname and affiliation):** Are the external organizations that the EPA is partnering with posted publicly?
    - **(Online chat) Christina Baghdikian (U.S. EPA):** Formal agreements between CCTE and others are listed here https://www.epa.gov/chemical-research/collaborative-agreements-computational-toxicology-research.
- **(Online chat) Marc Williams (Defense Health Agency):** Could I ask Dr. Thomas if there are exemplar Case Studies where NAMs have been successfully integrated into the risk assessment process?
    - **(Online chat) Monica Linnenbrink (U.S. EPA):** Hi Marc! Thank you for your question. Here are some examples. EPA using NAMs for Endocrine Disruption Screening Program https://www.epa.gov/sciencematters/epa-research-contributes-using-alternatives-screen-chemicals-endocrine-disruption and Developmental Neurotoxicity - https://www.epa.gov/sap/use-new-

[2] https://www.epa.gov/chemical-research/new-approach-methods-nams-training

approach-methodologies-nams-derive-extrapolation-factors-and-evaluate-developmental. TSCA New Chemicals Research Collaboration https://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/new-chemicals-collaborative. More information about these examples and additional examples are available in this report https://ntp.niehs.nih.gov/sites/default/files/2024-09/2022-2023_ICCVAMBienRpt_FD_508.pdf.

- **(Online chat) Maria Hegstad (Inside EPA):** If I recall rightly, the current (2021) NAMs workplan was written for activities through 2024. Is another NAMs workplan update in the works?
    - **(Online chat) Monica Linnenbrink (U.S. EPA):** Thank you for the question, Maria! We are focused on completing the deliverables in the current work plan. As we complete the deliverables, we will discuss the next steps for the EPA NAMs work plan.
- **(Online chat) Anonymous virtual attendee:** I may have missed it - if so, I apologize - is there an update on progress with the New Chemicals Collaborative Research Program?
    - **(Online chat) Monica Linnenbrink (U.S. EPA):** Since EPA launched the program in February 2022, a public meeting in April 2022 provided an overview of the program and gave the public the opportunity to provide feedback. A 2023-2026 research plan was reviewed by the Board of Scientific Counselors in October 2022 with a final report posted in early 2023. EPA responded to the feedback from this review and it is available https://www.epa.gov/system/files/documents/2023-12/2022_october_bosc_nccrp_report_final_eparesponse_0.pdf. EPA is currently moving forward with the implementation. To date, the program has advanced multiple research objectives, including publications of research evaluation of chemical and analogue approaches, cheminformatics-driven selection of chemicals to include in an *in vitro* NAM proof of concept, and the beginning of screening work on that proof-of-concept.

Questions answered following the conference included:

- **(Verbal, in-person) Jon Arnot:** Are there opportunities for looking at toxicokinetics of avian species, *in vitro* biotransformation in particular?
    - **(Post meeting, written) Rusty Thomas**: There are opportunities for developing avian NAMs, particularly in toxicokinetics, and cross-species extrapolation to avian species through application of the EPA Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS). SeqAPASS is intended to be used to extrapolate chemical toxicity knowledge/data from one species to others. This extrapolation is termed cross species extrapolation and is based on the current understanding of a chemical-protein or protein-protein interaction in one species and the collection of lines of evidence indicating a similar interaction in another species is likely to occur. The results from SeqAPASS provide evidence of protein conservation to be used for predictions of chemical susceptibility or pathway conservation across the diversity of species. This is important as the majority of species will never be tested in the laboratory for toxicity though researchers and regulators are interested in understanding the potential for chemical impacts on all species that have potential for exposure in the environment. More information available online: https://seqapass.epa.gov/seqapass/
- **(Online chat) Kristie Sullivan (Institute for In Vitro Sciences):** Can you talk a little more about the reporting templates? What tools are they envisioned for? How were they developed? How will they be communicated to companies and developers?
    - **(Post meeting, written) Rusty Thomas:** Reporting templates currently being leveraged by EPA include the OECD Harmonized Template 201 (OHT 201) for recording non-apical effects, such as those obtained from *in vitro* testing; OECD GD 211 to describe non-guideline *in vitro* test methods that are included as assay information within the CompTox Chemicals Dashboard; and the OECD 'Omics Reporting Framework (OORF) and associated template to report the results from *in vitro* and *in vivo* transcriptomics studies.
- **(Online chat) Jennifer Sass (Natural Resources Defense Council):** Can Dr. Thomas re-show the slide with the list of projects that are now ongoing? I am curious as to which of those have components that include identifying and communicating uncertainties.
    - **(Post meeting, written) Rusty Thomas:** It is not clear whether this question is related to the ongoing NAM partnership activities or the ongoing case studies evaluating application of NAMs. In general,

identifying and communicating uncertainties constitute a component in most of the projects and case studies as the uncertainties depend on the context of use.

Nicole Kleinstreuer (NICEATM): ICCVAM Validation Report and NICEATM Activities

Dr. Nicole Kleinstreuer presented updates from NICEATM and ICCVAM on activities related to biomedical research, validation, and adoption of human-based NAMs. The presentation highlighted activities such as: Complement-ARIE, a collaboration with key partners including the U.S. Food and Drug Administration (FDA) and EPA; the Method Developers Forum (MDF) as a proactive effort to implement new technologies and provide opportunities for NAMs developers to interact with relevant stakeholders; and ongoing NICEATM projects such as updates to the Integrated Chemical Environment (ICE) and various computational tools.

Summary

- Dr. Kleinstreuer presented the ICCVAM and NICEATM activities in the context of the NAMs confidence continuum, which includes developing methods in basic research for biomedical applications that can then go through validation and qualification before being adopted and implemented into regulatory policy.
- She announced that new funding will be available under the Complement-ARIE program. The program will invest approximately $300 million over the next decade in the development, standardization, validation, and implementation of human-based NAMs. Specific goals include understanding human health and disease outcomes across diverse populations, providing insights into specific biological processes and disease states, supporting regulatory use through validation and standardization, and making biomedical research more efficient and effective by complementing existing animal models.
- Dr. Kleinstreuer provided an overview of NICEATM and ICCVAM. She explained how ICCVAM, a collaboration of federal agencies, was established by Congress to reduce reliance on animal models and promote NAMs-based human-relevant approaches. NICEATM is housed at the National Institute of Environmental Health Sciences (NIEHS) and provides operational and scientific support to ICCVAM workgroups. She briefly described how ICCVAM workgroups welcome federal partners to become workgroup members. Workgroups are formed around a specific scope and charge. For example, the validation workgroup drafted and published a new guidance document on validation, qualification, and regulatory acceptance of NAMs.
- Dr. Kleinstreuer introduced the MDF, which facilitates early and frequent interaction between method developers and end users. She described the process of how the MDF works where research partners in the federal government and regulated industries articulate their needs around a particular endpoint and put out a call to method developers to articulate how their NAMs address those needs. The first forum was held in summer 2024 and focused on carcinogenicity. More are planned for spring 2025.
- Dr. Kleinstreuer summarized several ongoing NICEATM projects, noting that the list covers a broad portfolio of work. Examples include developing computational models and workflows to democratize access to computational tools. The efforts she highlighted included OPERA (Open (Quantitative) Structure-activity/property Relationship App) version 2.9, Collaborative Acute Toxicity Modeling Suite (CATMoS) (outputs of which are intended to be the basis of a forthcoming science policy guidance to consider a CATMoS-predicted LD50 value as a line of evidence for potentially waiving the rat study), an open-source chemical grouping workflow in KNIME to take a more informed approach to identifying test compounds that will provide the highest value of information (VOI) in new assays, and using machine learning and large language models (LLMs) for automating study data curation to generate robust and reliable reference data for validation.
- Dr. Kleinstreuer provided an overview of the recent updates and uses of ICE. This included example applications, such as determining risk characterization of triazole fungicides and associating *in vitro* molecular targets to human biomarker alterations.
- Dr. Kleinstreuer concluded by presenting the in-progress work on a new interactive database of validated/qualified NAMs, Collection of Alternative Methods for Regulatory Application (CAMeRA), which is in development with an Advisory Committee to the Director (ACD) workgroup. She mentioned an upcoming call for feedback from the scientific community about what should be in CAMeRA and how it should be designed.

There were no questions answered during the conference.

Questions answered following the conference included:

- **(Online chat) D'Ann Williams (Johns Hopkins University):** Does CATMoS include synergistic or multiple compounds in the toxicological assessment?
    - **(Post meeting, written) Nicole Kleinstreuer:** CATMoS does not provide information on potential synergistic effects of ingredients within mixtures but has been favorably assessed by external groups for its ability to predict the LD50 of formulated products by combining the individual component predictions with the GHS (Globally Harmonized System of Classification and Labelling of Chemicals) additivity equation (e.g., Chushak et al. 2020 https://pubs.acs.org/doi/abs/10.1021/acs.chemrestox.0c00256).

Alison Harrill (U.S. EPA): Building Confidence in NAMs via Validation Standard Setting in a Revised OECD GD 34

Dr. Alison Harrill presented on updating OECD Guidance Document (GD) 34 to include modern *in vitro* and *in silico* approaches, emphasizing the need for standardized protocols and benchmarks. The presentation also highlighted the role of international collaboration, the Mutual Acceptance of Data (MAD) system in ensuring data acceptance across participating countries, and the use of Value of Information (VOI) frameworks to contextualize the socioeconomic benefits of new testing methods. The goal is to streamline the validation process and build scientific support for new methods.

Summary

- Dr. Harrill introduced OECD GD 34 on Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment. Efforts to revise the GD started in 2023 to address changes in NAMs technologies and best practices for validation and have been led by the Joint Research Centre (JRC), the Netherlands, and key contributors from the U.S., including Dr. Harrill, Nicole Kleinstreuer, Warren Casey (NIEHS), and Charles Kovatch (EPA).
- The OECD defines validation as a process based on scientifically sound principles, establishing the reliability and relevance of a test method for a specific purpose, and emphasizes flexibility, adaptability, and performance demonstration using reference chemicals. OECD Test Guidelines (TG) are based on Mutual Acceptance of Data (MAD), meaning all participating countries agree to use data from an official TG for their risk assessment. Building international scientific support requires consensus on the validation program for a method.
- Dr. Harrill described how a working group is being used to build international scientific support for revisions to GD 34. These are focusing on establishing readiness criteria, modernizing approaches to transferability studies, and special considerations for Defined Approaches.
- Dr. Harrill emphasized the need to update GD 34 because it currently has repetitive language and outdated steps, such as pre-validation and validation steps, which the team aims to streamline. The proposed new, streamlined process has seven steps: standardizing materials, assessing assay relevance and performance benchmarks, technical characterization assessment, within-lab and between-lab reproducibility studies, qualification to assess reliability and relevance for context of use, finalization of the test protocol, and recommendations for or against proposed regulatory use.
- Dr. Harrill then described how validation efforts are typically performed for OECD TGs starting with transferability studies.
    - Transferability studies are performed across multiple labs to assess variability, with fewer labs required for *in vitro* studies compared to *in vivo* studies, and reference chemical lists are used to assess method performance. She also noted that not all OECD TGs have undergone method reliability studies because they predated GD 34.
    - Data from TGs covering vertebrate and invertebrate species show differences in the number of chemicals used in validated studies, with *in vitro* human health studies requiring more chemicals than *in vivo* studies, likely due to a lack of appropriate benchmarks.
- Dr. Harrill shared that EPA is focusing on case studies to establish benchmarks for reliability and relevance, considering questions about between-lab validation versus standardization, human relevance compared to gold standard tests, and reliability of assay results compared to traditional approaches. EPA is assessing

cross-species concordance using rodent data as surrogates for human data, with pharmaceutical drug data used to compare doses and effects across species.
    - Dr. Harrill presented how EPA is using pharmaceutical data to assess the qualitative (hazard) and quantitative (dose matching) concordance across species since they contain directly linked human and rodent data. For this effort, data were curated from new drug applications (NDAs) submitted to FDA.
    - She described how comparisons between human and rodent doses show that there is not a strong concordance between human and rodent data, while rat and mouse data are more dose concordant with each other than with human data.
    - Qualitative concordance, such as matching effects across species, shows low positive predictive value but high negative predictive value, which is beneficial for clinical development programs.
    - Dr. Harrill mentioned how cross-species concordance was also assessed for pharmaceutical data by the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ Consortium) in 2016, and those conclusions matched EPA's current conclusions.
- Dr. Harrill next focused on how to use this information to create benchmarks for human relevance for environmental chemicals. She described how the ToxRef *in vivo* dataset was used to compare rodent values from repeat-dose studies with NAMs to understand variability as a benchmark for NAMs.
    - A study by Katie Paul-Friedman and colleagues calculated root mean squared errors for point of departure (POD) estimates across repeated studies, specifically for understanding reproducibility of organ-level effects in animal studies. She said these results can also be used as a benchmark for NAM variability.
- Dr. Harrill presented how EPA's published VOI framework can be used as a decision-making framework to contextualize trade-offs in uncertainty around the POD, costs, and time to decide between a choice of methods that can lead to informing the decision.
    - Dr. Harrill presented a case study on how the VOI was applied to the EPA Transcriptomic Assessment Product (ETAP) to answer the following question: Given that additional toxicity testing data may be beneficial, which toxicity testing methodology and assessment process provides the most value?
    - Dr. Harrill showed a comparison between the ETAP and a two-year chronic testing modality. It showed that ETAP takes about one year, whereas the traditional method takes several years, including the time for toxicity testing and assessment.
    - Dr. Harrill emphasized the benefit of the EPA VOI consideration of socioeconomic factors to assess return on investment (ROI), such as public health costs and productivity loss, with economists estimating costs for various health conditions like asthma and developmental disorders. She concluded by noting how delays in mitigating exposure to toxic chemicals result in accumulating health care costs, whereas timely mitigation can save significant public health resources; the VOI framework helps balance public health costs and benefits, with case studies showing that the new method (ETAP) often provides the most benefit in various scenarios.

Questions answered and comments made during the conference included:

- **(Online chat) Susanne Kolle (BASF)**: What was the source of the data presented for the validation?
    - **(Verbal, in-person) Alison Harrill:** The data source for this information is the OECD secretariat.
- **(Verbal, in-person) Chris Vulpe (University of Florida):** When comparing *in vitro* tests and rodent tests, you talked about specific repeats of the same study and investigator. Was genetic variability that comes with using human cells over rodents considered when analyzing these data?
    - **(Verbal, in-person) Alison Harrill:** The *in vivo* concordance data that we collected were from published studies across a variety of labs, which means there is likely some genetic diversity within the dataset because there were likely multiple strains used. Even if the inbred strains were standardized, there is some diversity in outbred stocks. Therefore, there is some genetic variation in this dataset (across studies) even though it may not be the same amount of variation found among human populations. Genetic variability has not been systematically assessed for many chemicals.
- **(Verbal, in-person) Chris Vulpe (University of Florida):** Do you expect more variability in general in human studies? Is it a fair comparison when determining a comparator group?
    - **(Verbal, in-person) Alison Harrill:** It depends on the study design and on the mode of action of the chemical. Some clinical studies are quite large (later stage trials can be hundreds of thousands of

patients), so the expectation of precision around points of departure will be robust, but there are other outside variables to consider that may impact the outcomes. These could include gene-environment interactions that lead a subset of individuals to respond differently.

- **(Online chat) William Irwin (U.S. EPA):** From the EPA RATE [Risk Assessment Training and Experience] training on validity, reproducibility is different from accuracy. Olson et al 2000 pharma data from 12 companies also had some interesting concordance data based on many pharmaceuticals tested in both animals and humans (gold standard), where the goal was to predict hazards in humans.
    - Citation referenced: Olson, H. et al, "Concordance of the Toxicity of Pharmaceuticals in Humans and in Animals," Regulatory Toxicology and Pharmacology, Volume 32, Issue 1, 2000, Pages 56-67, https://doi.org/10.1006/rtph.2000.1399.
- **(Online chat) Richard Becker (American Chemistry Council):** Regarding VOI - we recently published "Commentary: Value of information case study strongly supports use of the Threshold of Toxicological Concern (TTC)." it's open access -- at https://www.sciencedirect.com/science/article/pii/S0273230024000357. The return on investment for the TTC was immensely greater than either the traditional repeat tox animal method and the ETAP considered by EPA in their ETAP VOI report.
    - **(Online chat) Monica Linnenbrink (U.S. EPA):** Thank you Rick for sharing the commentary. and we also appreciate you serving as an expert on BOSC [Board of Scientific Counselors] panel reviewing the VOI. As I recall, your feedback during the expert review process included support for the use of TTC. EPA reviewed recommendations received through the BOSC process and the final VOI report is now available - https://www.epa.gov/etap/value-information-voi-case-study-etap.

Questions answered following the conference included:

- **(Online chat) Morne van der Mescht (Environment Agency, U.K.):** Compared to the cost of not testing a chemical, what is the socioeconomic cost of making a mistake using a method with greater uncertainty?
    - **(Post meeting, written) Alison Harrill:** The VOI framework that has been developed does not specifically address a scenario in which the chemical is not tested; instead, the current framework offers metrics that can allow a decision maker to choose the best option between two different testing methods.
- **(Online chat) D'Ann Williams (Johns Hopkins University):** How do some of these modeling studies agree with methods used by other countries, such as REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) in the EU (European Union)?
    - **(Post meeting, written) Alison Harrill:** Thank you for this question. While the VOI case study focused on the ETAP methodology, we can certainly extend the analysis framework to other types of testing methods as a future direction.
- **(Online chat) Elaine Faustman (Institute for Risk Analyses and Risk Communication):** Have you done your comparisons between human epidemiology studies and NAMs for environmental chemicals of focus for EPA?
    - **(Post meeting, written) Alison Harrill:** This is a much smaller dataset for comparison but could certainly be done. One advantage of comparing to clinical trial data is that more of the experimental variables are controlled or at least accounted for via study design and subject exclusion criteria.
- **(Online chat) Clive Roper (Roper Toxicology Consulting Limited):** In animal studies, there are many other variables, such as strain, food, water, bedding, handling… Some OECD TGs ask for rodent, let alone strain of rat or mouse. These add to huge variability and are not controlled.
    - **(Post meeting, written) Alison Harrill:** Experimental conditions leading to environmental variability are certainly an important source of interstudy variability in dose-effect estimates.
- **(Online chat) Elaine Faustman (Institute for Risk Analyses and Risk Communication):** Another response to the question of human variability is the research on the comparison across studies.
    - **(Post meeting, written) Alison Harrill:** Thank you for your comment.
- **(Online chat) William Irwin (U.S. EPA):** Human tumor clonal cells can have high reproducibility, but many tumor cells have DNA chromosomal aberrations that make human relevance a concern. "Are they still 'human'?" is a question with these profound chromosomal alterations.
    - **(Post meeting, written) Alison Harrill:** Better characterization of genetic stability of cell lines used for toxicology studies is indeed an important consideration for testing.

Exposure NAMs

Kristin Isaacs (U.S. EPA): Development and Application of Exposure New Approach Methodologies in EPA's ExpoCast Project

Dr. Kristin Isaacs discussed the ways in which NAMs are used in EPA's Exposure Forecasting (ExpoCast) Project to expand the availability of exposure data for evaluation of thousands of chemicals in commerce. The presentation provided a broad overview of key approaches and outcomes.

Summary

- Dr. Isaacs introduced her presentation on how integrated research and development in high-throughput hazard, TK, and exposure data can link exposures that that cause an effect with exposures that occur in the world to enable risk assessors to more rapidly address public health challenges.
- Overlaying exposure and hazard distributions allow for ranking or binning chemicals in terms of priority. Chemicals in which hazard and exposure distributions are orders of magnitude apart may be a lower priority because there is less risk, whereas those with overlapping distributions but high uncertainty might represent a medium-level risk, and overlapping distributions with low uncertainty might represent a higher risk.
- ExpoCast aims to more fully characterize and represent the exposure distributions to reduce uncertainty. Within complex exposure pathways, contact between receptors and the environmental media is often difficult to measure directly, so exposure NAMs are needed for collecting additional data, extrapolating to fill data gaps, making predictions of exposure, and integrating models to predict potential risk and prioritize chemicals.
- Dr. Isaacs described recent efforts to curate data on how chemicals are used in commerce by collecting general use information from public documents. A new public web tool called the Chemical Exposure Knowledgebase allows for interactive evaluation or exploration of the curated chemicals and product information. The Multimedia Monitoring Database (MMDB) includes harmonized information from different data sources mapped to harmonized chemical identifiers across approximately 30 media categories for more than 3,000 chemicals. These datasets are available through Application Programming Interfaces on EPA's CompTox Chemicals Dashboard[3] so the public can build reproducible workflows using the data.
- Dr. Isaacs described how machine learning NAMs are being used to fill gaps for chemicals with limited data, such as the quantitative-structure-use-relationship model, which predicts uses of chemicals.
- Non-targeted analysis (NTA) is being used to identify and evaluate chemicals in environmental and biological samples. EPA is investing in developing tools, databases, and workflows for rapid analysis of a range of environmental samples for chemicals of interest. Dr. Isaacs noted that the current focus is to look at how to quantify concentrations of chemicals in media using quantitative NTA or qNTA, such as by developing methods for selecting appropriate calibration surrogates for each study and quantifying uncertainty from those surrogates.
- Dr. Isaacs described how multiple high-throughput exposure models can be combined in a consensus framework through the Systematic Empirical Evaluation of Models (SEEM). Similar to meteorological predictions, she noted that one can get a better answer when looking across different models and weighting them according to performance. The various exposure models can cover different pathways, chemistries, and assumptions and can be evaluated and calibrated with known data to come up with a consensus approach using Bayesian regression.
- Machine learning and quantitative structure-activity relationship (QSAR) models are used to predict TK data to help inform bioactivity to exposure ratios that then inform risk evaluations, such as for candidates for chemical prioritization under TSCA.
- Dr. Isaacs summarized by stating that exposure, hazard, and TK data are critical inputs to risk-based screening and decision-making.

Questions answered during the conference included:

- **(Verbal, in-person) Sayak Mukherjee (Batelle)**: What overlap did you see between your databases and the ICE data?
    - **(Verbal, in-person) Kristin Isaacs:** The EPA team collaborates with the NIEHS team leading the ICE

[3] https://comptox.epa.gov/dashboard/

project. ICE IVIVE and PBPK tools leverage EPA's High-throughput Toxicokinetics (httk) package[4] to provide users with an approachable, easy-to-use interface for running analyses.

- **(Online chat) D'Ann Williams (Johns Hopkins University):** How do you manage predicting exposures in environmental areas where there are limited or no monitoring data? For example, near industrial animal production facilities?
    - **(Online chat) Jessica Wignall (ICF):** We hope Kristin's presentation provided examples of how to predict exposure when there is little to no exposure data. More information about how to predict exposure is available on EPA's website - https://www.epa.gov/chemical-research/rapid-chemical-exposure-and-dose-research
- **(Online chat) Maria Hegstad (Inside EPA):** Dr. Isaacs mentioned the bioactivity exposure ratio approach she described has been used in screening chemicals for prioritization under TSCA. Can anyone provide more details on that? Was the ratio approach used to select any of the candidates for the two most recent rounds of TSCA prioritization?
    - **(Online chat) Monica Linnenbrink (U.S. EPA):** The identification of 2014 TSCA Work Plan chemical substances included consideration of human health hazard as well as information on exposure potential or bioactivity exposure ratio. The approach is described in the EPA Report Proof-of-Concept Case Study Integrating Publicly Available Information to Screen Candidates for Chemical Prioritization under TSCA https://www.ncbi.nlm.nih.gov/books/NBK605713/pdf/Bookshelf_NBK605713.pdf (description starts at the bottom of page 20).

Questions answered following the conference included:

- **(Online chat) Nikhil Chivukula (The Institute of Mathematical Sciences, Chennai):** In the environment, we are exposed to a variety of chemical stressors. How can we correctly associate an adverse effect with a particular chemical or a group of chemicals in such scenarios?
    - **(Post meeting, written) Kristin Isaacs:** The tools we are building in ExpoCast do not necessarily link exposure to any specific endpoint. However, the data that we are building support an increased understanding of potential exposure sources and pathways, which can ultimately be linked with effects in epidemiological studies. EPA has also proposed a framework, aggregate exposure pathways (AEPs), that facilitates the linking of exposure data with adverse outcome pathway (AOP) information.
- **(Online chat) D'Ann Williams (Johns Hopkins University):** Are you using industry-derived data as the basis of these models? If so, are you validating those data?
    - **(Post meeting, written) Kristin Isaacs:** In our exposure modeling efforts, we rely on publicly available data about how chemicals are used in commerce (including levels in consumer products). Some of these data are provided by manufacturers. However, we know that reporting of intentionally added ingredients cannot necessarily provide a complete picture of product content. In ExpoCast, we are also developing analytical non-targeted methods to further examine product content. We have published several papers in this area, including efforts to characterize chemicals in recycled materials.
- **(Online chat) Elaine Faustman (Institute for Risk Analyses and Risk Communication):** If you had to identify two classes of compounds that pose the most difficulty in predicting exposures, what classes would they be and why? How are you addressing these challenges?
    - **(Post meeting, written) Kristin Isaacs:** There are classes of chemicals that are difficult due to their chemistries and those that are difficult due to lack of information about their sources and release. PFAS compounds are both: Our predictive models do not work well for PFAS structures (although CCTE [EPA Center for Computational Toxicology and Exposure] has recently published new structural descriptor sets that may help), and we have very little specific information about the types of PFAS used in consumer products due to confidential business concerns (oftentimes these compounds are reported on safety data sheets as "proprietary fluorosurfactants"). Other challenging compounds are UVCBs (unknown or variable composition, complex reaction products, or biological materials). There are challenges in characterizing their specific chemical components and estimating associated exposures. In ExpoCast, we are also using NTA to develop chemical "fingerprints" for specific

[4] https://www.epa.gov/comptox-tools/high-throughput-toxicokinetics-httk-r-package

consumer products, and we have proposed expanding this approach to UVCBs. We aim to develop a "representative" composition of different UVCBs to be used in exposure assessments when no other specific information is available.

Kyle Messier (NIEHS): Integrating Geospatial Exposure Models with NAMs to Evaluate Health Risks from Environmental Chemicals

Dr. Kyle Messier presented how classical geospatial methods and modeling can be integrated with NAMs to evaluate health risks from environmental contaminants. The presentation covered the exposome, geospatial exposomics, GeoTox software (designed to facilitate source-to-outcome modeling in geospatial exposomics), and some best practices in software and computational development for addressing complex challenges.

Summary

- The exposome is the exogenous counterpart to endogenous processes like the genome, transcriptome, microbiome, and metabolome. Dr. Messier stated that the goal is to have exposure and risk assessments incorporate both endogenous and exogenous factors.
- Most current methodologies pertaining to exposomics have been focused on internal exposomics. Dr. Messier discussed the strengths of the standard methods using non-targeted analytical chemistry approaches for novel exposure discovery. This method can have high chemical throughput, be individualized, and feed into other 'Omic measurements. Some limitations include limited sample sizes, cost, batch and lab effects, and the difficulty in understanding the source or timing of the exposure.
- Geospatial exposomics is reframing geospatial exposure modeling as a source-to-outcome cascade with sequential and necessary steps to result in an individual or population health outcome. There are two overarching frameworks that address the source-to-outcome modeling.
    - The biological modeling portion is covered by AOP, which tracks biological mechanisms from molecular targets to population outcomes.
    - The geospatial portion is covered by AEP, which is a comprehensive analysis of source, external exposure, and internal exposure (including transformation products) and links to AOPs.
    - Where the AEP ends is where the AOP begins.
- Dr. Messier introduced GeoTox as a new user-friendly software for source-to-outcome modeling that is currently available on GitHub and will be available soon on the Comprehensive R Archive Network (CRAN). The software has been tested, uses an object-oriented methodology, and is extensible to integrate with other TK models and data sources.
- Dr. Messier reviewed an example of geospatial risk mapping of chemical mixtures in air using the Congestion Mitigation and Air Quality (CMAQ) data and H2A histone family member X (*H2AX)* Histone Modification assay.
- He provided another example of a multi-assay risk approach for mapping chemical mixtures with 200+ assays based on key characteristics of carcinogens (KCCs). The model quantifies the multi-chemical and multi-assay risk across the population. He presented several North Carolina maps with county risk levels summarized across all of the KCC assays and some maps filtered for specific modes of action.
- Dr. Messier acknowledged several limitations of geospatial exposure models due to geospatial exposure data availability, population uncertainty, TK uncertainty, complexity of mixtures, and data analysis. New methodologies are needed to address these limitations.
- Dr. Messier reviewed some software and computational best practices for reproducibility by ensuring tools and data are well documented and tested. He emphasized the need to ensure compatibility while developing pipelines for complex analysis of environmental health risks.

Questions answered during the conference included:

- **(Online chat) Marc Williams (Defense Health Agency):** Are you also leveraging the National Aeronautics and Space Administration's (NASA's) technically advanced chemical detection technologies, such as highly sensitive photo-acoustic sensors for detecting sub-parts-per-billion levels of chemicals of interest at Langley Research Center (this has important military applications). NASA also offers early warning rapid detection capabilities of chemical precursors using highly sensitive equities and approaches aligned to environmental monitoring, homeland security, chemical "accidents," etc.
    - **(Verbal, in-person) Kyle Messier:** We are developing some models for criteria air pollutants and hazardous air pollutants. We do use NASA data, which serve as a tremendous resource for earth

observations. I don't know the specific one in this question. We pull in the chemical transport model with hundreds of species of air pollutants, and there are also more direct measurements from satellite observations. In the same place you find the GeoTox package[5], you can find our R package. We currently pull in 15 data sources and want to expand and democratize ease of access in R to large-scale data products. We use NASA products and moderate resolution imaging spectroradiometer (MODIS) and have made it easier to pull in global and national spatial data.

- **(Verbal, in-person) Rusty Thomas (U.S. EPA):** Could you comment further on the biggest sources of uncertainty and sensitivity in this work? If propagated, what are the total uncertainty and sensitivity in various counties? What is the biggest source of uncertainty and how big is that uncertainty when propagated?
    - **(Verbal, in-person) Kyle Messier:** Good question. Our first paper gave the unsatisfying answer of "everything." There was no one dominant figure; with the geospatial aspect, uncertainty can vary by study area. If we're bringing in National Health and Nutrition Examination Survey (NHANES) data, that could be different in another country without those data. We saw in the first analyses and here with the GeoTox that everything is contributing. There's no one area to target. We just keep moving on exposure modeling and TK. We could put limits on modeling chemicals though the entire pipeline that meets an overall variability threshold, but instead we have mostly focused on maintaining many chemicals. It also depends on the assay endpoint and how different the risk map could look.

Questions answered following the conference included:

- **(Online chat) Anonymous virtual attendee:** So, the dose-response data received are for the main drivers of the mixtures?
    - **(Post meeting, written) Kyle Messier:** Sensitivity analyses show all aspects of the GeoTox pipeline can contribute to the overall response and variability of the mapped mixture response. The dose-response parameters certainly play a significant impact but so do the external concentration, TK, and physical and behavioral factors.
- **(Online chat) Elaine Faustman (Institute for Risk Analyses and Risk Communication):** At the start, you mentioned the various mixture models available. I did not see what models you used in your platform or how you would choose between models?
    - **(Post meeting, written) Kyle Messier**: We use independent action and generalized concentration addition (GCA) with a 2-parameter hill model for each chemical. Future work will integrate our related work on reflected GCA, which allows for 3-parameter hill models in mixture modeling.

Sin Urban (Maryland Department of Health): Leveraging Non-Targeted High-Resolution Mass Spectrometry to Reveal the Complete PFAS Fingerprint in Maryland

Dr. Sin Urban's presentation focused on a NAM using high-resolution mass spectrometry (HRMS) and NTA to identify unknown PFAS in Maryland's drinking water. He also discussed an example of using NTA to investigate a potential poisoning.

Summary

- The mandate of the Environmental Sciences Division of the Maryland Department of Health (MDH) Laboratories is to try to limit or prevent harmful chemical exposures that have the potential to cause disease. The division consists of up to 72 scientists in 15 different sections employing radiochemistry, microbiology, and analytical chemistry to conduct surveillance testing and develop new methods.
- In 2020, Maryland's Department of the Environment initiated a multi-agency, data-driven response to assess the presence of PFAS in the state. MDH Laboratories were asked to be partners since no labs had PFAS testing capabilities at that time.
- MDH Laboratories implemented, automated, and accredited targeted drinking water methods: EPA Methods 537.1, 533, and 1633.
- MDH tested all the community water systems in Maryland for the presence of PFAS. Positive detect samples were re-sampled for confirmation and source investigation.

[5] https://niehs.github.io/GeoTox/

  - Legacy PFAS were commonly detected in drinking water, likely from firefighting foams, and the source was predominantly groundwater and not surface water.
  - Dr. Urban shared a figure that illustrated a geographic pattern of PFAS detection that is related to geology. The southern part of Maryland has confined aquifers that are protected by bedrock or clay, whereas northern Maryland has unconfined or semi-confined aquifers.
  - They concluded that 63 water systems (14%) were above the maximum contaminant levels (MCLs).
- Dr. Urban secured a grant from the Centers for Disease Control and Prevention (CDC) through the Association of Public Health Laboratories (APHL) for a High Resolution Mass Spectrometer specifically for environmental testing. The MDH Laboratories collaborated with Dr. Tim Buckley's group at EPA to develop NTA workflows. The goal was to analyze drinking water samples from the 63 water systems and use NTA to identify all PFAS.
  - Dr. Urban presented a diagram of the data analysis workflow and highlighted the importance of quality assurance/quality control (QA/QC) at the data acquisition step and use of an EPA-developed web app to assess QA/QC parameters. Lastly, he pointed out two steps that use vendor-specific libraries and academic tools like FluoroMatch for identifying unknown spectra.
  - He noted they had 100% tracer recoveries and 1,029 unique chemical features in drinking water.
  - The HRMS NTA was able to detect all 14 PFAS detected using the targeted EPA Method 533, including five of the six regulated PFAS. GenX was not detected.
  - The qNTA results achieved sub-part-per-trillion sensitivity, which was comparable to the targeted method.
  - An additional 28 unknown PFAS were detected with corresponding Distributed Structure Searchable Toxicity Substance Identifiers (DTXSIDs) identified for all but one.
  - Nine of the 14 targeted PFAS and six unknown PFAS were detected in all the contaminated drinking water samples.
- Dr. Urban summarized his findings by noting the NTA workflow is effective in identifying PFAS, Maryland drinking water contains triple the number of PFAS previously thought, and NTA identified ubiquitous and rare unknown PFAS in contaminated water systems. NTA can be used for source identification, comparison to other states, and health implications.
- Dr. Urban additionally shared a case study in which a Maryland resident was poisoned by diet nuts (Nuez de la India) that were bought online and were suspected to be yellow oleander seeds.
  - The Maryland Poison Center identified the poisoning and recommended chelation therapy, which ultimately saved the patient's life.
  - The Poison Center reached out to MDH Laboratories for further investigation, which included a targeted FDA method and NTA to chemically fingerprint the product.
  - Using HRMS, the chemical fingerprint of the nuts was a near perfect match to the yellow oleander seeds, although they were marketed as candlenuts.
  - FDA extended a consumer warning, and there are ongoing efforts to prohibit selling the product in the U.S.

Questions answered during the conference included:

- **(Online chat) Anonymous virtual attendee:** Are all those new PFAS compounds detected in contaminated water using NTA precursors? Or are some of these new detections environmental transformation products of more well-annotated PFAS?
  - **(Verbal, in-person) Sin Urban:** We do not know yet. It is very early in the process, but it is a powerful approach. We want other states to join in on this, so this is a teachable moment.
- **(Verbal, in-person) Miles Crockett (CCTE):** There are a few different areas to improve sensitivity of NTA protocols, including quality and size of spectra libraries, accuracy and power of analysis. Which areas do you think have the best avenues toward improving detection?
  - **(Verbal, in-person) Sin Urban:** In my experience, we got better detections than we expected. We used a brand-new instrument. The one used for diet nuts was an old one. That was the biggest difference.

Carsten Prasse (Johns Hopkins University): Human and Environmental Exposure Framework for Biosolids

Dr. Carsten Prasse's presentation focused on developing a framework for assessing contaminants in biosolids and their potential risks.

Summary

- Dr. Prasse introduced biosolids as the solid byproducts from sewage treatment. Approximately 5 million dry metric tons are produced every year in the U.S., and half of that is applied to land, with 25% being used in agriculture as fertilizer. He reviewed some benefits of using biosolids as fertilizers, such as improving soil quality, nutrient richness, and diverting waste from landfills or incineration.
- The current federal regulation of biosolid quality focuses on 10 heavy metals and pathogens with no regulations for organic contaminants like PFAS.
    - Since biosolids contain many unknown organic compounds, there is a need to develop approaches that aid in the identification of toxic compounds in complex mixtures.
- Dr. Prasse presented an illustration of the project's objectives to characterize the occurrence, fate, transport, and risks of novel biosolid-associated organic contaminants (BOCs) using different NAMs and HRMS.
- He collected 16 U.S. and three Canadian biosolid samples, and specimens were analyzed and processed using various extraction methods and custom R scripts for QA/QC.
    - To align with the national effort, the compounds of interest included those detected in at least 80% of biosolid samples.
    - Dr. Prasse shared that detected features were reduced to 451 after QA/QC.
    - Using the Schymanski criteria, 92 compounds were identified with high confidence (levels 1 and 2), with 58 compounds not previously reported in biosolids. Most (77.4%) of the detected compounds were excluded because of the lack of known structures.
    - Endogenous compounds, pharmaceuticals, and industrial chemicals were the most common functional use categories of the detected compounds.
- In collaboration with ORD, Dr. Prasse used the Hazard Comparison Module (HCM) to identify chronic and acute data for the 92 identified compounds.
    - Dr. Prasse described metrics such as hazard, quality, quality-adjusted hazard, and completeness scores that were calculated and used to prioritize the compounds for further evaluation (e.g., ketoconazole, clorophene).
- A target list of compounds underwent further investigation to explore environmental fate and transport.
    - Dr. Prasse presented preliminary results from a biodegradation study using soil and biosolid mixtures and shared that 18 of 31 compounds were degraded by more than 15%. Ongoing work is focused on identifying and assessing transformation products formed during degradation. These transformation products can be incorporated into the database and hazard screening tool.
    - His team is also evaluating whether compounds in biosolids are taken up by vegetables grown in soil treated with biosolids.
- Dr. Prasse briefly introduced a pilot study focused on the characterization of exposure scenarios and quantification of exposure factors unique to biosolids workers.
- He summarized his presentation by noting that this framework, based on HRMS and hazard assessment tools, is a promising approach for compound prioritization in complex mixtures.
    - He noted that the limited number of compounds in reference mass spectral databases is a major challenge.

Questions answered during the conference included:

- **(Verbal, in-person) Chris Vulpe (University of Florida):** It seems like a lot of these compounds are manufactured. I'm worried about compounds that are anthropogenic. It seems like there's no requirement on providing mass spectrometry data on anthropogenic compounds. That seems like it could be a requirement when a new compound is made. They could have those data to look at.
    - **(Verbal, in-person) Carsten Prasse:** I love that idea. To my knowledge, this is not part of any regulation. Many of these chemicals have been around a long time, so we don't have HRMS data with legacy compounds. It would help to have them, but to my knowledge, they are not required.
- **(Online chat) Lisa Golding (Commonwealth Scientific and Industrial Research Organization, Australia)**: Were the concentrations of the compounds identified by NTA in biosolids quantified?
    - **(Verbal, in-person) Carsten Prasse:** Great question. We are working on some quantitative non-target analyses – that is the next step for these 40 compounds. We plan to ask utilities for additional samples to quantify concentrations.

- **(Verbal, in-person) Sin Urban (Maryland Department of Health):** I thought pesticides had to be registered and standards had to be submitted; I wish we had that for other chemicals. We have this rich regional sampling. Are there differences in regions or commonalities for biosolids?
    - **(Verbal, in-person) Carsten Prasse:** We haven't looked at that aspect yet. There are a lot of data on what is present in biosolids. In addition to municipal sources, wastewater treatment plants also receive industrial wastewater. There are a lot of differences, but that is a less regional and more facility-to-facility difference.
- **(Online chat) Anonymous virtual attendee:** If biosolids are contaminated with toxic and potentially toxic compounds, why are they still being used on agricultural fields?
    - **(Verbal, in-person) Carsten Prasse:** I want to make this clear: We don't yet know whether there are any risks associated with biosolids. EPA provided funding for groups to evaluate whether there are compounds present. Johns Hopkins is evaluating biosolids because they are important fertilizers and, if used, we then don't need to apply synthetic fertilizers. This provides a valuable resource and diverts waste from incineration and landfilling, which might be required in some states to address PFAS. We are in the beginning of assessing these materials. I am not trying to say that there is risk, but we don't know if there is with the samples we are looking at.

Annette Guiseppi-Elie (U.S. EPA): Session Wrap-Up

Dr. Annette Guiseppi-Elie expressed appreciation for the afternoon filled with presentations of thoughtful and applicable research. She thanked Dr. Maureen Gwinn for opening the workshop and Dr. Rusty Thomas for outlining the five goals and providing an excellent summary of the progress made. She acknowledged Drs. Nicole Kleinstreuer and Alison Harrill for presenting their efforts to encourage the use of NAMs by building scientific confidence and enhancing validation processes.

Dr. Guiseppi-Elie shared that the exposure session was included on the agenda because it was a topic of interest identified by external stakeholders when surveyed for potential NAMs conference agenda topics. She emphasized that a key takeaway from Dr. Kristin Isaacs' presentation was the need for exposure assessments to be on the same footing as toxicity assessments. She agreed that exposure is not trivial and is essential for risk assessment. Dr. Guiseppi-Elie highlighted the development of various tools and models for high-throughput (HT) exposure evaluations and mentioned the importance of case studies in summarizing and applying the research.

She noted that she was unaware of software packages that were under development to model AOPs and AEPs and looked forward to trying out GeoTox's application and any future collaborations. She discussed the importance of addressing mixtures and geography as part of the cumulative impacts in risk assessments and the need for reproducible pipelines and best practices in software development (as presented by Dr. Messier).

She praised the practical application of research in identifying unknown PFAS in Maryland's drinking water from Dr. Urban's presentation and emphasized the importance of collaboration with EPA ORD and other partners. She expressed gratitude for collaborative researchers in the STAR grant program, like Dr. Prasse, to help leverage external activities.

She encouraged attendees to reach out to other researchers and explore further collaborations and stressed the need for coordinated efforts and leveraging partnerships across federal agencies, academia, and other areas. She concluded by thanking all the presenters and attendees for their engagement.

Day 2 Summary

Welcome and Opening

Krystle Yozzo (U.S. EPA) and Monique Perron (U.S. EPA)

Dr. Krystle Yozzo welcomed participants back and commented on the discussion from Day 1, which covered the NAMs Work Plan and the use of NAMs to quantify exposures. She noted that Day 2 will include talks about 'omics in the morning and about IVIVE after lunch. Dr. Yozzo introduced Monique Perron, who works for EPA's OPP. Dr. Perron expressed that she was excited about the sessions and shared that external and internal survey results indicated that 'omics was a topic of interest for the conference.

'Omics

Connie Mitchell (Health and Environmental Sciences Institute, HESI): Collaborative Vision for 'Omics-Based Chemical Testing

Dr. Connie Mitchell presented current 'omics projects in progress at HESI, with a focus on the collaborative nature of the projects, as well as an emphasis on the use of these 'omics data in decision-making. Dr. Mitchell specifically focused on two ongoing 'omics projects within the Emerging Systems Toxicology for the Assessment of Risk (eSTAR) Committee. The projects discussed in this talk, as well all products generated by HESI, are freely available in the public domain.

Summary

- Dr. Mitchell introduced the goals of the HESI eSTAR committee, which focuses on the development of innovative translational tools and using 'omics technologies for decision making. eSTAR is using transcriptomics to answer specific scientific questions.
- She highlighted the promise of 'omics techniques for not only largescale data generation and the potential for mechanistic insights but also the challenges associated with using large 'omics datasets in decision making.
- Dr. Mitchell highlighted two key goals for 'omics data, including using them as biomarkers for pathways and using them to derive PODs.
- She presented data from a longstanding project at HESI, TGx-DDI, which is a transcriptomic biomarker established to determine whether an agent is DNA damage-inducing (DDI). TGx-DDI is an *in vitro* biomarker designed to complement the standard battery of assays currently used to measure genotoxicity, which are sensitive but generally lack specificity and are often unable to provide mechanistic insights.
    - TGx-DDI was developed in human cells using 28 prototype chemicals known to be either DDI or non-DDI. This resulted in the identification of 64 genes that were highly correlated with DDI potential. Further, this human-relevant biomarker demonstrated high specificity, and results have been consistent across cell lines.
- Dr. Mitchell discussed potential applications for TGx-DDI, including drug-screening efforts when traditional genotoxicity tests report contradictory findings or chemical safety testing to provide a higher-throughput format for hazard identification, determination of chemical potency, or dose-response evaluation.
- HESI was funded by FDA, as well as by other contributions and materials from partner organizations, to conduct a ring trial across four different laboratories that will help establish a standard operating procedure (SOP) for the use of TGx-DDI.
- Dr. Mitchell introduced another HESI project, Error Corrected Sequencing, which is currently in the early stages of development. The goal of this project is the successful detection of key events in tumorigenesis, particularly clonal expansion, prior to the onset of gross histopathological indicators. Error Corrected Sequencing is an *in vivo* refinement, with the goal of providing a quicker alternative to standard two-year carcinogenesis studies in rodents.
    - Error Corrected Sequencing is an ultrasensitive sequencing method for DNA that can help to detect rare mutation events, with a particular focus on cancer driver genes.
    - Dr. Mitchell presented the process by which this tool will be developed in order to gain confidence for regulatory acceptance, including testing it with benchmark compounds with known properties (tumor-inducing chemicals vs. non-tumor-inducing chemicals), testing it with a panel of genes, and running pilot studies to determine whether Error Corrected Sequencing can be used to detect clonal expansion after 90 days of exposure with chemicals known to induce carcinogenicity at the 2-year timepoint.

Questions answered during the conference included:

- **(Verbal, in-person) Alison Harrill (U.S. EPA)**: My question is regarding the TGx-DDI biomarker qualification process. I can recall how painful this was because it was one of the first biomarkers to go through the process. To my understanding, there have been lessons learned and thus changes to the qualification process to move away from subjective metrics and toward more objective measures like those discussed during yesterday's workshop sessions. Can you talk about the evolution of the process and how this can inform EPA's approach to new NAMs?

    - **(Verbal, in-person) Connie Mitchell:** A key consideration in the decision was how this can be done in a way akin to clinical trials (or at even higher bars). We looked at factors such as specificity and sensitivity and rates of false positives and negatives. Determining the adequate levels is still in discussion, but the history of this work is always of interest and will be helpful for OECD validation or Integrated Approaches to Testing and Assessment (IATA). This is one of the first examples for genomic biomarkers, so it encountered many hardships, but there have been others developed since.
- **(Online chat) Sue Fenton (NC State University):** In your team's work on the DDI project, have you found that the cell type you use in testing matters? Are you getting similar outcomes across different types of liver cells, for example?
    - **(Verbal, in-person) Connie Mitchell**: They are generally good, and details can be found in the papers referenced and distributed.

Logan Everett (U.S. EPA): Development and Application of Transcriptomics in EPA

Dr. Logan Everett presented a summary of transcriptomics work at the EPA, representing a large collaborative team of scientists. He emphasized that high-throughput transcriptomics is meant to be the first tier of EPA ORD's tiered testing strategy to screen large numbers of chemicals which currently lack data to sufficiently identify biological perturbations. Dr. Everett highlighted two major goals for these types of data include use for the determination of overall POD values, where we see any kind of perturbation in cells, and for making predictions regarding mechanistic targets that could be combined with other targeted NAMs.

Summary

- Dr. Everett discussed the large-scale transcriptomics work going on at EPA in which TempO-Seq is being leveraged to evaluate up to 20,000 genes in a way that is ideal for large-scale screening and more cost-effective than RNA sequencing or microarray.
- Evaluating up to 20,000 genes, with hundreds or thousands of chemicals, yields a large volume of data, which then requires: (1) analysis for changes across treatments to identify dose-response trends and (2) integration of signals across sets of genes related to specific pathways. Analysis of coordinated changes across a set of genes in a pathway is important for boosting signal-to-noise ratio and increasing biological interpretability.
- Dr. Everett emphasized that even across widely accepted data analysis approaches, there are many different protocol parameter decisions that can impact the results of the study. Therefore, the context for analyzing data is always very important, and data analyses should always be tailored to the experimental question.
- EPA developed an analytical approach for estimating changes in gene expression using transcriptomics, and dose-response modeling is then conducted on those data. For this analytical approach, the code is available on GitHub and all components are based on open-source tools.
    - In this approach, sequencing data for one chemical at a time is normalized to fold change data and is then fed into gene set enrichment analysis, which is used to generate an enrichment score for up- or down-regulation of a gene set at each concentration of the chemical. These data are then used in concentration-response modeling to determine whether the chemical exhibits a dose-response relationship for impacting a gene set.
    - The workflow has been applied to over 12,000 chemicals in three cell lines, and all data regarding each active gene set "signature" hit for each chemical are publicly available on the CompTox Chemicals Dashboard.
- Dr. Everett described a 2021 publication outlining the pilot study of this data analysis approach, which analyzed 44 well-characterized chemicals with a large volume of existing targeted HT screening data available in ToxCast[6] (Toxicity Forecasting). For most chemicals, this publication demonstrated that this transcriptomic data analysis method could serve as an alternative to the combined results of hundreds of targeted HT assays, with these signature-based POD values in high agreement with HT assay results.
- Dr. Everett described the derivation of a fifth percentile biological pathway altering concentration ($BPAC_{05}$), using the fifth percentile of the active signatures. Data indicated the more potent chemicals with known specificity for a molecular target tended to have a $BPAC_{05}$ that was greater than the median value.

[6] https://www.epa.gov/comptox-tools/toxicity-forecasting-toxcast

    - With this information, signature annotation was used to determine whether any signatures were associated with particular molecular targets, and Dr. Everett showed that this approach was successful in linking many chemicals to their known targets.
- Dr. Everett also described the use of connectivity mapping to cluster chemicals based on target receptor similarity, and this clustering correlated well with the transcriptomic signatures. Further, building new target-specific gene signatures and incorporating them into the modeling led to the accurate prediction of chemicals that were likely to be potent and dose-responsive for a particular target.
- The next steps for this work will include screening more cell types, benchmarking analysis methods, and harmonizing them across different use cases for transcriptomics.

Comments made and questions answered during the conference included:

- **(Verbal, in-person) Ivan Rusyn (Texas A&M University):** You showed a perfect dose-response curve going up, but very few chemicals show this, and most will have nonmonotonic curves that crash as cells are dying. How does the ToxCast pipeline deal with that? I saw there was a cytotoxicity threshold on the web interface, but how do you deal with that when you are modeling?
    - **(Verbal, in-person) Logan Everett:** On the web interface, the cytotoxicity thresholds are based on ToxCast data from the cytotoxic burst. In screening, we always have some type of cytotoxicity data alongside the transcriptomics, and we mask out any concentration that either killed more than 50% of cells or had more than 50% of the LDH (lactate dehydrogenase) signal before any concentration-response modeling on the transcriptomic data (which should cut down on non-monotonic responses).
    - **(Online chat) William Irwin (U.S. EPA):** Good question. However, an IC50 [half-maximal inhibitory concentration] value is regularly not a sensitive threshold, such as 50% cell death by LDH data.
- **(Verbal, in-person) Ivan Rusyn (Texas A&M University):** It is encouraging that the high-throughput transcriptomics (HTTr) PODs correlate with the *in vitro* PODs; you alluded to the fact that this one assay can replace hundreds of assays. But working up towards the same data across 100 cell types would be the same cost as 100 assays. Can you clarify?
    - **(Verbal, in-person) Logan Everett:** That is a good question, and we don't know how many cell types we need to use to have the answer, but hopefully it will be 10s and not 100s.
- **(Verbal, in-person) Ivan Rusyn (Texas A&M University):** I suggest considering the use of machine learning applications with these data.
- **(Verbal, in-person) Sayak Mukherjee (Battelle):** I know there is cost involved in molecular target discovery. Have you tried to ground it in other multi-omics approaches?
    - **(Verbal, in-person) Logan Everett:** Yes, we are using some cell painting and transcriptomic data on the same set of chemicals for some cell types and looking into which targets we can predict more accurately with those data together versus which targets are predicted more accurately with cell painting versus transcriptomics. Other than cell painting, we do not have another approach that is cost effective enough to also use.
- **(Verbal, in-person) Sayak Mukherjee (Battelle):** Are you looking at actual gene-gene correlations?
    - **(Verbal, in-person) Logan Everett:** We are computing gene set enrichment analysis on each concentration for each gene set and then doing concentration-response modeling on those enrichment scores. We are getting a concentration-response or enrichment score for each signature.
- **(Verbal, in-person) Sayak Mukherjee (Batelle):** If it was only one timepoint?
    - **(Verbal, in-person) Logan Everett:** Yes, we evaluated three timepoints in the pilot and got very similar results, but we did not see much of an advantage of investigating at multiple timepoints.
- **(Online chat) Richard Currie (Syngenta):** Do you think we will have to generate new reference signatures for each cell line?
    - **(Verbal, in-person) Logan Everett:** Maybe. It's possible. For cell lines that are more well studied, there will be less of that. For cell lines without a lot of transcriptomic data, yes, we will need new reference signatures. That may just mean we need to focus on screening a bunch of reference chemicals first.
- **(Online chat) Anonymous virtual attendee:** Do these results mean that EPA will be moving away from ToxCast and toward HTTr in the future?

    - **(Verbal, in-person) Logan Everett:** I think we already were and to some degree some ToxCast assays are not even commercially available anywhere, and we can't keep screening that way. We are not completely moving away from these methods—we still need targeted testing for orthogonal confirmation—but we are moving away from strict tiered testing. Maybe we do a narrower set of chemicals, and we could do some follow-up testing with other targeted testing. We have already moved away from looking at lots of chemicals in lots of targeted assays.
- **(Online chat) Hesbon Nyambego (Exxon Mobil):** Do we think we are losing any information by not pursuing conventional RNA-seq in lieu of TempO-Seq? I'm asking because global RNA-seq is now very cost-efficient.
    - **(Verbal, in-person) Logan Everett:** We are losing information, but is it actually useful for toxicogenomics? We cannot tease apart isoforms or slice variants, but from the standpoint of overall PODs, I'm not convinced those extra levels of information add anything. For target prediction, I don't think we need those data. For understanding the toxicity of the chemical, yes, there is more that we are missing, but for toxicogenomics and our goal in this screening context, no.

Jessica LaRocca (Corteva Agriscience): Use of 'Omics to Inform Development and Safety of New Pesticide Active Ingredients

Dr. Jessica LaRocca presented on the application of 'omics to inform the development and safety of new pesticide active ingredients using NAMs. She detailed the practical application of NAMs, including the use of computational models to predict toxicological outcomes, and the integration of *in vitro* and *in vivo* data. She introduced the use of cell painting toxicophenomics to predict PODs and presented validation data comparing transcriptomic PODs with apical PODs, demonstrating high concordance and supporting the reliability of the method.

Summary

- Global regulatory agencies' requirements for human health rely heavily on animal studies, which can consume a lot of time and resources. NAMs can be used for predictive toxicology studies to reduce animal use, while maintaining product safety.
- Dr. LaRocca reviewed the use of transcriptomics to predict PODs for risk assessment by leveraging previously harvested tissue from existing studies to generate 'omics data.
- AOPs are typically anchored with specific adverse effects, and transcriptomics are used in a hazard-agnostic manner to predict any apical effect preceded by molecular changes.
    - The POD can be determined by using no observed effect level (NOEL) or benchmark dose (BMD) approaches.
    - The transcriptome point of departure (tPOD) estimates the lowest dose for a "concerted change in gene expression."
    - Dr. LaRocca presented findings that showed concordance between tPODs derived from short-term studies and apical PODs from long-term studies.
    - Dr. LaRocca reviewed the ETAP 5-day toxicogenomic standardized study design, which can be used to inform target organs and PODs for risk assessments and has incorporated this design into Corteva's development pipeline to increase confidence during the early molecule discovery phase.
- Cell painting toxicophenomics can be used to analyze changes at the cellular level using high content imaging assays and to predict PODs. This approach can be used in drug discovery or predictive toxicology.
    - Dr. LaRocca explained how HepaRG liver cells were selected for predictive toxicity because the liver is the most common target organ for agrochemicals.
    - She presented results of *in vitro* cell painting data with *in vivo* PODs demonstrating high concordance.
    - She shared additional results from the application of machine learning to cell painting data in order to predict Ames classification and hazard. Machine learning allowed them to meaningfully leverage the massive amounts of data generated by cell painting.
- Dr. LaRocca mentioned an ongoing collaborative project with HESI and the 'omics for Assessing Signatures for Integrated Safety (OASIS) Consortium, which aims to integrate various 'omics technologies to improve predictive toxicology.
    - The mission is to gain confidence in the combination of cell painting, transcriptomics, and proteomics for safety assessment using hepatotoxicity as a use case.

- Dr. LaRocca concluded with the aspirational goal of including NAMs in discovery programs and regulatory applications.

Questions answered during the conference included:

- **(Verbal, in-person) Douglas Wolf (WolfToxRisk LLC)**: I have a question about confidence building and implementation in transcriptomics. There was a previous publication having to do with using benchmark dosing modeling transcriptomics, and there were two major stakeholders: the research and development (R&D) program and senior leadership. If you think about the application of what you're doing in the *in vivo* space with 1-, 2-, or 5-week studies, you can do predictive risk assessment, and it is not substantially different than the typical. Internal stakeholders are very risk averse, so how do you convince them to make these development decisions without needing to do other studies? Another population to keep in mind is the regulatory community and consider what conversations are being had to get this information out to the broader community. Some uncertainty factors, like database uncertainty or sub-chronic to chronic uncertainty, should go away. Where are these conversations happening outside of this workshop?
    - **(Verbal, in-person) Jessica LaRocca:** Thank you for your question, but the data have to speak for themselves. We are not the only organization routinely generating transcriptomic data. The evidence that is there is useful and is being included in reports and being submitted for regulatory agencies to see. For regulatory application, they have to take a global view since every product goes to many agencies around the world, so there could be a situation where you have adequate data for a molecule and present it to an agency, but they have other studies to address still. It will take time, data, and evidence of acceptability to get the process started.
- **(Online chat) Charlie Stevenson (Cruelty Free International):** How did you calculate/where did you get your figures for the number of animals used in the mammalian *in vivo* studies for toxicology requirements?
    - **(Verbal, in-person) Jessica LaRocca:** I'd like to thank my colleague, Joel Enriquez, who put it together by looking at internal protocol and SOPs being used. For the developmental and reproductive toxicology (DART) study, only adults were included but they do have the numbers that would include the offspring. There is another publication that was recently accepted and should be available to the public in the next couple of weeks; it will list these data in detail as well as the animal tracking benchmarks for Corteva.
- **(Verbal, in-person) Ivan Rusyn (Texas A&M University):** I agree that it is important to benchmark these approaches both at the end user and government agency. The thing about the internal data that struck me was that the rank correlation was not adequate to use these assays to rank compounds taking them forward. How might you expand the ranking of compounds going forward, and is that a benchmark for you or not? Guessing the POD is one thing, but since you are deciding alternatives that will require rank prioritization, is that a concern to you?
    - **(Verbal, in-person) Jessica LaRocca**: The example in my presentation is just one way of looking at it. One methodology that is very simplistic but works well is ranking by biological potency, like micromolar and nanomolar points of departure. This is just one piece of a larger puzzle to predict the profile of the molecule, while considering the pharmacokinetic profile.
- **(Verbal, in-person) Ivan Rusyn (Texas A&M University):** Is this being communicated as such to make it clear the rank correlations could be interpreted differently by others? How you communicate and integrate this information is important to know.
    - **(Verbal, in-person) Jessica LaRocca**: Agree, this is one data point out of many.
- **(Verbal, in-person) Rusty Thomas (U.S. EPA):** Following up on the comments made by Doug Wolf, regarding the POD, the issue isn't determining the POD, the issue is that regulatory frameworks would have to change to accommodate that in decision making. North America seems more accepting of this as a tool due to our focus on dose-response and risk compared to other parts of the world, where their decision process is classification, labeling, and other processes. This tool and this approach are less amenable. Uncertainty factors like how to derive risk or toxicity value – we have worked through with EPA buy-in to accommodate uncertainties in typical risk assessment so it can be carried over from transcriptomic datasets. Getting consistency among large regulatory frameworks is still something we are working toward and is a crucial part of applying this internationally.
    - **(Verbal, in-person) Jessica LaRocca**: I agree that it would be good to run a short set of studies, especially for a class of chemistry that is particularly well characterized by subsequent molecules.

For hazard-based classification, it would not fit that purpose, and it may not be possible to have transcriptomics predict every specific hazard known. What could happen is that this would be part of a comprehensive alternative data package. Continuing these conversations with multiple stakeholders is crucial to understanding what they need to give them confidence.

Questions answered following the conference included:

- **(Online chat) Anonymous virtual attendee:** Can you use 'omics to screen pesticides? Do you send your 'omics data to EPA/EFSA (European Food Safety Authority) for pesticide registration?
    - **(Post meeting, written) Jessica LaRocca:** For high-throughput screening, *in vitro* toxicogenomics and *in vitro* cell painting are excellent tools to enable decision-making processes and gain an initial assessment of bioactivity. We incorporate toxicogenomics data into some of our Good Laboratory Practice *in vivo* studies, which are included in our reports. I envision a future in which tPODs may be used in risk assessments compared to traditional apical endpoint NOAELs.
- **(Online chat) William Irwin (U.S. EPA):** Berberine is fluorescent. How is that artifact handled in the data consideration?
    - **(Post meeting, written) Jessica LaRocca:** We have not seen issues with this. The plates are washed and fixed before imaging.
- **(Online chat) Barbara Birk (BASF SE):** How do you distinguish in the cell painting approach between an unspecific toxic effect and a specific effect? Do you start with a range-finder study first?
    - **(Post meeting, written) Jessica LaRocca:** I view these phenotypic changes as helping to determine bioactivity, not necessarily identifying specific adverse tox effects. We do not run a rangefinder for cell painting studies.
- **(Online chat) Rahul Date (JRF):** You briefly mentioned HepaRGs were tricky to handle. Can you please explain more?
    - **(Post meeting, written) Jessica LaRocca:** Typically, HepaRGs in culture need to be grown to very high confluence, which makes the imaging and processing more difficult. We altered the culture conditions to better enable cell painting and plan to publish this method soon.
- **(Online chat) Rahul Date (JRF):** How was the rat hepatocyte response in comparison with HepaRG?
    - **(Post meeting, written) Jessica LaRocca:** We do not have this information yet.

Chris Martyniuk (University of Florida): Knowledge Gaps and Opportunities for 'omics Data in Environmental Assessments

Dr. Chris Martyniuk's presentation addressed the challenges and opportunities of using 'omics data in environmental assessments.

Summary

- Dr. Martyniuk introduced his lab, which focuses on HT toxicity testing, primarily with zebrafish and field work to understand the impact of chemicals on the environment and human health by analyzing changes at the molecular level.
- He reviewed the challenge for regulatory agencies with approximately 100,000 chemicals on the market and only approximately 500 chemicals with known hazards and exposures, representing an extensive data gap.
- He emphasized the importance of NAMs in risk evaluations for existing chemicals to help prioritize chemicals and evaluate toxicity.
- He discussed the benefits of the zebrafish model for high-throughput toxicity testing, such as reduced cost, rapid development, and the capacity of high experimental replication.
- Dr. Martyniuk's research focuses on defining AOPs for contaminants of concern. He highlighted the opportunities for 'omics in risk characterization and emphasized a focus on mitochondrial dysfunction, which is a key event in various diseases.
- One disadvantage of the zebrafish model is that zebrafish are not ecotoxicologically relevant.
- He reviewed several challenges of using field-based 'omics studies to understand impacts on populations, such as the influence of abiotic factors (e.g., temperature, pH, salinity) and the individual organism, temporal, and spatial variability that can influence gene expression.

- Dr. Martyniuk presented results from two field studies from the Grand River and Shenandoah River.
    - Fish and water were sampled from the largest watershed in Canada, the Grand River, which receives discharges from 30 sewage plants.
        - Findings from the transcriptomic analysis found unique profiles differentiating reference and polluted sites and temporal changes in transcriptomic profiles before and after sewage treatment plant upgrades.
        - Improvements in sewage treatment processes led to a reduction in the expression of stress-related genes, indicating a positive impact on fish health.
    - The second field study used mobile laboratories to assess the health of fish in the Shenandoah River and to understand the impact of environmental stressors.
        - Fish were sampled over multiple years, observing variability in multiple factors, including condition factor, gonadosomatic index, and vitellogenin levels.
        - Agricultural and industrial discharge was identified as a significant influence on fish health.
        - Integrating 'omics data with physiological measurements can provide a comprehensive assessment of fish health; however, doing so requires complex data analysis techniques.
- Dr. Martyniuk shared a critical review paper defining the role of 'omics in assessing ecosystem health and the need to establish normal 'omics profiles to better understand deviations caused by environmental stressors.
- Accounting for the timing of sample collection is important to account for temporal variability.
- Future directions include development of tools for better environmental monitoring and data integration, use of confidence intervals to manage variability, and integrating 'omics data with physiological responses to provide a comprehensive understanding of environmental impacts.

Comments made and questions answered during the conference included:

- Dr. Martyniuk was introducing the zebrafish AOP framework his work focuses on. The AOP starts with molecular initiating events that cause mitochondrial dysfunction because mitochondrial respiration disruption is a "hallmark signature" of human and wildlife diseases.
    - **(Online chat) William Irwin (U.S. EPA):** Great point, mitochondrial function is important for free radical generation and ATP [adenosine triphosphate] production for biological work (protein synthesis, motion, ion pumping, etc.) consistent with the Laws of Thermodynamics.
- Dr. Martyniuk was describing the variables that could influence 'omics datasets from the field versus laboratory studies, and the challenges with understanding chemical-specific responses with data variability.
    - **(Online chat) Matt Baur (Western IPM Center)**: The stated goal of many of the NAM projects discussed seems to be increased efficiency. But it seems an important goal should be the ability to better protect human and environmental health from potential toxicants.
- **(Verbal, in-person) Douglas Wolf (WolfToxRisk LLC)**: Could there be survivor bias in the population being sampled as they have adapted to the negative environment and may not have as large of a response as metropolitans? In addition, what needs to be done to ensure these approaches are informed with mitigation strategies that are in compliance with the Endangered Species Act?
    - **(Verbal, in-person) Chris Martyniuk:** Survivor bias may be a possibility, but I do not have a good sense of the underlying mortality events happening in these natural environments. Data from those that are alive and living in these habitats are typically what are collected. I believe there have not been any significant mortality events in these areas of research. Fish populations have stayed consistent and stable leading to the assumption that it's a sublethal exposure at these sites, and exposure to chemicals is low. In regard to the Endangered Species Act, I am working with collaborators who deal with endangered native species who try to understand chemical impacts in those specific species. Getting them on board with the 'omics technology is a huge step and will require much more work. 'Omics haven't been accepted for environmental modeling programs nationally, so it has been a challenge to get them incorporated as it is difficult to demonstrate that changes in a gene or protein

are related to an adverse health population response. While there is progress being made, there is still much work to be done in this area.

Questions answered following the conference included:

- **(Online chat) William Irwin (U.S. EPA):** For field studies, can the temporal changes in 'omics also be due to changes in food sources over the seasons?
    - **(Post meeting, written) Chris Martyniuk:** Absolutely. The nutrient input into the system and food availability can also alter the transcriptome (or 'omics) response and can mask any effect of chemical load in macroenvironments.
- **(Online chat) Laura Langan (University of South Carolina)**: You mentioned some factors, but what have you thought about regarding the incorporation of differing strains (zebrafish being famous for wide strains as are other species who have adapted to environmental pollutants)? Likewise, what about light-dark cycles' impact on variation in expression profiles? We know these are very infrequently reported in studies, even in the lab.
    - **(Post meeting, written) Chris Martyniuk:** Photoperiod will influence transcriptional responses, but its relative contribution to the overall response, as you have pointed out, has gone largely unreported in both the lab and the field. Indeed, different strains have been shown to respond differently to chemical exposure, which reflects the role of genetic variation in the overall response of the transcriptome. Elucidating the role of individual single nucleotide polymorphisms in mediating chemical-induced 'omics responses is critical moving forward.

Matthew Meier (Health Canada): Fostering Transparency and Reproducibility Using the OECD 'Omics Reporting Framework

Dr. Matthew Meier presented on fostering transparency and reproducibility using the OECD 'Omics Reporting Framework (OORF). He reviewed the development and testing of reporting modules and several case studies. Future directions include machine-readable formats and centralized data repositories.

Summary

- Dr. Meier provided background on how 'omics data can play a critical role in NAMs-based testing.
- Many advantages of toxicogenomics include exploring different levels of biological systems, rapid and cost-effective data generation, and reduction in animal use. Toxicogenomics can be implemented along with other data streams in decision-making.
- Some challenges to applying 'omics in risk assessment include the lack of transparency in data generation and processing, lack of standardization in study parameters and reporting, and lack of case studies and guidance describing acceptable practices.
- Dr. Meier introduced the OORF, which is publicly available as of last year and which has the goal of developing a framework for the reporting standards of 'omics data to ensure that all the information required to understand, interpret, and reproduce results is available.
- The structure of the OORF consists of several modules.
    - The Study Summary Reporting Module describes a subset of reporting elements to provide a high-level overview of a regulatory toxicology and 'omics experiment, focused on key objectives.
    - The Toxicology Experiment Reporting Module captures and reports the key descriptors of an *in vivo* or *in vitro* toxicology study from which samples are derived for 'omics analysis.
    - Data Acquisition and Processing Reporting Modules capture and report descriptions of the 'omics assays, data acquisition, and associated data processing prior to statistical analysis. These modules are unique to each 'omics data type.
    - Data Analysis Reporting Modules capture and report descriptions of the statistical analysis that is often undertaken in an 'omics study, for example, for the purposes of discovering differentially abundant transcripts or metabolites.
    - Application Reporting Modules integrate various data streams for regulatory applications, such as chemical grouping and biomarker analysis.
- Dr. Meier presented several case studies of completing an analysis using the OORF with a test dataset and having a third party reproduce the analysis to assess concordance.
    - One trial for the enrichment analysis reporting module included using provided code to reproduce the analysis for the hexabromocyclododecane exposure dataset with RNA-Seq results.

    - The initial results showed high concordance (99.5%) with some discrepancies due to software versions and database updates. This highlighted the importance of precise documentation and configuration management.
- Dr. Meier noted that there are ongoing efforts to expand the OORF to include new technologies and applications.
- He mentioned that the goal of making the OORF machine readable is to improve data management and integration. Additional training and implementation can facilitate regulatory acceptance and practical application of the OORF.
- Dr. Meier highlighted that the OORF increases the interoperability of 'omics approaches by providing a framework and guidance for use. The focus now is on case studies, implementation, and training.

Questions answered during the conference included:

- **(Verbal, in-person) Logan Everett (U.S. EPA):** Is there a central repository to put the completed OORFs?
    - **(Verbal, in-person) Matthew Meier:** There is not, and this is a concern I share. With the International Collaboration on Cosmetics Safety application, it's an open-source code base, so a strategy may be to have institutions deploy their own instance, as this will be much easier. Some concerns are who owns the data, who wants what to be made public (and at what time), as well as who is going to pay for and host all of this. One solution may be that there is an international method of doing this. Starting the institutions with a standardized process to populate these fields will be helpful if there is a solution for centralized data storage in the future.
- **(Verbal, in-person) Ivan Rusyn (Texas A&M University):** There is a study titled "Standardizing Global Gene Expression Analysis between Laboratories and across Platforms" that was published in 2005 that tried to address the same problem being looked at in your presentation. While some institutions have done work on standardizing the data, this seems to be an effort in and of itself instead of something to be addressed by each agency. It's important to make this standardization so it can be sustainable and used for many years.
    - **(Verbal, in-person) Matthew Meier:** I agree that regulatory agencies will have to decide on their own what parameters are critical, and it poses the question of whether a risk assessor is going to take all that information and use it. Even if they may not use all data, it is at least recorded. The focus of the OORF has been to make sure the information needed to reproduce an analysis is recorded, and taking the last step may not be obvious at this time, but I do not want to speak on behalf of risk assessors.
- **(Verbal, in-person) Ivan Rusyn (Texas A&M University):** I want to talk about an example in ETAP in which the protocol is written and is supposed to be followed, then peer reviewed. Focusing on the context in which these are used can lead to more practical applications. I encourage thinking in terms of more practical solutions, instead of adding tasks to risk assessors.
    - **(Verbal, in-person) Matthew Meier:** I agree this is a challenge, but I think the bigger challenge is deciding what needs to be included since there is so much discussion involved, especially at the OECD level where other countries are involved. The decisions being made constitute a possible starting point, but there is room to grow when it comes to integrating the data across agencies.
- **(Verbal, in-person) Rusty Thomas (U.S. EPA):** I suggest this is not a unique problem. There are 90-day studies that are standardized and reported in harmonized templates, but regulatory agencies will interpret them differently. At the OECD national level, there is disagreement on how to do a consistent risk assessment across regulatory jurisdictions. The intent of the reporting template was to harmonize that reporting, and then each jurisdiction can use that information within their regulatory framework. This is not a unique problem, and you continue to run into this issue whether it be in the United States, Canada, or Europe. Harmonization has not been accomplished for toxicology, so expecting it for toxicogenomics is unrealistic.
    - **(Verbal, in-person) Matthew Meier:** Thank you.
- **(Verbal, in-person) Gina Hilton (PETA):** I agree with the points brought up by Rusty and Ivan. I want to add that this framework is just step one or two in the process. While I appreciate the comments being made on sustainability and making it more digitally accessible, an area worth bringing additional attention to is making the OECD trainings applicable to a regional context and making sure that regulators are in a prepared position when they start receiving this type of information, instead of saying it's too much data or they don't understand the language. Having an OECD storage shed is going to be an important step in developing this workgroup. Ensuring global communication will be important in this development.

Questions answered following the conference included:

- **(Online chat) Claudio Zanettini (FDA):** As you mentioned, there are ways we can maximize replicability by reporting software versions (e.g., using renv in R or Conda in Python) and running analyses in shared containers. One of the challenges, however, is the lack of incentives from journals to reward the publication of replicable code. Even when analysis code is shared, it is often system specific and challenging to rerun, with little to no review of its usability. How can we foster a change in this publishing environment?
    - **(Post meeting, written) Matthew Meier:** Great question. I've seen examples in which journals attempt to set requirements for transparent methods sections, and while the spirit of this approach is good, the end result is that reviewers, editors, and authors all have to be responsible enough to carry out this mandate. It is too easy to circumvent these requirements. I think the OORF could be an answer to some of this but only within the limited scope of toxicology experiments that have used 'omics. I could envision a scenario in which 'omics-related toxicology journals may take an interest in accepting a completed OORF (or at least some similar "checkbox" type of document indicating whether the individual elements have been accounted for in the publication). However, as a researcher, this sounds like an amount of additional overhead that would disincentivize submissions to a journal that requires it.
- **(Online chat) Anthony Reardon (Health Canada):** Could we take advantage of the availability of artificial intelligence or similar programs to automate the process of filling out the OORF?
    - **(Post meeting, written) Matthew Meier:** Very interesting question. I think one of the biggest advantages with LLMs in the context of these types of repetitive tasks is to create small, lightweight "agents" – that is, not a full Chat GPT-type model but a small, purpose-built/fine-tuned model that could help with specific tasks. So, some examples might be validating the input of a form and ensuring that it captures the intended elements or, as you allude to, taking elements from a graph-type structure of pre-filled information and using that structured data to populate the OORF (or, to go the other way and convert structured data into free-form text, create a methods section from a filled OORF). Retrieval augmented generation workflows could also be useful for this sort of task. Unfortunately, in the context of OECD, I think these applications would be somewhat in the distant future because of the requirements for oversight, governance, and mutual acceptance. These challenges are exponentiated by the number of agencies involved in a given initiative. Tying this to the previous question, maybe a good application would be ingesting manuscripts and attempting to extract and fill in the elements required for reporting. This could be worth a try; although I predict it would be far from perfect, it could save time. The problem is whether one would still have to have a human validate all of those LLM-based interpretations.

Monique Perron (U.S. EPA): Session Wrap-up

Dr. Monique Perron highlighted the main themes discussed throughout the day, including the integration of 'omics technologies, the importance of transparency and reproducibility, and the application of NAMs in a regulatory context. Dr. Perron highlighted the importance of knowing what one wants to do with their data within the decision context, whether that is ecotoxicology, human health, or something else. She briefly summarized the presentations, including the use of cell painting for predictive toxicity as presented by Dr. LaRocca, the challenges and opportunities of using 'omics data in environmental assessments and field studies as presented by Dr. Martyniuk, and the OORF as presented by Mr. Meier. Dr. Perron emphasized the importance of collaboration across disciplines to advance toxicity and risk assessments and the development of a framework for reporting. She expressed excitement for the work presented in this session. She emphasized the importance of training and effective communication to facilitate the adoption of new methodologies by regulatory agencies and stakeholders. She concluded by thanking the speakers and attendees for their participation and engagement.

In Vitro to In Vivo Extrapolation

John Wambaugh (U.S. EPA): Recent Developments in High-Throughput Toxicokinetics Including Confidence Assessment

Dr. Wambaugh presented on open-source tools and data available through the EPA in support of enabling HTTK and IVIVE approaches to enable the conversion of *in vitro* concentrations showing bioactivity as a POD to an external

administered dose for a chemical (mg/kg/day). These derived POD data can be used in risk evaluation and predictive toxicology.

Summary

- Dr. Wambaugh shared that EPA has made *in vitro* POD data openly available for approximately 10,000 chemicals, exposure forecast data available for approximately 400,000 chemicals, and HTTK data available for approximately 7,500 chemicals through the open-source, freely available CompTox Chemicals Dashboard.
    - The HTTK data are a combination of chemical-specific TK data derived from *in vitro* measurements and generic physiologically based pharmacokinetic (PBPK) modeling. EPA's R package for calculating HTTK data ("httk" package) is openly available, and the ADME [absorption, distribution, metabolism and excretion] IVIVE tab of the CompTox Chemicals Dashboard shows HTTK predicted values.
- There are many approaches to IVIVE, which is traditionally done using a chemical-specific scaling factor that predicts the administered dose based on steady-state blood concentrations. The httk R package incorporates probabilistic methods to predict this scaling factor which accounts for human variability and uncertainty in measurement. This higher throughput HTTK approach is fast and appropriate for screening thousands of chemicals.
- Dr. Wambaugh presented some of the work that has been done with the EPA httk R package, including updating the httk-pop human variability simulator to incorporate the most recently available NHANES data. The httk package has also been used to develop a model to simulate human gestational exposures and has been used to measure *in vitro* gut permeability using Caco-2 cells.
- A tool called "invitroTKstats" has been developed, allowing reproducible data analysis using a standardized workflow across laboratories, which also allows the estimation of chemical-specific measurement uncertainty.
- Dr. Wambaugh discussed HTTK quantitative structure-property relationship (QSPR) models, which use *in silico* methods to predict TK parameters such as intrinsic hepatic clearance, plasma protein binding, and oral absorption. Each model has an associated domain of applicability, which indicates for each chemical how reliable the model prediction is. There are nearly 10,000 QSPR predictions available on the CompTox Chemicals Dashboard.
- The HTTK Confidence Assessment database is also publicly available to help determine how well HTTK can predict ADME for a particular chemical. The CvTdb is a curated database of *in vivo* TK data, which has been used to analyze the confidence of HTTK predictions. Dr. Wambaugh shared that when comparing *in vitro* HTTK data to *in vivo* derived data, there is about a 16-fold error in extrapolation, which is the same as the amount of error when extrapolating from a combination of QSPRs to *in vivo* values.
- HTTK has also been used to analyze *in vitro* distribution of chemicals to understand where a chemical partitions to in a dish and to ultimately determine the concentration of a chemical reaching cells.
- Dr. Wambaugh shared that these tools are beginning to be suitable for use in decision-making processes through the combination of chemical-specific data, HTTK model data, and open-source tools for HTTK analysis and confidence characterization.

Comments made and questions answered during the conference included:

- The following comment referred to Dr. Wambaugh's updates to the httk package that allow for predicting gut absorption after oral chemical exposure, which can be used within the paradigm that less gastrointestinal absorption leads to less toxicological risk. However, the comment was recorded at the end of the presentation.
    - **(Online chat) William Irwin (U.S. EPA):** Pharmaceutical data has shown that some chemicals may have fairly common gastrointestinal tract toxicity with little or even no gastrointestinal absorption.
- **(Online chat) Tim Anderson (U.S EPA):** Can you talk more about how this steady state concentration captures the variability in the human population? Do you think an additional uncertainty factor (UF) for IVIVE would be necessary?
    - **(Verbal, in-person) John Wambaugh:** Good question. Right now, the NHANES goes down to age 3 in children. For human variability, we are pretty confident that we are capturing physiological variability. There are populations that are missing certain enzymes – we do simulate that, although we don't know which enzymes are missing so we are making an assumption. For that population, I feel good

that we are capturing variability. For the gestational model, it is a trick to get it to work for pregnant females, so we don't have variability greatly simulated for the average pregnant female in the model – nor do we have good simulation of the changes in enzyme induction as one grows up. We are actively working to include that. For adults, we are comfortable with the variability; for the pediatric population, one may need to consider more factors.

- **(Verbal, in-person) Tharacad Ramanarayanan (Syngenta):** I wondered about oral absorption. I understand if your endpoint is oral for a dietary study; however, if people are starting to use these data for other non-dietary (particularly dermal) exposures, how do you deal with that?
    - **(Verbal, in-person) John Wambaugh:** One of the things that's fun and challenging is that every feature we add to the httk R package you can turn off, so if you liked the way it was before an update you can turn that update off. We are also working specifically on a dermal model to approximate dermal over oral absorption, but that's not available yet – maybe 2 years from now. So, when we add a feature, you can turn it off, if you don't like how it's set up. We have a hard time making every permutation someone could be interested in available on the Dashboard outputs, but everything is available to flip on and off and make different approximations on when using the httk tool programmatically.
- **(Verbal, in-person) Sayak Mukherjee (Battelle):** I'm curious about the reported values in the Dashboard. Which model do you use?
    - **(Verbal, in-person) John Wambaugh:** We use a couple of them. There's a column in CompTox that says which model we are using. There is a tab that says "PBTK [physiologically-based toxicokinetic] model." The goal is to add more models so one can choose which to use.
- **(Verbal, in-person) Sayak Mukerjee:** When you develop these models, how do you decide on their complexity? For example, the gestational model was very complex.
    - **(Verbal, in-person) John Wambaugh:** Our guiding principle is no more complex than we have the data to support. We have an entire paper statistically evaluating each equation in the gestational model against *in vivo* data for each of the included terms. In a lot of cases, we don't have *in vivo* data. Moving from adult female (pre pregnancy) to second trimester is just a linear model between the two life stages, which was the simplest model we could get. We always try to build to the evaluation data that we have, so that's why generally the models we have are simple but with the gestational model we couldn't get away with that.

Questions answered following the conference included:

- **(Online chat) Jingjie Zhang:** I like the httk package and use it for prediction, but I do not have 100% confidence in the results. Is the model accurate at the level of order of magnitude, or for example 40%?
    - **(Post meeting, written) John Wambaugh:** The accuracy of EPA's httk depends on the chemicals for which it is used and what you are trying to predict. Wang 2010 (https://doi.org/10.1124/dmd.110.032177) showed that it could predict AUC within a factor of 3 for pharmaceuticals. For non-pharmaceuticals we think it predicts AUC within 20-fold, C_{max} within 6-fold, and clearance within 60-fold. Wambaugh 2015 (https://doi.org/10.1093/toxsci/kfv118) looked at how the accuracy varied with chemical type.

Fabian Fischer (University of Rhode Island): Translation of In Vitro Effect Concentrations: Equilibrium or Kinetic Distribution Models?

Dr. Fabian Fischer focused his talk on the use of equilibrium versus kinetic distribution models for the translation of *in vitro* effect concentration data and how these different types of models can provide insights into what is happening to cells in a multi-well plate system. Dr. Fischer discussed the challenges presented by the huge number of registered toxic chemicals that are released into the environment and the increasing complexity of these chemicals compared to traditional persistent organic pollutants, making previously developed QSARs less applicable to these chemicals.

Summary

- Dr. Fischer discussed the importance of assessing freely dissolved concentrations (C_{free}) when studying chemical exposures and toxicity, as the free fraction is important in bioavailability and disposition of the

chemical in complex systems. Once C_{free} is predicted or measured, only toxicodynamic information is needed to understand toxic effects.

- Dr. Fischer presented work focused on evaluating C_{free} in a multi-well plate and performing quantitative IVIVE (QIVIVE) to directly relate unbound concentrations to humans.
- Two types of models can be used to understand chemical distribution in a complex system: (1) equilibrium mass balance models, which assume equilibrium between partitioning compartments, with only the partition coefficient needing to be defined; and (2) kinetic (rate-limited) models, which incorporate permeability and transfer rate information and can be very complex.
- In this work, Dr. Fischer used *in vitro* reporter gene assays to visualize chemical-receptor interactions and to generate an effect or activity concentration (10% activation of the receptor). Using these data, equilibrium mass or kinetic models are then applied to calculate the C_{free} effect concentration, which can be compared to C_{free} concentrations measured *in vivo*.
- Variables that can interfere with accurate *in vitro* calculations of C_{free} include fetal bovine serum (FBS) content, chemical volatility, adsorption to plastic wells, diffusion of chemicals into plastic over time, and uptake of chemicals into the cells that changes the C_{free} over time. Different cell lines also exhibit different capacities to metabolize chemicals.
- Dr. Fischer described his collaborative work using a simplified mass balance model to quantify the effective C_{free} dose in high-throughput screening *in vitro* assays.
    - Dr. Fischer described using fluorescence microscopy to measure cell uptake of chemicals *in vitro* in real time. This work demonstrated that with more FBS content in the assay, the concentration of chemical in the cells decreases but the intracellular equilibrium is established more quickly, which favors the application of the mass balance model.
- Dr. Fischer also described investigation of plastic sorption using a kinetic model because there is diffusion into the well plates over time and there is a known rate-limiting step if FBS is removed. These models correlated well with the depletion of C_{free} observed in multi-well plates.
    - In solution, FBS was found to keep chemical concentrations stable over time because chemical that was absorbed into the plastic was resorbed back into solution by the FBS.
- Without FBS in a cell culture medium, hydrophobic chemicals were found to be more unstable, leading to uncertain exposure conditions and requiring more complex modeling. However, when there is FBS in the well, an equilibrium mass balance model is favored. Mass balance models can also be useful in the calculation of cell and membrane concentrations.
- Dr. Fischer discussed ongoing work developing a kinetic model, including all data he has generated over time, evaluating volatilization, multi-well plate sorption, FBS sorption, headspace, cell uptake, etc. While these data are less informative in assays that contain FBS, this kinetic model will be important in evaluating newer cell-free or organoid systems without FBS.

Questions answered during the conference included:

- **(Verbal, in-person) Jennifer Brennan (U.S. EPA):** I was at SETAC (Society of Environmental Toxicology and Chemistry), and equilibrium modeling was applied to a serum-free assay (OECD Test Guideline 249). What would you say to those using that model? Should they be using the kinetic model?
    - **(Verbal, in-person) Fabian Fischer:** Yes, in serum-free assays, most likely the chemical will be depleted by well plate absorption and other processes.
- **(Verbal, in-person) Jennifer Brennan (U.S. EPA):** I used to do tissue cultures, and, in my experience, it seems like those who are doing these assays are trying to move away from 10% FBS due to the cost and the fact that it is not animal free.
    - **(Verbal, in-person) Fabian Fischer:** You can use artificial nutrients, such as artificial serum albumin. It should work in a similar way.
- **(Verbal, in-person) Ivan Rusyn (Texas A&M University):** I'm curious about the data available to calibrate models and compare between models. What is the universe of chemicals that actually have been measured in media and cells, not just fluorescent chemicals? There was a lot of talk about modeling, but how many actually have measured data?
    - **(Verbal, in-person) Fabian Fischer:** For binding to FBS and medium in general, we have data for more than 100 chemicals. The chemical space is diverse. For the neutral organics, there are well-

calibrated QSARs as well

- **(Verbal, in-person) Ivan Rusyn (Texas A&M University):** I was asking about the number of chemicals that have been either actually modelled or measured. How many chemicals are available to test model performance on?
    - **(Verbal, in-person) Fabian Fischer:** For taking the media concentration, they have quite a lot, 20–25 including complex chemicals that don't follow the neutral organics profiles. For uptake into the cells, they measured 12 or 13. Because of quenching, they had a hard time calibrating with intracellular concentrations.
- **(Verbal, in-person) Ivan Rusyn (Texas A&M University):** This could be an opportunity for EPA to provide more data.
    - **(Verbal, in-person) Fabian Fischer:** Agreed.

Questions answered following the conference included:

- **(Online chat) William Irwin (U.S. EPA):** For membrane partitioning, did you apply the Nernst Equation, especially for cationic molecules? Quenching is common for Nernst molecules.
    - **(Post meeting, written) Fabian Fischer:** Thank you for the question. For membrane partitioning, we used either experimentally derived membrane-water partition coefficients from various sources (see Fischer et al., 2017) or predicted values. For predicting neutral chemicals, we applied poly-parameter linear free energy relationships (PP-LFER; Endo et al., 2013), whereas for ionic compounds, we used COSMOmic (Bittermann et al., 2014). COSMOmic accounts for the phospholipid bilayer's anisotropy by virtually dividing the bilayer into layers and sequentially calculating the Gibbs free energy of the chemicals between a bulk water layer and each phospholipid layer.

Shane Hutson (Vanderbilt University): Impact of Chemical-PDMS Interactions on IVIVE from Microphysiological Systems

Dr. Shane Hutson presented on the interactions of chemicals with materials used in NAMs and microphysiological systems (MPS), including ways to estimate and account for the interactions.

Summary

- Many devices like organs-on-a-chip for toxicological research are made of polydimethylsiloxane (PDMS), which is very hydrophobic and poses a chemical binding issue. Chemicals diffuse into the bulk of it and do not just bind to the surface.
    - In environmental toxicology, there are a lot of hydrophobic chemicals, many of which are toxic because of their hydrophobicity.
    - Researchers can either try to mitigate this issue or measure and model it.
- Dr. Hutson described experiments that were used to calculate partition coefficients and diffusion constants of a range of compounds (e.g., dyes, pesticides), such as using a disc of PDMS floating in a solution and measuring the change in concentration over time. For chemicals that were insufficiently soluble, values were extrapolated from experiments with high amounts of a cosolvent.
    - While the results help inform parameter estimates, there are not enough data to develop a robust model for predicting parameters from the chemical structures (e.g., logK does not always correlate with logP).
- Additional experiments included visualizing diffusion of materials to confirm diffusion constants, including those for several dyes. Values from these experiments confirmed values from the earlier experiments.
- Structurally similar compounds sometimes had experimental values that differed by orders of magnitude, precluding the use of read across without further information.
- The previously described experiments were completed in a static solution, whereas MPS have a constant flow rate. Experiments and modeling showed that MPS never reach a constant concentration with the PDMS, and diffusion constants of the compounds determine how steep the gradient with the PDMS is.
- Mitigation strategies include replacing PDMS with styrene-ethylene-butylene-styrene (SEBS) copolymer, but experiments showed that materials would partition into the SEBS material strongly but would not diffuse as quickly throughout. Using higher concentrations of serum provides a more stable environment to prevent chemical partitioning into PDMS.

Comments made and questions answered during the conference included:

- Dr. Huston introduced the challenges of using PDMS, which is what organ-on-chip devices are usually made with. He presented the 10 test chemicals used to produce the data he was presenting (1 of which was fluorescein).
    - **(Online chat) William Irwin (U.S. EPA):** For live cell membranes, fluorescein partitioning is also influenced by the pH gradient and for hydrophobic cations by the membrane potential.
    - **(Online chat) Anonymous virtual attendee:** Recommendations already exist for replacing PDMS. Any chosen plastic (or glass) has the potential for test article binding, but PDMS should be avoided to avoid additional complexity. A good example is:
        - Tomlinson et al, (2023). Considerations from an international regulatory and pharmaceutical industry (IQ MPS Affiliate) workshop on the standardization of complex in vitro models in drug development. Advanced Biology 9:e2300131. https://doi.org/10.1002/adbi.202300131
- **(Verbal, in-person) Jon Arnot (ARC Arnot Research and Consulting):** I'm not familiar with all the test chemicals, but is amodiaquine an ionizable chemical? I thought it might disassociate. Was that a bit of an anomaly?
    - **(Verbal, in-person) Shane Huston:** Good question. I thought we looked at it, but I don't remember and will follow up.
    - **(Verbal, in-person) Jon Arnot:** I know there was an ionizable functional group, so I wasn't sure.
- **(Verbal, in-person) Lena Smirnova (Johns Hopkins University):** Two questions: (1) If you add FBS, which is what *in vitro* folks tried to avoid, would you have too many unknowns, and that would defeat the purpose of delivering the chemical to the cell? (2) Should they be worried about media components, also to be absorbed?
    - **(Verbal, in-person) Shane Huston:** Both of these are great questions. For the first one, you are right: Putting in serum or bovine serum albumin (BSA) would reduce availability to cells, but it stabilizes the concentration in solution; the concentration is known better, but it does reduce the availability. For the media component, that is a concern. We have looked at estrogens and estrogen-like compounds. We lose a lot of those, so if those are important growth factors, they're going to be different from what the nominal concentrations are.
- **(Verbal, in-person) Fabian Fischer (University of Rhode Island):** The whole idea of adding FBS or BSA is to understand the factor needed to increase the total dose to reach a similar freely dissolved dose. It is not creating more unknowns; it is more stability and easier control over the test system.
    - **(Verbal, in-person) Shane Huston:** Good point.
- **(Verbal, in-person) Jennifer Brennan (U.S. EPA):** Does molecular weight have something to do with logP or hydrophobic chemicals not behaving as expected?
    - **(Verbal, in-person) Shane Huston:** For the partition coefficient, we don't see any pattern for molecular weight. For the diffusion constant, there is a drop-off with rhodamine B, which is a compound with the highest molecular weight in the test set, so that is possible.
- **(Online chat) Vikrant Singh (California Department of Pesticide Regulation):** Can a liquid, light molecular weight equivalent of PDMS be used as a quick surrogate for evaluating the binding constants?
    - **(Verbal, in-person) Shane Huston:** Good question. You probably could use it to estimate binding or partition coefficient, but it won't give information about diffusion constants. When you are trying to model what happens in an actual device in which spatial dependence matters, you're going to be missing a key parameter.

Questions answered following the conference included:

- **(Online chat) Sudip Mondal (vivoVerse, LLC):** I am interested in understanding the material property that changes due to different kinds of fabrication techniques/parameters used in MPS platforms. How will that affect the parameters?
    - **(Post meeting, written) Shane Hutson:** We have looked at the impact of two PDMS treatments: high temperature annealing and surface plasma oxidation. We found that annealing the PDMS caused small but measurable changes in the partitioning and diffusion of a test chemical, indole. The partition coefficient for indole decreased by 7% after annealing, and the diffusion constant of indole in PDMS dropped by 27%. As for plasma oxidation, it is unfortunately reversible on time scales

comparable to the disc-soak and membrane experiments. Oxidizing the PDMS surface did reduce partitioning somewhat, but it is hard to be quantitative because the degree of oxidation changed over the course of the experiments.

Jon Arnot (ARC Arnot Research and Consulting): Developing Internal Thresholds of Toxicological Concern

Dr. Jon Arnot's presentation provided an overview of a paper published as part of EPA's ExpoCast Special Issue and the applications of the new internal thresholds of toxicological concern (iTTCs), intended to be a conservative threshold for the protection of human health.

Summary

- The threshold of toxicological concern (TTC) has been around for 20 years and is considered a pragmatic risk assessment tool with the objective of establishing a human exposure threshold for all chemicals, below which human health has a very low probability of appreciable risk. This leverages existing toxicology data and structural information for substances with little or no data. Dr. Arnot explained that there are three classes of chemicals: low, moderate, and high toxicity, which are plotted together on a cumulative curve; the 5th percentile is then taken, and this number is divided by 100 to derive the TTC.
- Initial TTCs were established based on oral exposure, and recent work has explored inhalation and dermal exposure TTCs. However, environmental exposures may involve multiple exposure routes.
- The overall objective is to develop a few different iTTCs for whole-body and blood concentrations.
- Dr. Arnot explained that the general approach is to develop and apply 1-compartment physiologically based kinetic (1Co-PBK) models for mammals followed with a tiered approach using available NAMs data to parameterize the models. These models can be applied to the Monroe TTC database of 613 chemicals, and human iTTCs will be determined by applying these models.
- Dr. Arnot reviewed the PBK model concepts, including chemical uptake and elimination rates, elimination rate processes, and allometric scaling. He described how the PBK models require measurements or predictions for TK parameters, such as biotransformation rates and physicochemical properties, which are critical parameters for TK.
- Dr. Arnot reviewed the top-down (beginning with *in vitro* whole-body data and calculating half-lives) and bottom-up (beginning with *in vitro* data and using models to estimate whole-body values) methods for estimating biotransformation rates. With each approach, QSARs can be developed for predicting biotransformation rates like intrinsic *in vitro* clearance or hepatic clearance or whole-body elimination.
- He reviewed the three tiers to parameterize 1-compartment PBK models for iTTC, which included calculating total elimination rates for 91/613 chemicals (Tier 1), *in vitro* biotransformation rates for 120/613 chemicals (Tier 2), and *in silico* QSAR prediction for the remaining chemicals.
- Dr. Arnot shared a case study using iTTCs as default parameters in the Exposure and Safety Estimation (EAS-E) Suite and examples of comparing iTTCs with *in vitro* NAM data extrapolated from blood concentrations and comparing iTTCs. This included approximately 100 case study endocrine-disrupting chemicals (EDCs) and Carcinogenic, Mutagenic, or Toxic for Reproduction (CMRs) chemicals from the Canadian Domestic Substances List (DSL).
- Using the external exposure risk-based priority setting, they employed the high throughput exposure data from SEEM2 and applied the external TTC of 1.5 ug/kg/d.
    - Effective concentrations (EC_{50}s) data from EPA SEEM3 were used to obtain terminal elimination half-life QSAR predictions and calculate whole-body concentrations.
- Dr. Arnot summarized his presentation by acknowledging that the TTC concept has been incorporated in some regulatory frameworks but does not replace required toxicity testing or chemical-specific risk assessment. TTCs and iTTCs are functions of the selected datasets and describe the tail of the distributions. New iTTCs were whole-body (0.5 nmol/kg) and blood (0.1 nmol/L). The new iTTCs can be readily converted to chemical mass iTTCs by multiplying by the molecular weight. iTTCs are useful for prioritizing large numbers of data-poor chemicals and can be applied to bioaccumulative chemicals.
- Dr. Arnot described further work to expand the iTTC chemical domain, establish chemical class approaches, and develop IATAs to address uncertainty.

Comments made and questions answered during the conference included:

- Dr. Arnot was defining TTCs and decision-tree approaches for determining TTCs. He noted that he was going to focus on iTTCs for this presentation.
    - **(Online chat) Clive Roper (Roper Toxicology Consulting Limited):** You may find this reference useful for dermal TTC:
        - Williams et al. (2016). Assessing the safety of cosmetic chemicals: consideration of a flux decision tree to predict dermally delivered systemic dose for comparison with oral TTC (threshold of toxicological concern). RTP 76; 174-186. http://dx.doi.org/10.1016/j.yrtph.2016.01.005.
- **(Verbal, in-person) Rusty Thomas (U.S. EPA):** What overlap of chemicals was found between the TTC case study and the iTTC study conducted?
    - **(Verbal, in-person) Jon Arnot:** I have not looked at that yet as this was a way to explore how these could be applied and to demonstrate the capacity of the QSARs developed to get to internal doses for many data-poor chemicals rather than looking at exposure and hazard together in a risk-based context.

Alicia Paini (EFSA): Existing OECD Guidance on Toxicokinetics, In Vitro-to-In Vivo Extrapolation, and Exploring Future Opportunities

Dr. Alicia Paini is a Senior Scientific Officer at EFSA. She emphasized the global movement toward NAMs and the need for developing reliable NAMs to interpret toxicity data. This presentation provided an overview of the current OECD guidance on PBK modeling and the development of new guidance for IVIVE, particularly for developmental neurotoxicity (DNT). Dr. Paini focused her talk on the current OECD guidance for TK and IVIVE and on efforts to improve these methodologies to increase acceptance of these data in a regulatory context.

Summary

- Dr. Paini emphasized the need to develop NAMs for the interpretation of toxicity data and highlighted the challenges in the translation of these data to a regulatory context due to the reluctance of regulatory bodies to accept these data without thorough validation or understanding of the models.
    - Dr. Paini reviewed OECD GD 331, published in 2021, which aims to provide a robust framework for the application of PBK modeling in risk assessment.
    - GD 331 is composed of five different elements, which provide: (1) a scientific workflow for PBK model characterization/validation; (2) knowledge sources on *in vitro* and *in silico* methods; (3) an assessment framework to evaluate the PBK model based on content, implementation, and validity; (4) a template for documentation and clear model reporting; and (5) a checklist for evaluation of PBK model applicability.
    - The OECD GD emphasized the importance of sensitivity and uncertainty analysis and stated the goal was building a high performing model when *in vivo* data were not available. The GD also reported methods for validating models using analog data.
    - The assessment framework was designed to evaluate PBK models based on their content, implementation, and validity to ensure that models are fit for their intended regulatory purposes.
    - The GD provides a standardized template to facilitate consistent reporting of PBK models and help bridge the communication gap between model developers and risk assessors.
    - The GD includes a checklist to ensure all critical aspects of the PBK model have been thoroughly evaluated.
    - The GD details the necessary input parameters for PBK models, including methodologies for obtaining these parameters and techniques for scaling them appropriately. The GD's guidelines for conducting sensitivity and uncertainty analyses are crucial for understanding the robustness of the model's predictions. The document outlines approaches using analog data to predict outcomes for chemicals that lack existing data.
- Dr. Paini introduced the OECD recommendations established by a working group in 2022 to address the kinetic and exposure components missing from the DNT *in vitro* testing battery (IVB). The group's objectives were to convert *in vitro* concentrations associated with bioactivity to external exposure levels and to derive PODs that correspond to *in vivo* doses.
    - The GD described three main approaches for QIVIVE.

1. An *in vitro* POD is translated to an external dose using PBK modeling, credited to Dr. Barbara Wetmore (U.S. EPA).
2. *In vitro* concentration-response data are translated into *in vivo* dose-response data using PBK modeling, from which an external POD can be derived. This approach is credited to Dr. Jochem Louisse (EFSA).
3. A forward dosimetry approach using a PBK model to predict the internal concentrations resulting from a specific external dose or a range of external doses. The predicted internal concentration can then be compared to the *in vitro* effect concentrations.[7]

- Dr. Paini explained the tiered approach proposed in the guidance for selecting PBK models based on the availability of data and the specific purpose of the model. It included a compilation of data from existing models, particularly those related to pregnancy and gestational stages, to help parameterize new models.

- Dr. Paini highlighted the need for a general IVIVE GD that extends beyond DNT. She emphasized the importance of developing guidance for translating nominal concentrations to free concentrations in *in vitro* assays. Additionally, she mentioned ongoing OECD proposals to revise GD 331 and to develop test guidelines (TGs) for hepatic clearance and fraction unbound.
- Dr. Paini concluded that the GD's aim is to provide a structured approach for PBK modeling and IVIVE, as well as facilitate their acceptance and application in regulatory contexts. She encouraged collaboration among regulatory authorities, researchers, and industry to advance the use of these methodologies.

Questions answered following the conference included:

- **(Online chat) Deborah Ramsingh (Health Canada):** Could the QIVIVE guidance under development for the DNT IVB have application/relevance to other *in vitro* test systems not part of the DNT IVB?
  - **(Post meeting, written) Alicia Paini:** Thank you for your question. Indeed, the principles and the QIVIVE approaches listed in the presentation and reported in the QIVIVE DNT IVB OECD document are applicable to any endpoint. Ideally, one needs to collect different information and probably (maybe) less complex than a gestational PBPK model; thus, the tier workflow still holds but to parametrize it the requirements are different. As such, each endpoint will have different aspects in model development that should or should not be included and considered. A general QIVIVE guidance is needed and could be proposed at the OECD level, could be a point to expand on in the current OECD 331, or could be a document on its own.

Rusty Thomas (U.S. EPA): Session Wrap-Up

Dr. Rusty Thomas recalled working at the Hamner Institute with Drs. Harvey Clewell and Mel Andersen around the time of the origins of IVIVE TK. Dr. Clewell was excited about a SimCyp workshop and his work with Dr. Cecilia Tan on reverse dosimetry of epidemiology studies. Dr. Andersen would frequently bring up the topic of his 2007 report. This sparked discussion about using some of the concepts of SimCyp, IVIVE, and reverse dosimetry to gain dose context of the ToxCast toxicity testing. Despite Dr. Andersen's initial skepticism, Drs. Clewell and Thomas believed in the potential of these ideas, leading to a series of publications demonstrating how *in vitro* concentrations could be translated into useful tools for risk assessments. He expressed excitement and satisfaction with the progress made over the last 15–20 years in developing and improving IVIVE and *in vitro* TK approaches. Dr. Thomas thanked Dr. Wambaugh for providing an overview of the current activities and advancements of the IVIVE approaches and emphasized the importance of incorporating uncertainty into these models for expansion to more complex models. Dr. Thomas highlighted Dr. Hutson's presentation from an engineering perspective of translating simple static systems to flow-through systems and more complex 3D models. He pointed out some of the iTTC approaches and the application for screening and identifying chemicals. He touched on Dr. Paini's presentation about the importance of translating science into the regulatory decision context and the regulatory applications of these scientific advancements.

[7] Christian Maass, Stephan Schaller, André Dallmann, Kathrin Bothe, Dennis Müller, Considering developmental neurotoxicity in vitro data for human health risk assessment using physiologically-based kinetic modeling: deltamethrin case study, Toxicological Sciences, Volume 192, Issue 1, March 2023, Pages 59–70, https://doi.org/10.1093/toxsci/kfad007

Dr. Thomas expressed excitement about the progress made in these fields and hopes to share more success stories at the next NAMs workshop. He thanked all of the speakers for connecting the different concepts that go into the IVIVE and *in vitro* TK fields and identified what we need to work on next.

Meeting Closing

Rick Keigwin (U.S. EPA): Meeting Wrap-Up

Dr. Keigwin expressed his gratitude for the opportunity to speak and acknowledged the hard work of the conference participants. He highlighted the importance of collaboration and camaraderie among the participants, including those working late hours in Europe like Dr. Paini. He reflected on his nearly 35-year career at EPA and the evolution of tools and methods used in risk assessment. Dr. Keigwin emphasized the significance of integrating scientific advancements into risk management to enhance efficiency, protection, and cost-effectiveness. He stressed the importance of continuing dialogue with skeptical people to achieve broader acceptance of this work and integration of these scientific advancements. He expressed appreciation for the attendees and the collaborative efforts. He thanked the organizing committee and wished everyone safe travel.

Appendix A: Acronym List

ACD	Advisory Committee to the Director
ADME	absorption, distribution, metabolism and excretion
AEP	aggregate exposure pathway
AOP	adverse outcome pathway
APCRA	Accelerating the Pace of Chemical Risk Assessment
APHL	Association of Public Health Laboratories
BMD	benchmark dose
BPAC	biological pathway altering concentration
BSA	bovine serum albumin
CAMeRA	Collection of Alternative Methods for Regulatory Application
CATMoS	Collaborative Acute Toxicity Modeling Suite
CCTE	Center for Computational Toxicology and Exposure
CDC	Centers for Disease Control and Prevention
C_{free}	freely dissolved concentrations
CMAQ	Congestion Mitigation and Air Quality
Complement-ARIE	Complement Animal Research in Experimentation
CRAN	Comprehensive R Archive Network
DART	developmental and reproductive toxicology
DDI	DNA damage-inducing
DNT	developmental neurotoxicity
DSL	Domestic Substances List
EAS-E	Exposure and Safety Estimation
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
eSTAR	Emerging Systems Toxicology for the Assessment of Risk
ETAP	EPA Transcriptomic Assessment Product
EU	European Union
ExpoCast	Exposure Forecasting
FBS	fetal bovine serum
FDA	U.S. Food and Drug Administration
FY	Fiscal Year
GCA	generalized concentration addition
GD	Guidance Document
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
HCM	Hazard Comparison Module
HESI	Health and Environmental Sciences Institute
HRMS	high-resolution mass spectrometry
HT	high-throughput
HTTK	high-throughput toxicokinetics
HTTr	high-throughput transcriptomics
IATA	Integrated Approaches to Testing and Assessment
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICE	Integrated Chemical Environment
IQ Consortium	International Consortium for Innovation and Quality in Pharmaceutical Development
iTTC	internal threshold of toxicological concern
IVB	in vitro testing battery
IVIVE	in vitro to in vivo extrapolation
JRC	Joint Research Centre
LDH	lactate dehydrogenase
LLM	large language model
MAD	Mutual Acceptance of Data
MDF	Method Developers Forum

MDH	Maryland Department of Health
MMDB	Multimedia Monitoring Database
MODIS	moderate resolution imaging spectroradiometer
MPS	microphysiological system
NAMs	new approach methods
NASA	National Aeronautics and Space Administration
NASEM	National Academies of Sciences, Engineering, and Medicine
NDA	new drug application
NHANES	National Health and Nutrition Examination Survey
NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NOEL	no observed effect level
NTA	non-targeted analysis
NTP	National Toxicology Program
OASIS	'Omics for Assessing Signatures for Integrated Safety
OECD	Organisation for Economic Cooperation and Development
OHT	OECD Harmonized Template
OORF	OECD 'Omics Reporting Framework
OPERA	Open (Quantitative) Structure-activity/property Relationship App
OPP	Office of Pesticide Programs
ORD	Office of Research and Development
PBK	physiologically-based kinetic
PBPK	physiologically-based pharmacokinetic
PBTK	physiologically-based toxicokinetic
PDMS	polydimethylsiloxane
PFAS	per- and polyfluoroalkyl substances
POD	point of departure
PP-LFER	poly-parameter linear free energy relationships
QA	quality assurance
QC	quality control
QIVIVE	quantitative IVIVE
qNTA	quantitative non-targeted analysis
QSAR	quantitative structure-activity relationship
QSPR	quantitative structure-property relationship
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
ROI	return on investment
SEBS	styrene-ethylene-butylene-styrene
SEEM	Systematic Empirical Evaluation of Models
SeqAPASS	Sequence Alignment to Predict Across Species Susceptibility
SETAC	Society of Environmental Toxicology and Chemistry
SOP	standard operating procedure
STAR	Science to Achieve Results
StRAP	Strategic Research Action Plan
TG	test guideline
TK	toxicokinetic
ToxCast	Toxicity Forecasting
tPOD	transcriptome point of departure
TSCA	Toxic Substances Control Act
TTC	threshold of toxicological concern
UF	uncertainly factor
UVCBs	unknown or variable composition, complex reaction products, or biological materials
VOI	Value of Information