Beyond Science and Decisions Workshop XIII

Location: Institute for the Advancement of Food and Nutrition Sciences
740 15th ST NW, STE 600, Washington DC, 20005

February 15, 16 & 17, 2022

A HYBRID IN-PERSON AND VIRTUAL EVENT

Workshop Chairs:
Mark S. Johnson, U.S. Army Public Health Center
Michael Dourson, TERA
Wendelyn Jones, IAFNS

Workshop Coordinator:
Christen Williams, TERA

Workshop Rapporteurs:
Virunya Bhat, TERA

Workshop Report – Final

Contents

Beyond Science and Decisions Workshop XIII Introduction

OVERVIEW

Since 2010, the Alliance for Risk Assessment (ARA: see here) has sponsored a workshop series titled Beyond Science & Decisions: From Problem Formulation to Comprehensive Risk Assessment. Building on the ideas of the National Academy of Sciences' (NAS) Science & Decisions: Advancing Risk Assessment (2009), twelve previous workshops brought together over 60 organizations seeking to advance the NAS recommendations (see here).

Over 50 presented case studies have thus far contributed to an expanding compendium of practical, problem-driven approaches for "fit for purpose" risk assessments. As a resource for regulators and scientists, the compendium details key considerations for applying relevant dose-response or exposure assessment methods, whether novel or evolving, and links these methods to specific problems faced by risk assessors and risk managers in a variety of organizations (e.g., local, regional and federal governments, academia, private sector).

OBJECTIVES

- Use a multi-stakeholder approach to share information supporting the development of practical problem-driven risk assessment methods
- Highlight effective and meaningful context-specific problem formulation approaches that apply an appropriate level of detail
- Promote hazard identification, dose-response, exposure assessment, and risk characterization methods, such as those that:
    - Reflect relevant biology (including dose-dependent transitions) and mode of action
    - Describe assumptions, strengths, and limitations of relevant data and address key considerations in risk assessment and decision-making
    - Use new approaches to advance the 3'Rs (replace, reduce, and refine animal use in research)
    - Consider population and human variability, including underlying disease and exposure conditions
    - Address quantitative or qualitative uncertainty analysis.

Please note that the following report of Workshop XIII is only a **summary** of points raised during the workshop and is not intended to be a complete discussion of all issues.

Welcome and Opening Statements

Wendelyn Jones, the Institute for the Advancement of Food and Nutrition Sciences Executive Director welcomed participants to the meeting, went over housekeeping items and provided a brief overview of the IAFNS.

Michael Dourson, Toxicology Excellence for Risk Assessment, welcomed the 200+ registrants and participants to the meeting and provided a brief overview of the ARA Steering Committee (see here), the Beyond Science and Decisions Science Panel (see here), and the Risk Assessment Advisory Committee, which includes state, federal, industry, and NGO representatives.

Mark S. Johnson, U.S. Army Public Health Center, was introduced as the Chair of the Workshop by Michael Dourson. Dr. Johnson noted that he would be keeping the meeting running on time due to the full agenda. He introduced Rusty Thomas as the keynote speaker.

Keynote Talk: NAMs Paradox: When an Unstoppable Force Meets an Immovable Object

The keynote talk was given by Russell Thomas **(**U.S. Environmental Protection Agency, Director, Center for Computational Toxicology and Exposure), and the presentation is available on the *ARA* website.
.

Highlights

- There is a paradox of an unstoppable New Approach Methodologies (NAMs) force and the immovable regulatory systems and processes.
- U.S. EPA proposes a seven-step plan to address the paradox:
    1. Continue to innovate and improve NAMs, e.g., develop complex organotypic models to evaluate tissue/organ effects, address known limitations
    2. Accept that most chemicals non-selectively interact with biological systems and that bioactivity for non-selective chemicals can be a useful surrogate for potential adverse effects
    3. Assemble NAMs into a practical testing framework, published as CompTox Blueprint (ToxSci 169, 2019)
    4. Understand how to benchmark approaches, e.g., how do the in vitro models compare to *in vivo* models or other models, quantifying variability and uncertainty of *in vivo* models
    5. Grapple with the issue of protection versus prediction with current models and NAMs
    6. Evaluate regulatory flexibilities and develop a fit-for-purpose scientific confidence framework, November 2021 U.S. EPA NAMs workplan
    7. Quantify trade-offs of uncertainty, cost, and time in toxicity testing methods, e.g., 6-20 years, $$$ with smaller uncertainty (2 orders of magnitude) versus <1 year, $ with bigger uncertainty (4 orders of magnitude), via a Value of Information (VOI) analysis described by Hagiwara et al., Risk Anal, 2022.

Discussion and Comments from Panelists and Observers

Panelist 1: Which is more prescient, a computational versus a traditional approach if you segregated the results of traditional studies to assess any influence by dose, route and/or by time?

Russell Thomas response*:* This is difficult to say. Some chemical behaviors are better described by concentration versus time (CxT) whereas some chemicals are better described by their Cmax.

Panelist 2: Since there are so many NAMs, how is EPA benchmarking these?

Russell Thomas response*:* The U.S. EPA is developing an agency-wide framework for NAMs that will be flexible and not method-specific.

Panelist 3: I am a big fan of value of information (VOI) analysis. Was the delayed timeframe identified in the diethylhexyl phthalate (DEHP) VOI analysis impacted by litigation?

Russell Thomas response*:* The analysis was not necessarily focused on the specific action(s) that caused the delay. We were more interested in what the delay(s) cost.

Research Case Study 1

Developing Confidence in NAMs data for risk assessment: *C elegans* as a case study

This case study was presented by Piper Hunt and Suzanne Fitzpatrick, U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition (CFSAN), Office of Applied Research and Safety Assessment (OARSA), and the presentation is available on the *ARA* website.

Highlights:

Part I: Piper Hunt

- *C. elegans* is transparent so it makes a good model for visualizing organ/site-specific transgene expression, such as to study oxidative stress induced by lead acetate, sodium arsenate, or their combination.
- Every cell from fertilization to adulthood has been mapped, with many pathways conserved across *C. elegans* and mammals, e.g., can use *C. elegans* to study developmental toxins in collaboration with the NTP, and it is also extensively used to study human neurodegenerative conditions, such as Parkinson's and Alzheimer's.
- Can *C elegans* predict human responses?
    - E.g., Cadmium induces ultrastructural damage in the intestinal epithelium of *C. elegans.*
    - *C. elegans* lethality assay versus rodent LD_{50} values are concordant (r=0.885, n=17 chemicals, excluding 4 acidic chemicals).
    - There was 52% concordance with *C. elegans* that can be conducted in <1 week with one technician compared to 58% concordance with rat/mouse ToxCast results (Boyd, Smith et al., 2016, NTP study).
    - *C. elegans* can also detect mammalian neurotoxicants, e.g., anticholinergic effects, see European Food Safety Authority (EFSA) April 2018 External Scientific Report, Literature Review and Appraisal on alternative neurotoxicity testing methods.
- There is a need for standardized protocols and Good *C. elegans* Culture Practice methods (Hunt et al., 2017).
- *C. elegans* is primarily an oral toxicity model, but users must understand their limitations, e.g., versus zebrafish to study developmental toxicity.
- Qualification studies are needed to define fit-for-purpose and chemical domains of applicability.
- *C elegans* can be used to develop adverse outcome pathways (AOPs) and integrated approaches to testing and assessment (IATA) for neurodegenerative diseases.

Part II: Arsenic in Rice (Piper Hunt)

- The FDA has a Closer to Zero Plan that addresses lead, arsenic, cadmium and mercury.
- FDA has been using *C elegans* to study inorganic (e.g., NaAsO2) arsenic versus dimethylarsinic acid (DMA) induced developmental delay and hypoactivity. NaAsO2 is a far more potent inducer of developmental delay and oxidative stress than DMA.
- *C elegans* concurred with epidemiological studies that found DMA to have less hazard concern compared to inorganic arsenic.

- Gene expression and transgene expression studies looking at *C. elegans* exposed to inorganic and organic arsenic indicate that oxidative stress and immune response pathways might be the mode of action for developmental delay.
- Arsenic-3-methyltransferase (As3MT) was considered the methylase in mammals for metabolizing inorganic arsenic, though recently, other human methylases have been found to be important for metabolizing arsenite. *C. elegans* does not have a close homolog for As3MT, but its genome encodes ~200 methylases and one of these could be a functional ortholog.

Discussion and Comments from Panelists and Observers (Part I)

Panelist 4: Also need standards to interpret test results in addition to conducting the studies. Has the Organization of Economic Cooperation and Development (OECD) been involved in developing standard protocols?

Piper Hunt response: One thing to remember is that *C. elegans* eat microbia, but OECD protocols use *E. coli*. Secondary metabolism in the nutrient media is a consideration. Ours is not a widely used protocol. The culture media is expensive, with 48 ingredients and requirements to be sterile. OECD methods may not be necessary or appropriate for predictive toxicology applications. OECD has two Test Guidelines with *C. elegans* for assessing soil and water toxicity, but not for the purposes that we are using the model for.

Panelist 5: How confident are you that their cuticle is impermeable and thus it only characterizes oral exposure?

Piper Hunt response: Evidence of cuticle absorption occurs with solvents such as dimethyl sulfoxide (DMSO) vehicle. For water-soluble compounds, it is not an issue. Our laboratory is developing methods to dose lipid-soluble chemicals via nano-emulsion.

Panelist 6: I work a lot with *C. elegans*, which has critical issues with their cuticle. There are many *C. elegans* strains, some with permeable cuticles. Researchers must use high concentrations because of the cuticle, and they also need to measure what concentration is in the worm as well as show that the concentrations in the worm are the same as in humans. Our laboratory has done this with mercury.

Piper Hunt response: We are worried about the mutant strains with permeable cuticle having other unknown differences; also want to ensure that effects are due to chemical toxicity rather than xenobiotic metabolism, therefore we do not use a feeder organism. By dosing in nutrient media rather than non-nutrient buffer, we ensure that the worms are eating normally.

Panelist 7: What is a ring trial? Do *C. elegans* have stem cells?

Piper Hunt response: A ring study is a multi-laboratory study with blinded test chemical. Correct, *C. elegans* adults do not have stem cells other than in their reproductive tract for the production of germ cells.

Panelist 2: This is not a good model for cardiotoxicity.

Piper Hunt response: Agree.

Discussion and Comments from Panelists and Observers (Part II)

Suzanne Fitzpatrick: FDA needs new tools to ensure the food supply is safe. FDA is proud of these efforts as well as some ongoing NAMs efforts in zebrafish.

Panelist 8: The concordance and ranking were for what measures?

Piper Hunt response: Concordance was for ranking hazard concern based on EC_{50} values for morbidity/mortality and locomotion.

Panelist 6: Though I am not a risk assessor, *C. elegans* has translational value for humans, e.g., for studying metal toxicity, oxidative stress, etc. One has to be cautious when determining its predictive value in risk assessment, since it has no heart, no blood brain barrier, no liver, etc. It has only 952 somatic cells and 302 neurons. *C. elegans* has 19K genes compared to the human genome of 24K. *C. elegans* does not have alpha-synuclein protein that is involved in Parkinson's Disease and this context is important for studying arsenic. Other differences exist, such as an important transporter, SLC3810, is lacking in *C. elegans* when studying neurodegenerative disease. *C. elegans* also has different P450 than humans, e.g., some CYP isoforms are lacking and thus researchers must measure internal toxicant concentrations. The life cycle of *C. elegans* is 3 days and they live for 21-23 days. There are no females, only males and hermaphrodites. Our laboratory exposures are 30 minutes thus we need high exposure concentrations. There is room for more refined studies in *C. elegans*. The model has strengths, such as its conserved genetics that can be probed with functional assays. Certain behaviors (such as seen in children) are dependent on dopaminergic neurons.

Panelist 4: A charge question to the panelists was how to get better acceptance of this model in absence of ring trial? Why not a ring trial? OECD guideline acceptance is a very difficult process.

Panelist 6: Different test substance exposure times/windows are very different due to the rapid developmental timeline.

Panelist 3: Both standard operating procedures (SOPs) and reporting standards are needed and should include FAIR (findability, accessibility, interoperability, and reusability) principles. What *C. elegans* DOES and DOES NOT address needs to be conveyed (e.g., domain of applicability) and what other types of assays cover the gaps.

Panelist 7: Use mixtures as a case study as an example of the utility of this model, such as the oil dispersants; use a battery of short-term assays that cover the domain.

Panelist 1: What if the results from this battery are not confirmative?

Panelist 7: This is why a battery is needed; any one system is inadequate.

Panelist 3: The National Institute of Environmental Health Sciences (NIEHS) Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) at its recent meeting emphasized that in vitro NAMs should move to human-relevant approaches that recapitulate human biology such as recently published for ocular toxicity [Clippinger et al (2021) 10.1080/15569527.2021.1910291]. This context is important to convey.

Panelist 1: More problems arise when the NAMs are negative, compared to if they are positive. Understanding the mode of action or mechanism is important, including when comparing the results with other systems.

Panelist 4: Even with all the caveats, this is a better model than *Salmonella*.

Panelist 6: It is relatively easy to do studies in sensitive populations. There is an RNAi library that can be used to make mutant or knock out strains in a few hours. There are constraints when using *E. coli* due to their differences in P450s.

Piper Hunt response: FDA uses nutrient media to avoid constraints with *E coli.*

Panelist 9: Do the trade-offs (e.g., cost, uncertainty reduction, timeliness) justify use of *C. elegans* in regulatory decision making?

***Suzanne Fitzpatrick response*:** FDA is not proposing direct use in regulatory decision making. The ring trial would help resolve questions around intra-laboratory variability. This is ONE of the tools in the toolbox. Similarly, FDA would not make a regulatory decision based on one animal study alone.

Panelist 9: Fifteen studies conducted in parallel can be achieved in the same time and space requirements of an animal study.

Panelist 9: Are the methylation polymorphisms associated with specific populations?

Piper Hunt response: Where there is high arsenic in the environment, there is selection for more robust methylation of inorganic arsenic.

Panelist 4: Yes, there are certainly polymorphisms associated with cancer. I am not sure about developmental effects.

Panelist 10: Nice case study. Within an Integrated Approach to Testing and Assessment (IATA) (purpose-driven) framework, the purpose is specified: e.g., screening, etc. This case study would be a good example IATA case study.

Suzanne Fitzpatrick response: An IATA would start simple and apply no more than what is needed for answering the question.

Panelist 11: Very interesting presentation. As an epidemiologist, 200K worms is statistical power! With that degree of statistical power, any small difference will be statistically significant. How do you determine if a difference is biologically meaningful if virtually any difference is statistically significant? Finally, with this degree of enhanced statistical power, one might be able to use lower test concentrations.

Piper Hunt response: FDA has been looking to identify biologically meaningful change versus simply statistically significant change. It is also important to remember that, like a single rodent litter, a single well of a multi-well plate containing 1,000 *C. elegans* is still an 'n' of one. So, the 200K worms = an 'n' of 200.

Observer: How can academicians know what should be included in a published manuscript to facilitate use in a risk assessment?

Piper Hunt response: See the recent publication on reporting standards ToxRes, e.g., staging/ synchronization of the animals.

Observer: Is there another laboratory species that can be used to compare with *C. elegans* results?

Panelist 3: Reinforcing the need to include the rationales for the elements of the SOP, consistent with FAIR principles, not just what you did, but why you did it.

Panelist 5: There is a real opportunity with large sample size to use lower concentrations and also refine understanding of the internal dosimetry. The field of hormesis has consistently taught us of likely differentials in low-dose responses (stimulation) versus inhibition (toxicity) at higher doses. The vast majority of environmental chemical exposures are very low relative to typical doses tested in mammalian toxicity test systems. *C. elegans* is an opportunity to reframe the interpretative risk context of testing conducted at high doses/concentrations versus potential responses observed at doses/concentrations in the range of actual human exposures. Importantly, characterization of a wide range of dose response should be coupled with an understanding of internal dosimetry in worms as compared toto human internal dosimetry.

Panelist 7: The fact that *C. elegans* is missing some organ systems is less impactful.

Piper Hunt response: The department has been investing in chemical analyses, but the equipment is expensive, and the technical expertise required is high. The ICPMS analyses required a million worms to be grown over several weeks to generate enough sample to analyze. The Aschner laboratory has a different focus on mechanistic insights whereas the FDA goal is to develop a model and assay(s) to define domain of applicability of the assay to determine if it is fit for purpose.

Panelist 5: Some laboratories are doing this with much smaller sample sizes.

Panelist 8: Would immunohistochemistry be helpful?

Panelist 6: Mammalian antibodies don't work well in *C. elegans*. Yes, one can get by with smaller than a million worms for ICP-MS. However, this should be considered n=1, such as with 20K worms and 6 replicates needed. Laser ablation ICP-MS doesn't work either. The resolution is not good. It will tell you whether it is in the head or the tail but not what concentration that the metal is.

Panelist 8: *C. elegans* can allow examination of inter-organ effects, such as for neuroendocrine effects.

Piper Hunt response: There is endocrine-like communication, but they do not have an endocrine system per se.

Observer: Since this model is being explored as a way to screen a large number of individual compounds and mixtures, wouldn't it be possible to have a tiered approach where positive results would be followed up to get things like internal concentration and whole nematode cell fractions (e.g., S9) to further explore the dosing and metabolism in a system where you can use lethal doses to explore metabolism the same way we already do with liver?

Piper Hunt response: Note that *C. elegans* is a whole organism with active metabolism, so S9 is unnecessary.

Observer: We have had success quantifying total body burden for various metals at the Duke Superfund Analytical Chemistry Core in ~1000 total *C. elegans* (lead, arsenic, cadmium).

Panelist 6: If there is a physiological concentration detectable within the worm, one could possibly proceed in a tiered approach.

Panelist 8: Consideration should be given to Type I and Type II errors and false positives, such as common in *Salmonella*.

Panelist 4: I and other genetic toxicologists prefer the term "irrelevant" positives when one is comparing Salmonella results to an endpoint in other organisms.
There is much enthusiasm for this case study; a lot of test concentrations were included with a lot of replicates and phenotypes. This model fits well along the continuum of models and tools that are translatable to humans. SOPs are needed that include guidance for interpretation.

Panelist 3: I echo the enthusiasm. The FDA needs to add elements that were included in the presentation to the written Case Study that was submitted. The presentation had more detailed and compelling context compared to the Case Study. Developing this into an IATA in a battery approach is better than a tiered approach. IATA is most useful for creating context and establishing confidence. Yes, reporting standards in addition to an SOP should be developed. Reporting standards are what advanced genomics. The rationale for the SOP is what needs reporting the most.

Panelist 10: There are many examples of IATA that are tiered with multiple assays. There is also a recent article providing input towards a reporting guideline for DART in C. elegans.
https://academic.oup.com/toxres/article/10/6/1202/6445109

Piper Hunt response: The Tiered part of the framework might refer to the decision matrix, not that one would prioritize one assay over another.

***Suzanne Fitzpatrick response*:** The FDA is working on a draft of an IATA for food safety testing and will welcome input when we get there- input from others always makes these documents better.

Panelist 3: Dosimetry to characterize internal dose (at least cellular uptake) in ALI versus direct application versus submerged culture has been CRITICAL for exposure alignment among inhalation test systems

Observer: Chemistry testing is expensive. It will be cheaper to just do the testing if you only do it when you have toxicity of concern. If you test 1000 compounds and 10 have doses that are concerning, then you can follow up with just those first.

Panelist 3: Yes, a BATTERY of assays in an IATA strategy. I would also include DOSIMETRY constraints discussed as part of the revisions.

Observer: We did a similar thing with p-glycoprotein related toxicity. Only the chemicals with reduced toxicity towards p-glycoprotein expressing cells were sent for more definitive flow-through studies (i.e., Caco-2).

Chair: Does the Panel see utility in revising and updating the Case study for inclusion on the website?

Panelist 1: Yes. The write up should be updated to emphasize the need to understand mechanisms or mode of action when designing a tiered approach.

Panelist 6: Agree, one might get to the same mechanism with a different model.

Panelist 3: Dosimetry is a constraint that should be articulated in the revised Case Study write up. For example, when is this model applicable, e.g., discussion of solubility considerations.

Michael Dourson: Thank you to all for a nice discussion. The draft workshop report will be provided to the speakers and panelists with the names of those who made the comment or response included, for the purposes of internal review. For the final report, the panelist names will be omitted.

Day 2

Wendelyn Jones welcomed participants to Day 2.

Michael Dourson noted that Mark Johnson and Michael Dourson will Chair today in the morning and afternoon, respectively, since Neeraja Erraguntla is unavailable.

Research Case Study 2

Biological Effect Action Level (BEAL) Versus Biological Exposure Index (BEI)

This case study was presented by Michael Taylor, Adriana Oller (NiPERA) and Ken Bogen (Versar). The presentation is available on the *ARA* website.

Highlights:

- A method is needed since NiPERA member companies did not know how to interpret the data showing urinary levels of nickel in workers. Workplace forms of nickel include water soluble and insoluble compounds and metal.
- Systemic effects expected to occur via all exposure routes while local effects occur via inhalation and skin contact.
- Main human health effects include respiratory toxicity and carcinogenicity (local lung effects), reproductive (systemic effects), and dermal (local skin effects. Main respiratory effects are related to repeated prolonged inhalation exposure; OEL set to protect workers from inhalation effects.
- Compliance with air standards is main risk management tool for Ni. It is not appropriate to use a BEI to protect from lung effects (local) since BEI is not linked to a health effect; best way is via air monitoring and compliance to OEL; urinary Ni can be useful to assess compliance with PPE.
- NiPERA generated a model where a health-based reference level for urinary Ni in workers (BEAL) can be incorporated into a framework (e.g., traffic light approach) to help companies interpret biological results and react appropriately.
- WHO oral Tolerable Daily Intake (2007) of 11 μg Ni/kg/day used as the reference value for the updated biokinetic model (Bogen et al. 2021 Human and Ecological Risk Assessment) that included a slope function and Michalis-Menten saturation kinetics for urinary excretion using data in human volunteers exposed to soluble Ni after fasting.
- Model predicted a median and 95% LCL urinary Ni output (BEAL) for each worker as if they were exposed to ingested soluble Ni at the fasting Tolerable Daily Intake level using each worker's body weight and urinary concentrations; worst case exposure assumption is continuous delivery (Mon-Fri 8 hour shifts or 12-hour shifts versus 6 minute pulse exposure at the start of each shift); interindividual variability model predicted 4.62-fold reduction below median (so must scale/reduce the measured value by 4.62-fold to accommodate interindividual variability as a conservative protective/safety factor).
- An example BEAL interpretation framework was proposed (e.g., red, yellow and green traffic light approach) as an alternate or supplemental approach compared to the Biomonitoring Equivalents (BE) approach of Hays et al., 2007.

- Questions for the Panel: Should the BEAL be indexed to the POD instead of the Tolerable Daily Intake and how does that affect the modeling that has already been done? How should NiPERA interpret the results considering uncertainty and high degree of conservatism in the BEAL approach based on occupational exposures instead of general population. Instead of total urinary output over time, is there a way to normalize urine parameters (e.g., density, creatinine, etc.,), so the entire urine volume does not need to be measured during urine collection?

Discussion and Comments from Panelists and Observers

Adriana Oller noted that the EFSA BMDL value is 1.3 mg Ni/kg bw and that the value on the slide during the presentation (13 mg Ni/kg bw) was a typo.

Panelist 3: What are the inhalable versus respirable values for OELs?

Adriana Oller response: In 2018, the European Chemicals Agency (ECHA) Risk Assessment Committee recommended 30 $\mu g/m^3$ as the limit for inhalable Ni and 5 $\mu g/m^3$ as the limit for respirable Ni.

Panelist 3: What was the analytical method used to measure urine Ni in the human biomonitoring work? Atomic absorption?

Michael Taylor response: Isotopic ^{61}Ni was measured via atomic absorption and other researchers have used inductively coupled plasma mass spectrometry (ICPMS).

Panelist 3: Were the dietary exposure data of Sunderman et al. considered too?

Ken Bogen response: Yes, a joint fit was considered using the Sunderman and NiPERA data. A 12-hr fast was employed, and it was oral exposure to soluble Ni in drinking water or added to food.

Adriana Oller response: Soluble Ni was added to the diet in a separate study within Sundermann's et al 1989 paper. Some studies including Sundermann's have used scrambled eggs. The TDI is 11-13 ug/kg per day and background dietary intake is ~2-3 ug/kg/day.

Panelist 12: Is the TDI level you are using put in occupational equivalent units?

Adriana Oller response: No. Animals were exposed to 1-2 mg Ni/kg/day, in addition to nickel in feed. So, the added levels were at least 1000-fold above dietary Ni in feed.

Panelist 7: Looking at the lower bound data. Cumulative probability considerations may apply.

Panelist 6: There are a lot of interactions between metals. How does this model consider other metals, e.g., manganese in diet? For some metals such as manganese there is not a good relationship between biological levels and health effects or deposition into tissues. How do other metals affect this model and how do you account for the duration of exposure, especially if urine levels are not indicative of tissue levels.

Ken Bogen response: The assumption for the model is that other metals are independent at these low levels of Ni exposure. The model assumes urinary levels are indicative of short-term kinetic equilibrium. If there are long term deposition reservoirs, there will be a longer half-time compartment.

Observer: A urine sample is not collected over 1000 hours.

Michael Taylor response: Ni ion is cleared rapidly from the blood and it is eliminated in urine (versus biliary clearance for manganese). Urine is considered a good biomarker for short-term integrated exposure to nickel.

Panelist 5: This is a good example of fit-for-purpose problem formulation- the method and model development were conducted in response to a risk assessment issue and need for a solution.
How does the dietary Ni background exposure compare to the 95% lower confidence limit for workers?

Ken Bogen response: There is a clear distinction between dietary Ni exposure and what the model predicts is the additional exposure due to occupational exposure, including consideration of 95% lower confidence limit.

Panelist 5: What was the background Ni in the diet study that the World Health Organization Tolerable Daily Intake determination was based on?

Michael Taylor response: Ni intake from basal diet is 1000-fold lower. In animal studies, Ni is given via gavage.

Panelist 9: Did you address creatinine clearance? What was the lognormal adjustment for?

Michael Taylor response: Normalizing Ni concentration to creatinine is one way we may avoid collecting the entire volume of the urine void and would make this approach easier to implement in existing industrial biological monitoring programs. We would like to discuss how to go from (i.e., normalize) total Ni excreted to a Ni concentration with the panel.

Ken Bogen response: The mixed lognormal model represents a weighted average. For BEAL determinations, we can compare the 95% lower confidence limit to individual data and apply the median to population exposure.

Panelist 3: How does the updated model differ from the Melo and Leggett (2017) model (doi: 10.1097/HP.0000000000000579)? Are there particle size considerations with respect to cellular uptake? The International Commission on Radiological Protection (ICRP) is looking to revise their Ni absorption factor for its human respiratory tract model (HRTM). NiPERA should mention the Melo and Leggett model for context.

Ken Bogen response: The Melo and Leggett study was published at the same time, and they reached a similar conclusion. NiPERA had data not available to Melo and Leggett. NiPERA explicitly modeled inter-individual variability not addressed by Melo and Leggett. NiPERA studies did not address particle size and neither did the Melo and Legget model, as both models are based on oral exposures.

Panelist 8: Were these 24-hour urine samples?

***Ken Bogen response*:** No. The model can be applied using any exposure pattern, usually short-term (<8 hrs).

Panelist 12: Is the WHO Tolerable Daily Intake adjusted for occupational exposure?

Panelist 9: How important is the bone compartment that was added in the modified model? Is the bone truly a one-way compartment forever during the whole lifetime?

Panelist 3: You mention the Melo and Leggett model (Melo and Legett, 2017) is more complex. What is the difference in structure? Is bone included? Other compartments?

Observer: To follow up on the previous question about bone being a one-way compartment, wouldn't pregnancy in calcium deficient women be expected to release nickel the same way it does with calcium and other sequestered metals (e.g., lead)?

Ken Bogen response: Lifetime exposure data are not available to assess bone. The bone compartment was required in order to fit the data well.

Panelist 3: Is there a bone compartment in the Melo and Leggett (2017) model?

Panelist 3: Provided some references in the chat string:

- Hack CE, Covington TR, Lawrence G, Shipp AM, Gentry R, Yager J, Clewell HJ. (2007). A pharmacokinetic model of the intracellular dosimetry of inhaled nickel. J Toxicol Environ Health Part A 70:445–464.
- Melo DR, Leggett RW. A Biokinetic Model for Systemic Nickel. Health Phys. 2017 Jan;112(1):18-27. doi: 10.1097/HP.0000000000000579.
- Efremenko AY, Campbell JL, Dodd DE, Oller AR, Clewell HJ 3rd. Time- and concentration-dependent genomic responses of the rat airway to inhaled nickel sulfate. Environ Mol Mutagen. 2017 Oct;58(8):607-618. doi: 10.1002/em.22139.
- Efremenko AY, Campbell JL Jr, Dodd DE, Oller AR, Clewell HJ 3rd. Time- and concentration-dependent genomic responses of the rat airway to inhaled nickel subsulfide. Toxicol Appl Pharmacol. 2014 Sep 15;279(3):441-454. doi: 10.1016/j.taap.2014.06.007.

Ken Bogen response: Yes, bone is included within the tissues and body sink compartments of their model (see Figure 4 from Melo and Leggett). Their model is basically identical to NiPERA's, with the main difference being that NiPERA used a larger dataset.

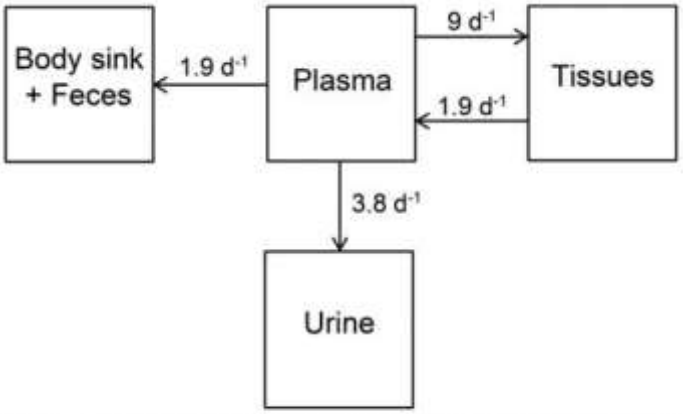

Fig. 4. A modified version of the model of Sunderman et al. (1989) that provides better agreement with results of the nickel tracer study of Patriarca et al. (1997).

Observer: Nickel goes into a compartment and stays there, whether you call it bone or another compartment.

Panelist 7: Good to see biomonitoring method being adapted to occupational exposure. At or below the Tolerable Daily Intake is green, if between Tolerable Daily Intake and the point of departure = yellow, and above the point of departure is red. The default intraspecies toxicokinetic subfactor is 3x and thus applying 4.62x is double dipping.

Panelist 3: The total sample size (n=24) from the three studies is small. Sunderman addressed 2 females and 2 males at each dose tested so there was a total of 4 of each sex. Industrial hygiene data should be able to provide exposures by process (e.g., refinery versus grinding, mixing bag etc). As mentioned earlier, the International Commission on Radiological Protection (ICRP) is also evaluating systemic nickel dosing. Really encourage exploration of that work. I also provided references for an interspecies biokinetics model earlier (Hack et al).

Panelist 10: This approach is inherently conservative, though I agree that it is a small sample size.

Michael Taylor response: We are fortunate to have human data and recognize the limitations of sample size. We recognize using the TDI is conservative for an occupational exposure. Despite the conservatism, only one worker in the pilot study was in the red zone. Could we be informed by the animal data and parallelogram approach how conservative the approach based on human data is?

Panelist 2: Has the contribution of cigarette smoke and food been considered?

Michael Taylor response: We have looked at worker exposure that included smoking status and diet. Smoking status did not impact the urinary levels. Urine levels are representative of total systemic exposure from diet and inhalation and cannot be attributed to any particular source.

Panelist 10: It would be helpful to provide some idea of the degree of conservatism for context.

Michael Taylor response: Agree, we can expand on the conservatism of using a TDI for occupational setting in our written case study. Regarding sample size, NiPERA intends to extend the pilot study to get a complete void sample at end of work week, end of day.

Panelist 9: Are you not going far enough in that this is an individual occupational exposure and NiPERA has long-term access to the worker? Instead of population level, could you look more at the individual level and consider creatinine also?

Michael Taylor response: NiPERA does look closely at each individual worker. How should these data be normalized?

Panelist 9: An individual with low dietary Ni might have higher occupational exposure that is not recognized in this approach.

Ken Bogen response: But as long as the sum of dietary and occupational exposure is below TDI, there is no health risk to workers from systemic exposure. As part of the on-boarding process, could possibly collect background urine samples; NHANES creatinine variability data are available; they may be a relationship between dermal sensitivity to Ni and urinary Ni output.

Panelist 11: Are the details on the Russian reproductive outcomes cohort study reliable? For example, what endpoints, what was the exposure, and what was sample size?

Adriana Oller response: It was a good, thorough study in female workers and published in several manuscripts. It was a collaboration between McMaster University, Norwegian Institute of Occupational Health, and Russian researchers. Exposure assessed based on urine levels. Urine levels were very high, as high as 120 ug/L. Reproductive outcomes were assessed. Conclusions were that there was no association between spontaneous abortion, malformation, etc and nickel exposure. This gives some idea of high urine levels with no effects. Sample size of women was ~600 to 1400 in Russian studies

Observer: These are the references Adriana is referring to of the Russian refinery cohorts:

- Chaschschin, V.P.; Artunina, G.P.; Norseth, T. Congenital defects, abortion and other health e ects in nickel refinery workers. Sci. Total Environ. 1994, 148, 287–291. [exploratory study that triggered later comprehensive research program]
- Vaktskjold, A.; Talykova, L.; Chashchin, V.; Odland, J.; Nieboer, E. Small-for-gestational age newborns of female refinery workers exposed to nickel. Int. J. Occup. Med. Environ. Health 2007, 20, 327–338.
- Vaktskjold, A.; Talykova, L.; Chashchin, V.; Odland, J.; Nieboer, E. Spontaneous abortions among nickel exposed female refinery workers. Int. J. Environ. Health Res. 2008, 18, 99–115.
- Vaktskjold, A.; Talykova, L.; Chashchin, V.; Nieboer, E.; Thomassen, Y.; Odland, J. Genital malformations in newborns of female nickel-refinery workers. Scand. J. Work Environ. Health 2006, 32, 41–50.
- Vaktskjold, A.; Talykova, L.; Chashchin, V.; Odland, J.; Nieboer, E. Maternal nickel exposure and congenital musculoskeletal defects. Am. J. Ind. Med. 2008, 51, 825–833.

Panelist 4: Why did you depart from Hays et al and use a Tolerable Daily Intake versus a point of departure approach? Can you derive a data-derived extrapolation factor (DDEF)?

Michael Taylor response: NiPERA was unaware of the Hays approach at the time the BEAL idea originated. We are now exploring the utility of the biomonitoring equivalent approach as well.

Panelist 12: Couple of questions: (1) If TDI is based on dermatitis, do you have data on the prevalence of this endpoint in this population of workers? How big is the problem you are trying to solve (relative to inhalation toxicity)? 2) If TDI is based on more chronic effect (e.g., repro) from lifetime exposures, do you account for the actual work history (work hours/days/years) of the individuals sampled or a general working lifetime? (3) Will you provide specific guidance on urinary sampling and analytical methods and personal data to ensure same methodology is being used across workplaces? Also, have you looked at exposure banding approach by NIOSH and AIHA for possible decision cutoffs? Does the Tolerable Daily Intake need to be adjusted for occupational exposure?

Michael Taylor response: The WHO (2005) Tolerable Daily Intake of 12 ug Ni/kg-day was based on human studies of sensitive individuals exposed orally to nickel and in 2007 WHO revised the number to 11 ug Ni/kg-day based on reproductive effects in animal studies, compared to EFSA 13 ug Ni/kg-day based on same animal studies. NiPERA does not see reproductive effects in workers. Workers are screened for dermatitis routinely. The Tolerable Daily Intake is a daily exposure that if below would protect against effects for a lifetime of exposure. BEAL approach generally reflects short-term exposure. We have a network of Industrial Hygienists across the member companies to help implement and standardized the process. Regarding Exposure Banding, we have not yet looked into it, but we can look into it.

Panelist 12: Your writeup suggests that the hand to mouth pathway may be important for workers. In your follow-up pilot study with workers (which I think you are now doing) will you collect some dermal wipe samples on fingertips, surfaces, etc.?

Michael Taylor response: Yes that is something we will certainly explore if we can implement.

Panelist 3: The following reference also provides information on influence of solubility on cellular uptake from other exposures (including in vitro) in addition to being another model for inhalation route: Goodman JE, Prueitt RL, Thakali S, Oller AR. The nickel ion bioavailability model of the carcinogenic potential of nickel-containing substances in the lung. Crit Rev Toxicol. 2011 Feb;41(2):142-74. https://doi.org/10.3109/10408444.2010.531460.

Panelist 12: You can compare the individual's work patterns and responses to each other.

Adriana Oller response: The prevalence of Ni dermatitis is higher in women. Only a small fraction of these women will react orally. They might react on their skin after oral exposure. In the latest EFSA opinion that includes a derivation for the sensitive population, the value is much lower than 13 ug/kg/day. However, the TDI is set at population level thus is based on reproductive effects.

Panelist 3: I echo the enthusiasm. The case study write-up needs to be updated. The model should consider that body weight is related to ventilation rate. Particle size is also important. Referring to the "traffic light" analogy, the model might need two sets of traffic lights due to solubility differences and inhalable versus respirable, so there may be a total of four sets of traffic lights. It would be interesting to take additional inhalable versus respirable samples to see if it distinguishes urine levels. There are also biokinetic models available for interspecies extrapolation.

Adriana Oller response: The respiratory effects need personal air sampling for workers' protection, but the integrated dose in the urine is what the BEAL is encompassing and is relevant for systemic effects.

Michael Taylor response: It is difficult to separate or speciate soluble versus insoluble worker exposures to utilize the two BEIs derived by ACGIH, since the workplace exposures are mixed. The BEAL can protect from systemic overexposure no matter what the exposure source, particle size or speciation is.

Panelist 3: The intracellular uptake is size-dependent, and solubility is compound-specific in both lung and systemic tissues. We cannot always assume that the ion is the toxic moiety. The industrial hygiene data should be able to help refine the approach– suggest that you take better advantage of it.

Michael Taylor response: Reproductive effects are seen in animals after oral exposure to soluble compounds, so in this case the toxic moiety is the Ni ion absorbed from the GI tract.

Panelist 7: The Haber et al publication has a more recent Tolerable Daily Intake (TDI) than the World Health Organization (WHO), though both are similar.

Adriana Oller response: The Haber et al. value was also reviewed by the TERA International Toxicity Estimates of Risk (ITER) process, and we considered the Haber 2017 assessment as well as WHO's 2005, 2007 and EFSA's 2020 TDI derivations in our assessment.

Panelist 7: The WHO/IPCS (2005) Chemical-Specific Adjustment Factor (CSAF) guidance indicates that a coefficient of variation (CoV) of 20% or less can be a metric to help determine what sample size is sufficient.

Panelist 8: Could NiPERA look at both approaches in the parallelogram, BE and BEAL?

Michael Taylor response: Yes, and the UF would be < 100 since the BEAL is an internal dose.

Panelist 7: For boron, some studies looked at natural boron in the diet of pregnant women and compared it to serum concentrations in animals to gauge and compare the interspecies uncertainty and differences.

Panelist 8: Would pregnancy be expected to release Ni in bone?

Michael Taylor response: Do not know. Not many pregnant women work in Ni refineries. The BEAL approach considers systemic exposure regardless of source.

Adriana Oller response: It is NiPERA Member Company Policy that when a woman becomes pregnant, she is removed from working in the refinery and reassigned to a different role without exposure.

Michael Taylor response: Pregnant rats do absorb more Ni like they do other divalent cations.

Panelist 9: Are there polyaromatic hydrocarbon (PAH) exposures in the Ni industry and are they monitored in urine? PAHs have similar problems. There will need to be back calculations going from urinary levels back to dietary exposure to avoid mistakes that the WHO JECFA panel made with cadmium.

***Steven Verpaele (NiPERA Industrial Hygienist) response*:** There are always mixed exposures in workplaces. Biomarkers are typically exposure-related (e.g., for PAHs), but the BEAL is based on a health standard. Exposure Banding methods have been considered to identify the exposure components of concern.

Panelist 5: In the Decision Tree for workers that test above the 95% lower confidence limit, is one of the actions that they are retested when they return from removal from workplace in order to determine if the elevated value was attributed to other than occupational exposure?

Michael Taylor response: Yes, this is considered, and if they still exceed the limit after not being in the workplace, then the exposure is not attributed to being from the workplace.

Panelist 3: Bone and kidney compartments in the Melo and Leggett (2017) model were a bit different in comparison to the NiPERA model.

Panelist 1: Did the NiPERA toxicokinetic studies in pregnant rat include Ni uptake in bone of pups?

Michael Taylor response: They were from a single dose study, and blood, urine, and reproductive organs were assessed, but not bone. In a single dose study, the bone is less important.

Panelist 4: Was the pilot study based on an end of shift void? Include the collection parameters in the pilot study write up. A well collected spot urine is not easy to collect.

Michael Taylor response: The urine sample is taken at the end of the shift, with the workers noting the time since last void. Workers took a shower at end of shift to, to minimize contamination.

Panelist 12: Dermal wipe samples could also be collected for reference to gauge contamination.

Panelist 1: When a worker exceeds the limit, what are the main anticipated contributors? Differences in shift exposure scenarios?

Ken Bogen response: The model prediction is conditioned on the worker exposure pattern. They are not independent. The worker exposure pattern was input into the model.

Michael Taylor response: We would certainly look into the scenarios of their exposures.

Panelist 6: Speciation doesn't matter once it is in the blood, but particle size does contribute to absorption. Is the model predictive of deposition of nickel into the lung that may not be absorbed.

Michael Taylor response: The Multiple Pathway Particle Dosimetry (MPPD) model might be a useful tool to estimate lung burden, but the current model only considers systemically absorbed Ni. This is the trade off with this model. However, routine air monitoring is the primary tool used protect from lung effects, and the BEAL model is an additional tool to assess systemic exposure and protect from any potential systemic effects.

Chair: There was a unanimous consensus to update the case study for inclusion on the ARA website.

Michael Taylor response: Thank you for the very valuable feedback.

Michael Dourson, Chair of the afternoon session on Ongoing Activities, provided an update on the ARA Workshop series, including a recent publication that resulted from a case study presented during the workshop series- "The Dilemma of perfluorooctanoate (PFOA) human half-life", published in Regulatory Toxicology and Pharmacology, Aug 2021. doi: 10.1016/j.yrtph.2021.105025. A "Hot Topics" session related to PFOA half-lives for the March 2021 Society of Toxicology Annual Meeting in San Diego has also been proposed.

Ongoing Activities

Activity 1: An industry perspective on strategies for integrated new approach methodologies (NAMs) for next generation risk assessment (NGRA)

This ongoing activity update was a prerecorded talk by Maria Baltazar, Unilever. The presentation is available on the *ARA* website.

Highlights

- Some key considerations were highlighted for integration of exposure and bioactivity for decision making.
- Examples of publications integrating exposure and bioactivity include the work by the Accelerating the Pace of Chemical Risk Assessment (APCRA) initiative (Paul Friedman et al., 2020 ToxSci 173 (1)) and the ab initio case studies, phenoxyethanol[1] and coumarin[2]; in addition, there are some examples of NAMs supporting hypothetical read-across NGRA case studies (e.g. caffeine and parabens) and NAMs used in a regulatory context, the chlorothalonil (Hargrove et al., 2021[3]).
- Key elements of next generation risk assessment (NGRA) include PBK modeling, *in vitro* pharmacology profiling, transcriptomics, and cellular stress pathways (ToxSci 176, 2020[4]).
- In addition to the key elements of NGRA, the coumarin case study included ToxTracker genotoxicity assessment, BioMap immunomodulatory screening assay, and metabolite identification and point of departure refinement steps in a metabolic competent cell model.
- Exposure and points of departure are plotted and used to derive Bioactivity Exposure Ratios (MOE/BER). One of the key questions in NGRA is how large the BER must be to conclude, with confidence, a new chemical at a given exposure is low risk. A systemic toolbox, which includes the key elements in NGRA and respective data analysis methods, and evaluation strategy was presented based on the principles of benchmarking chemical-exposure scenarios with known outcomes (i.e. chemicals that at certain exposures are either low or high risk based on historical safety decisions).
- An error model was developed to account for the uncertainty in PBK model predictions when parameters are available from in silico predictions only, in vitro data or human clinical trials. Similarly, Bayesian models to derive PoDs have been developed to account for uncertainty in the PoD estimation.

[1] https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV-CBC-MONO(2021)36%20&doclanguage=en

[2] https://pubmed.ncbi.nlm.nih.gov/32275751/

[3] https://www.liebertpub.com/doi/pdf/10.1089/aivt.2021.0005

[4] doi: 10.1093/toxsci/kfaa048

- The pilot study with 13 chemicals 27 exposure scenarios demonstrated that the systemic toolbox separates the high risk and low risk exposure-scenarios reasonably well, and that it might be possible to derive a protective BER threshold.
- Further evaluation with ~60 chemicals with high and low risk chemical exposure scenarios is ongoing. The chemical space across the 60 chemicals includes a wide range of toxicity mode of actions, chemistries, uses (i.e., cosmetics, pharmaceuticals, foods etc.) and exposure scenarios.

Colleagues from Unilever were not online for discussion, and due to time constraints, no comments were taken from Panelists or Observers.

Activity 2: NAM-based assessment of chemical disruption of spermatogenesis

This ongoing activity update was presented by Rebecca Clewell, 21CT (21stCenturyToxicology). The presentation is available on the *ARA* website.

Highlights

- A client was interested in whether reduced sperm count response observed in vivo was human relevant; spermatotoxicants can act directly or indirectly; Sought to identify *in vitro* models that recapitulate spermatogenesis in humans.
- Available *in vitro* assays covered most but not all stages; all differentiation steps occurring in testes are recapitulated in vitro; University of Georgia, Athens (UGA) human germ cell monolayer and Kallistem BioAlter rat and monkey *ex vivo* seminiferous tubule model (human tissue was not available due to Covid-19), including cell population analysis by flow cytometry in human, rat and monkey, which showed and RNA biomarker expression assessed in rat and monkey, which showed decrease in rat markers and increase in monkey markers.
- An *in vitro* to *in vivo* extrapolation (IVIVE) model was performed to estimated oral equivalent doses (OEDs) based on metabolism data collected in rat and human hepatocytes.
- Conclusion: The response in rat *in vivo* is unlikely to be human relevant since no germ cell toxicity was observed in a human model at up to an OED of 86 mg/kg-day or monkey up to an OED of 1376 mg/jg-day. There was a dose-dependent decrease in secondary spermatogenesis at the OED of 89 mg/kg-day. and above that occurred with reduced RNA expression in several markers of spermatocyte differentiation and somatic environment health. There were no dose dependent decreases observed in monkeys at an OED of up to 1376 mg/kg-day. These results suggest that the rat is uniquely susceptible to these effects.

Discussion and Comments from Panelists and Observers

Panelist 10: Has this been shared with European Chemicals Agency (ECHA)?

Rebecca Clewell response: Not yet. The study has been shared with ECHA, but we have not yet received feedback from ECHA

Panelist 6: How is the conversion from an *in vitro* concentration to an *in vivo* concentration conducted?

Rebecca Clewell response: The nominal concentrations were converted to oral equivalent doses (OEDs) with IVIVE and it was also confirmed that the actual concentrations were the same as nominal concentrations using analytical chemistry.

Panelist 7: An interspecies differences toxicodynamics subfactor chemical-specific adjustment factor (CSAF) could be calculated using these data.

Rebecca Clewell response: One of the benefits of the human stem cell model is that three different cell lines have been developed on different genetic backgrounds. Thus, there is also potential to look into intraspecies variability as well.

Observer: What is the end goal in regulatory assessment?

Rebecca Clewell response: Hazard classification is the issue, to avoid hazard classification based on effects that are not relevant to the human.

Activity 3: Key characteristics and mode of action/adverse outcome pathways in context

This ongoing activity update was presented by Bette Meek, University of Ottawa. The presentation is available on the *ARA* website.

Highlights

- Discussed mode of action and adverse outcome pathways as helpful organizing and integrating constructs across levels of biological organization and data streams and the utility of the AOP Wiki and Handbook.
- Strengths and weaknesses of these frameworks and their utility were noted, with one of the weaknesses being that the focus has been on well-studied chemicals, and another is the transparency of how inclusiveness/quality/relevance of data are taken into account (e.g., systematic review methods in chemical-specific assessments).
- Key characteristics (KCs) represent various combinations of individual or groups of key events in adverse outcome pathways not distinguished by level of biological organization. They've been helpful as organizing constructs for mechanistic data in systematic reviews for chemical assessments. However, they're also being used in hazard identification where they're unlikely to be discriminating due, for example, to their overlap and lack of specificity in some descriptions.
- Need to explore the possibility of strengthening both adverse outcome pathway (AOP) and KCC approaches, such as communicating their relationship to avoid continuing misunderstanding and miscommunication; explore more coordinated evolution to develop principles of good practice concerning their application in hazard identification and risk assessment. This seems an important area of focus to advance the incorporation of mechanistic data to support more predictive inference in hazard characterization and risk assessment.

Discussion and Comments from Panelists and Observers

Panelist 4: KCC is less transparent with respect to hazard characterization.

Bette Meek response: KCC are being used by the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) whereas in hazard identification; the International Programme on Chemical Safety (IPCS) of WHO has been influential in advancing frameworks for mode of action analysis.

Panelist 3: I reinforce the need for this; Julian Preston and Gary Williams first came up with KCC in 2005 (Preston R J, Williams G M (2005). DNA-reactive carcinogens: Mode of action and human cancer hazard. Crit Rev Toxicol 35:673–683) for DNA-reactive carcinogens based on the Hallmarks of Cancer [Hanahan D, Weinberg R A (2000). The hallmarks of cancer. Cell 100:57–70]. Preston and Williams presented the KCC as part of an actual process model and as a means by which to integrate considerations of biomarkers; the conversation has not changed for decades since considerations of biomarkers were introduced. Mechanistic models of pathogenesis are needed, whether they are called KCCs or AOPs.

Panelist 8: This type of thinking and construct is important and helpful.

Bette Meek response: Biological context is critical; consideration of key events in pathway descriptions and quantitation of key event relationships are important in interpreting mechanistic markers at lower levels of biological organization.

Panelist 5*:* The lack of consideration of dose response is a major limitation of KCC; *in vivo* internal dosimetry would be impossible to achieve for some of the *in vitro* concentrations flagging KCCs.

Bette Meek response: This is one of the important limitations of KCs and why their application in hazard identification is unlikely to be discriminating. Support for established patterns of dose-response and temporal concordance across different levels of biological organization are important components of formal weight of evidence considerations for AOPs/MOA.

Activity 4: ToxCalc! Automate routine calculations and reduce errors

This ongoing activity update was presented by Reena Sandhu, SafeDose. The presentation is available on the *ARA* website.

Highlights

- It is a free web-based tool being developed in collaboration with U.S. EPA, to work faster, provide transparency (e.g., a paper trail) and consistency in calculations performed by toxicologists and risk assessors.
- The calculators are multidirectional and will adjust for which variables are required for the conversions.
- Get in touch with Reena (reenasandhu@safedoseltd.com) if you or your colleagues want to get involved in its development. The code is located here: https://github.com/USEPA/toxcalc

Discussion and Comments from Panelists and Observers

Panelist 5: Thank you for filling a niche.

Panelist 7: Is EPA working with you on this?

Reena Sandhu response: The U.S. EPA will take up the project after Reena retires.

Panelist 9: The FDA Center for Food Safety and Applied Nutrition (CFSAN) I-RISK tool is a similar project for risk calculations, but it does not include dose conversions. I have been involved in its development. Some assessments, e.g., EFSA cadmium assessment had a preventable error in it.

Reena Sandhu response: I look forward to exploring I-RISK.

Panelist 3: Where is the updating of the software tool documented? Some departments in EPA are not harmonized in methodology. Who will update the tools based on the routinely updated Exposure Factors Handbook?

Reena Sandhu response: The build of the calculators will be prioritized based on authoritative recommendations.

Panelist 8: Is it a download?

Reena Sandhu response: No, it is web-based. Users can access it from a git-hub repository to launch it. SafeDose does not own this tool anymore. Sean Watford and Andy Shapiro at U.S. EPA may be able to assist interested individuals in getting access.

Activity 5: Next-generation Drug Safety Assessment with Organ-Chip Technology

This ongoing activity update was presented by Lorna Ewart, Emulate, and the presentation is available on the *ARA* website.

Highlights

- Organ-chip technology can be used to assess efficacy, safety, pharmacokinetics, and much more.
- Provided a consortium example based on 27 small molecules tested in liver-chip for DILI (Garside et al., 2014); Results were scored blindly by two experts, each from different organizations; 17 Phase I and II enzymes and transporter genes were also assessed to demonstrate metabolic competency; donor to donor variability was observed
- Model performance (sensitivity and specificity) was assessed by margin of safety = IC50/plasma exposure in human = threshold of 375 corrected for protein binding; spearman correlation 0.78; there were no false positives
- What does 87% sensitivity mean? Progressing drugs to clinic that are less likely to fail. 7.5x less drugs progressed to clinical that would have failed. If there is a positive in animal study and also in chip, then stop. If chip results are positive and animal study is negative, then consider further animal studies.
- In Summary:
    - The Liver-Chip met the model criteria set by the IQ MPS affiliate
    - The Liver-Chip has high sensitivity, specificity and Spearman correlation coefficient
    - These data demonstrate that the Liver-Chip has outperformed animal models and 3D hepatic spheroids in the prediction of drug induced liver injury
    - Further studies could be considered to increase the sample size of tested drugs that includes more non-toxic drugs as well as different therapeutic modalities
    - Building Liver-Chips with cells from rats or dogs could further enhance risk assessment during development of new therapeutics

Discussion and Comments from Panelists and Observers

Panelist 2: Do regulators accept this?

Lorna Ewart response: This is my job to get it accepted. We have a Cooperative Research and Development Agreement (CRADA) with five divisions at U.S. FDA. An Investigational New Drug (IND) submission has not happened with these data types yet. Post-meeting Note: Cantex Pharma announced that they have used organ-on-a-chip technology to support an IND application for repurposing of a drug for COVID-19. Here is the press release.

Panelist 2: How are you addressing batch to batch variability in the chip? Can you also other target organs on the same chip?

Lorna Ewart response: Australian company that manufacturers the chip has strict quality criteria to ensure that each chip is identical. They are generally for one organ each. If you want to sample tissue-to-tissue cross talk, you must remove effluent (body on a chip).

Panelist 3: The sensitivity and specificity slide did not include all 27 drugs. Why were only 18 drugs tested in one donor?

Lorna Ewart response: Time was a factor. The other compounds will be tested in the other donor.

Panelist 3: It is worthwhile to note that there is a current National Academy of Sciences, Engineering and Medicine (NASEM: https://www.nationalacademies.org/our-work/variability-and-relevance-of-current-laboratory-mammalian-toxicity-tests-and-expectations-for-new-approach-methods--nams--for-use-in-human-health-risk-assessment) committee evaluating expectations regarding variability in NAMs, including due to differences in donors. A recent EPA publication explored this for toxicodynamic variability among donor cells exposed via exposures (McCullough et al., 2021; 10.1093/toxsci/kfab128).

Panelist 8: Does the system emulate the metabolite formation?

Lorna Ewart response: Yes.

Observer: Toxicogenomics could identify idiosyncratic reactions *in vivo*. If there is a clean animal study in the pharmaceutical sector, no one wants to do anymore studies.

Lorna Ewart response: There is a slightly different pitch for the pharmaceutical sector. This is also why the economic study was conducted.

Activity 6: Framework Development for Evidence-Based Risk Assessment of Dietary Carcinogens

This ongoing activity update was presented by Christopher Bates and Andrew Maier, Cardo ChemRisk, supported by IAFNS. The presentation is available on the *ARA* website.

Highlights

- Overall goal is to establish a balance with ease of data application, approaches that adapt to advancing knowledge of cancer biology and toxicokinetics of ingested chemicals, and provide a lasting utility as knowledge and resources evolve.
- Exposure and hazard pathways constitute Framework Level A Problem Formulation/Scenario Description, different tiers for dietary exposure data quality.
- Framework Level B decision tree has 3 key questions, (1) Is the chemical a mutagen, (2) Is mutation from exposure to the chemical an early key event for cancer?, and (3) Are there sufficient high quality data to conduct a low dose tumor dose response analysis.
- Prioritization Matrix based on Tissue Dose (intermittent or sustained and high or low) and exposure scenarios (e.g., no cancer hazard/green, low sustained/high intermittent; high sustained/high intermittent, or only high sustained); Five example case study chemicals.
- Next steps will be to extend case studies to other food related chemicals such as aflatoxin, B-myrcene, propylene oxide.

Discussion and Comments from Panelists and Observers

Panelist 1: Low sustained/high intermittent exposure seems very different, why are they in the same category? Instinctively it seems like they should be two separate categories. Cumulative exposure considerations?

Christopher Bates response: Yes. Although low sustained and high intermittent are two separate categories of exposure patterns, they may both activate the same mode of action.

Andrew Maier response: The framework was designed considering different combinations of mode of action even though they might have different tissue exposure scenarios, e.g. direct acting mutagen is low sustained or high intermittent.

Panelist 7: Acrylamide, which is a direct acting agent, would fit this scenario of low sustained or high intermittent exposure.

Panelist 4: We were trying to avoid a binary genotoxic/non genotoxic decision, so we thought about mode of action binning; for example, mutagenic, receptor binding, etc.

Panelist 1: Temporal relationships are important, and these are not routinely considered.

Andrew Maier response: The second question is very important, (2) is mutation an early key event in cancer? The framework was built to address the many nuances.

Observer: Was low/medium/high quantified? E.g., aflatoxin, endogenous chemicals

Andrew Maier response: A key challenge was tissue dose surrogates, what is the sustained effective tissue dose? Future NAMs can help us answer these types of questions. It is high/medium/low relative to the active dose (e.g., EC50).

Panelist 7: What is a high activating dose in food?

Andrew Maier response: Chemicals in cereal would have an estimated intake dose and a high/med/low relative to active dose (e.g., EC_{50}).

Christopher Bates response: This is where deterministic or probabilistic models could help.

Panelist 1: Would this be helpful for comparing different types of exposures, such as subsistence fisherman versus customers purchasing fish from regular retailers? Fish caught at a single location present repeated dietary exposure (high or low contaminant levels) as the single source offers the sustained contaminant. Retail outlets change distributors, fish sources, etc. making it unlikely the consumer would repeatedly purchase fish with same contaminant profiles, but single contaminant incidences could be high.

***Christopher Bates response*:** Correct, because we can find out which exposures (and their associated tissue doses) are sufficient to activate the mode of action for the relevant intake patterns.

Panelist 10: The devil is in the details. Why the framework first, then the case studies after? Is it possible that due to limitations of the data, that we end up with only binary distinction of genotoxic/non-genotoxic?

Andrew Maier response: This is why pseudo-case studies were included early on, to make sure it was workable and to help identify data needs and gaps. Additional case studies are planned based on real world examples.

Day 3

Wendelyn Jones welcomed participants to the last day of the meeting.

Michael Dourson noted that Mark Johnson and Michael Dourson will Chair today in the morning and afternoon, respectively, since Suzanne Fitzpatrick is unavailable. Afterwards, several panelists had statements about the following case studies, as described below:

- **Rita Schoeny** worked on this case study and will thus recuse herself as a Panelist.
- **James Bus** worked on the afternoon case study and will thus recuse himself as a Panelist this afternoon.
- **Michael Dourson** worked with on a previous version of this case study and also worked on a U.S. EPA formaldehyde document in 2017, as well as with U.S. EPA, Health Canada, and CIIT on formaldehyde evaluations in the early 2000's.
- **Bette Meek** managed the Existing Substances program at Health Canada that was responsible for the evaluation of formaldehyde in the early 2000's.

Research Case Study 3

Use of Molecular Dosimetry to Identify Points of Departure for Potential Carcinogens with Both Endogenous and Exogenous Exposures
By Jim Sherman

Advances in Stable Isotope Labeling and Mass Spectrometry (SILMS) Technology and Use for Characterization in Molecular Dosimetry for Potential Molecular Targets in Target Organs
By Kun Lu

Use of DNA-Protein-x-Link Data in BBDR Modeling of Nasal Tumor Risk
By Rory Conolly

Case Study on Molecular Dosimetry: Use of USEPA Cancer Risk Assessment Guidelines, Sum Up, Bottom Up, Charge Questions
By Rita Schoeny

This case study was presented by Jim Sherman (Celanese) and co-authored by Rita Schoeny (U.S. EPA retired), Rory Conolly (U.S. EPA retired) and Kun Lu (University of North Carolina). The presentations are available on the *ARA* website.

Highlights:

- Jim Sherman provided an overview of SILMS molecular dosimetry and robust evidence for non-linear dose responses.
- Kun Lu provided an overview of endogenous and exogenous DNA adducts

- Methods for their measurement, and how to distinguish between and confirm whether the adducts are related to endogenous or exogenous exposures
- Using formaldehyde as a case study, the development and validation (e.g., accuracy and precision) of an analytical method to quantitate formaldehyde-specific DNA adduct biomarker was presented, along with inhalation study results with heavy isotope-labeled formaldehyde in rats at 1 and 5 days using this method.
- Molecular dosimetry study results for formaldehyde DNA adducts in the nasal epithelium of rats exposed to formaldehyde in single (6 hr) and repeated (28-d exposures) were also presented.
- In addition to nasal tissues, several systemic tissues (e.g., brain, liver, PBMCs, bone marrow) were assessed after 28-days of inhalation and not found to have exogenous adducts.
- Vinyl acetate monomer (VAM) is another case study example chemical that has been assessed in a similar fashion, by measuring N^2-ethyl-dG adducts. A non-liner dose response was similarly observed in the respiratory and olfactory epithelium.
- Summary:
  - Stable Isotope Labeling and Mass Spectrometry (SILMS) serves as a powerful method to differentiate and measure both endogenous and exogenous exposure-related DNA adducts in vitro and in vivo.
  - Molecular dosimetry data generated by SILMS are highly informative to improve risk assessment of carcinogens with both endogenous and exogenous sources.
  - Future studies may be needed to continuously demonstrate the utilities and power of SILMS for other chemicals/carcinogens of relevance.

- Jim Sherman provided additional context regarding dosimetry.
  - It was noted that where formaldehyde enters the body or where it is generated in tissues and/or within cells is an important determinant of toxicity. Inhaled formaldehyde does not reach nasal epithelial cells at low (environmentally relevant) concentrations of ≤0.3 ppm or underlying capillaries at <15 ppm. The molecular dosimetry is considered a Pre-Key Event prior to Key Event 1 (saturation of formaldehyde metabolism) in an IPCS mode of action framework for nasal tumor formation.
  - A point where adducts levels are not statistically significant or biologically relevant is subjective (where is homeostasis observed?) but could be based on more objective measures like some percentage of standard deviations and ratios of endogenous to exogenous adduct levels.
  - Use of SILMS technology in risk assessment has been supported by 32 EU member countries, and the European Commission Scientific Committee on Occupational Exposure Limits (SCOEL, 2016) committee adopted a mode of action-based NOAEC of 1 ppm for cancer at portal of entry. With assessment factors applied, an occupational exposure limit of 0.3 ppm was adopted.
- Rory Conolly presented an overview of the BBDR model for formaldehyde.
  - Focus of the presentation was on adduct implementation rather than a full overview of the BBDR model.
  - Based on nasal epithelial labeling index demonstrating a J shape, the hypothesis is that DNA protein crosslinks slow down the replicative machinery.
  - Endogenous and exogenous formaldehyde compete for intracellular metabolism. Thus, with nonzero exogenous exposures, the intercellular level of endogenous formaldehyde will increase.
  - The available data indicate that dG adducts have a dosimetric threshold around 0.3 ppm.
  - Summary slide of the Conolly presentation:

        - BBDR model maximizes used of dosimetry and relevant mechanistic data
        - Predict the full dose-response.
        - Current published version of the human BBDR model predicts essentially no cancer risk below about 2 ppm.
        - Model is complex, and EPA hasn't been willing to use for risk assessment
        - Risk assessment based solely on DNA adducts also likely to indicate little or no low dose risk.
        - More palatable for EPA?
        - How best to evaluate the trade-off between these different approaches?
    - The rat version of the BBDR model is currently being revised.
- Rita Schoeny discussed how these data could be used considering U.S.EPA Cancer Risk Assessment Guidelines
    - "Bottom up" approach evaluating incremental increase in cancer risk above the background risk (Starr and Swenberg, 2013, 2016) using monkey data was contrasted and "ground truthed" with U.S. EPA's much higher potency risk estimate, based on a linear slope factor.
    - Rather than linear or non-linear binary question, we should be asking if there is something better, e.g., a BBDR?
    - Charge questions for the Panel: What is the preferred approach for incorporating molecular dosimetry into quantitative risk assessment? (1) when the combined endogenous + exogenous is not statistically significant, (2) Determine when exogenous DNA adducts are less than or equal to a defined percentage of natural variability (3 Determine a mean level of exogenous DNA adducts that reaches a defined percentage of the mean level endogenous adducts, (4) Use a model, e.g., BBDR, then set a defined acceptable risk (e.g., 10^{-6} or a percentage of endogenous risk), (5) apply BMDL to a defined BMR, (6) other suggestions?

Discussion and Comments from Panelists and Observers

Panelist 3: EPA just purchased an orbitrap for conducting molecular dosimetry. Were samples from respiratory and olfactory epithelium of the lateral wall obtained?

Kun Lu response: The epithelial samples obtained during necropsy were not from the lateral wall separately, as epithelia from the lateral wall were combined with other nasal respiratory epithelia.

Panelist 2: Were these exposures two years?

Kun Lu and Jim Sherman response: The studies were 6 hours/day for either 1, 2, 6, 14 or 28 days, since the DNA adducts approach steady state with repeated exposures up to 28 days. The shorter duration studies were generally done as part of method development, kinetics and/or screening, while the longer duration exposures are more appropriate comparators for directly quantifying the ratio of exogenous to endogenous adducts when both were at steady state.

Panelist 5: Would a methylated compound like caffeine that is metabolically demethylated to formaldehyde and routinely dosed orally in humans be able to use this method?

Kun Lu response: Further discussions are needed to explore this possibility.

Panelist 5: Would large non-toxic doses of caffeine increase exogenous adducts? Can such compounds like caffeine be used to explore the potential cytotoxicity contribution of direct formaldehyde exposure relative to its *in situ* metabolic formation (like caffeine)? For example, methyl chloride rodent cancer bioassays were serendipitously being conducted at the same time and location as the formaldehyde cancer bioassays conducted by CIIT. Methyl chloride was later determined as a substantial metabolic formaldehyde generator but, despite this, was not a systemic rodent tumorigen. Although it was a very active glutathione depletor (the methyl chloride glutathione metabolite is the source of formaldehyde generation), and it was a weakly active cytotoxic agent as measured by increased cell proliferation. The methyl chloride data indicate systemically generated (including endogenous) formaldehyde is unlikely be to carcinogenic, as constrasted with exogenous formaldehyde that is highly cytotoxic at its respiratory site of contact.

Jim Sherman response: There is much different dosimetry with oral exposures in relation to inhalation exposures, such as much higher systemic exposures possible with caffeine due to systemic distribution before metabolism. As such, after an oral dose of caffeine in the range of human exposures, systemic formaldehyde adducts related to caffeine demethylation would be predicted if the caffeine were heavy isotope labeled.

Panelist 1: What would happen if the outer-most layers became cytotoxic over time? Is there a marker for cell health during the exposures since there is a gradient response? Why are humans anticipated to be less susceptible?

Jim Sherman response: The pathological changes in the rat nose are much more than metaplasia, the epithelium layers increase, and a lot of cell death is observed at these ultra-high exposures, that are not environmentally relevant concentrations, which caused cancer in laboratory animals. The nasal tumors were seen at concentrations that caused early mortality and exceeded the MTD in rats. Computational fluid dynamics has shown reduced exposure to cells in human nasal passages, which would likely reduce nasal cancer risk in humans when compared to rats exposed to the same formaldehyde air concentration.

Panelist 1: Is MTD defined better now, compared to the older studies?

Jim Sherman response: The regulatory definition of MTD has not yet improved very much but there has been a bit more concern for animal health and ethics. So, a new long-term inhalation study in rats using the previous tumorigenic concentrations may not be supported, due to animal rights issues related to excessive toxicity at formaldehyde concentrations ≥10 ppm. Human sensitivity assessed in computational fluid dynamic models at CIIT showed that rats have more exposure in lateral wall compared to human airways that has about half the air flow and thus half the exposure in humans compared to rats. Endogenous levels in rats and monkeys is similar but monkeys have half the increase in exogenous adducts compared to rats exposed to the same formaldehyde concentrations, for the same amount of time.

Panelist 1: It is mostly a morphological and biochemical assessment to assess human relevance.

Jim Sherman response: We assume this mode of action is relevant to humans at a certain high concentration.

Panelist 3: Why is DNA adduct formation not the molecular initiating event (MIE)?

Jim Sherman response: At a certain point it would be. It is a dose-dependent MIE.

Panelist 3: The rat as an obligate nose breather would notaccount for increased ventilation and anatomy differences, and the lower respiratory tract also has respiratory epithelium. Rather than empirical reliance on these measurements, consideration should instead be given to using the biologically based dose-response (BBDR) mode. VAM and VAM and acetaldehyde also have BBDR model structures available[5] that could be used to create context and useful comparisons.

Jim Sherman response: We would need much larger studies to achieve this objective. If it is not found in the nose or pharynx then it won't be in the lung. We would really like to do more studies if more funding can be obtained.

Panelist 3: Use BBDR modeling to address Chris's questions. Humans have different ventilation patterns, e.g., 15% of the populations breathes through its mouth routinely, and most people "augment" their need for oxygen with oral breathing upon exertion) and thus expose the lower respiratory tract. Only a mechanistic BBDR model can address these differences quantitatively.

Rory Conolly response: There is a lower respiratory tract cancer risk predicted when humans switch to oral nasal breathing with increased exercise or ventilation rate.

Panelist 10: Nice work, we really need an evolution of the existing Biologically Based Case Specific Model for formaldehyde to include the current science and mode of action data

Panelist 1: Due to the high 40x-fold different gradient flux influences cell turnover. Is the cell turnover driving the homeostasis?

Rory Conolly response: The tumors originate in the front of the nose where the highest flux occurs. The computational fluid dynamics (CFD) model was developed to describe this. There is a correlation between the DPC, labeling index, tumor formation. Cytotoxicity does not occur until high flux, 2-3ppm. The J shape dose response data were collected across several different sites in the nasal epithelium.

Panelist 8: Was the J shape the best fit? What is the signal to noise ratio? Does the model capture the variability?

Rory Conolly response: The J shape was the average of the many, many data points, not simply a statistical fit.

Panelist 10: What were EPA's objections to the model?

[5] Bogdanffy MS, Sarangapani R, Plowchalk DR, Jarabek A, Andersen ME. A biologically based risk assessment for vinyl acetate-induced cancer and noncancer inhalation toxicity. Toxicol Sci. 1999 Sep;51(1):19-35. doi: 10.1093/toxsci/51.1.19.
Hinderliter PM, Thrall KD, Corley RA, Bloemen LJ, Bogdanffy MS. Validation of human physiologically based pharmacokinetic model for vinyl acetate against human nasal dosimetry data. Toxicol Sci. 2005 May;85(1):460-7. doi: 10.1093/toxsci/kfi091.
Teeguarden JG, Bogdanffy MS, Covington TR, Tan C, Jarabek AM. A PBPK model for evaluating the impact of aldehyde dehydrogenase polymorphisms on comparative rat and human nasal tissue acetaldehyde dosimetry. Inhal Toxicol. 2008 Feb;20(4):375-90. doi: 10.1080/08958370801903750.
Zhu L, Pei W, Thiele I, Mahadevan R. Integration of a physiologically-based pharmacokinetic model with a whole-body, organ-resolved genome-scale model for characterization of ethanol and acetaldehyde metabolism. PLoS Comput Biol. 2021 Aug 5;17(8):e1009110. doi: 10.1371/journal.pcbi.1009110.

***Rory Conolly response*:** There is not a simple answer. There were 3 published critiques by EPA. Current work is trying to address some of the comments, but other comments miss the mark.

Panelist 1: The toxicology and regulator community should align on the context of the models, such that scenarios that are not relevant to human exposures should not be considered.

Rita Schoeny response: This "value of information" context is important.

Panelist 5: The technology is glitzy but don't miss the forest for the trees. There are two credible forks in the MOA pathway, adducts and cell proliferation, both are critical and need to be combined in the model.

Rita Schoeny response: There is a continuing discussion as what to do with DNA adduct data other than consider them a biomarker of exposure (e.g., Jarabek et al. 2009, and Pottenger et al. 2014 publications). We need to consider them a biomarker of effect too.

Panelist 5: The dose response curve would shift to the right with consideration of adducts. Must also consider proliferation as critical mode of action event too.

Panelist 3: Where the DNA adducts occur is important. A BBDR model such as with formaldehyde or VAM can answer this question as well as inform quantitative AOPs. Components of such models comprise the CFD model, physiologically based pharmacokinetic kinetic (PBPK) of tissue reactions, and a clonal growth model, to enable determination of the relative contribution of adducts, endogenous metabolism, and compensatory cellular proliferation in tumor pathogenesis. Such models can also help to acknowledge the limitations and assumptions of the approach. The only way to translate animal to human is with these sophisticated BBDR models.

Panelist 10: Health Canada also grappled with this. The cytotoxicity is rate-limiting and the point of no return. The quantitation of the response-response relationships between key events is important. DNA alkylation is the MIE and has little to do with quantitation of the response-response. It would be great to partner or engage with U.S.EPA to evolve the Biologically Based Dose Response (BBDR) model. Some of the Charge Questions are policy-based and/or relate to science policy or risk management decisions.

Panelist 7: Back many years ago, EPA's model did account for the J or hockey shape, the EU threshold approach is practical. The Bottom-Up approach was previously presented at an earlier ARA workshop. Will the answers be similar no matter which approach?

Panelist 9: With respect to Charge Question Option 4- use of a statistically significant difference approach seems like taking something that is extremely science-based then jumping off the science wagon. Some of the logic is not clear.

Jim Sherman response: It is an argument of "added risk" in that we do not need any added risk. But in homeostasis, there is no added risk. There are both science and policy decisions when determining acceptable risk. In the end, metrics that are useful to policy makers may need to be developed to improve the use of SILMS in a regulatory framework.

Panelist 9: If we believe that there is an added risk (i.e., small risk rather than zero), we need a path forward.

Jim Sherman response: It is a policy decision.

Panelist 11: The Bottom-Up approach is a nice reality check. There are a variety of ways to approach this, given the robust and elegant dataset. Using multiple approaches and comparing their results for reference might be useful or best path.

Rita Schoeny response: When the Charge Questions were formulated, we tried to look at all of the past perspectives. We tried to identify options that would be science-based, not perceived as unusual, and be reasonable, considering feasibility of data needs. Agree that a lot of the Charge Questions are policy.

Panelist 1: If focus on the endogenous/exogenous ratios, it is a moving target in terms of regulatory policy compared to a chemical without endogenous exposure. Should policies consider that?

Panelist 10: When previously employed at Health Canada, policies and risk management took into consideration factors such as endogenous exposure for which sources cannot be managed. This requires interface of risk assessors and managers as a basis for informed policy.

Panelist 7: We have practical methods to estimate risk and now we have the Stable Isotope Labeled Mass Spectrometry (SILMS) method to measure the level of exogenous exposure that does not enter the cells lining the nasal passages.

Panelist 5: One of the workshop series' objectives is to generate methods that can be extrapolated and used with other compounds. This adduct method is applicable much more broadly to a key assumption of risk assessment that genotoxic (i.e., DNA interacting) substances operate by a low-dose linear dose response. This basic assumption is likely flawed, based on the sensitive analytical method that can measure a single adduct per cell and also demonstrate that no increase in adducts were measured at the low concentrations/exposures.

Panelist 9: The charge questions here are very similar to nitrosamines in drinking water, endogenous and exogenous. The analogy is steering versus braking in a car.

Panelist 3: The model must account for <u>where</u> the dosimetry differences are occurring.

Wendelyn Jones instead of Mark Johnson will co-chair with Michael Dourson the final session.

Research Case Study 4

Cancer Risk Assessment for 1,3-Butadiene: Incorporating New Data and Methods

Bette Meek noted that she managed the Existing Substances program at Health Canada that was responsible for the evaluation of butadiene in the early 2000's.

This case study was presented by Christopher Kirman, Summit Toxicology, and the presentation is available on the *ARA* website.

Highlights:

- Almost 99% of all human exposures are via inhalation, primarily occupational exposure to rubber workers; NTP bioassay data served as the basis of past regulatory risk assessments (U.S. EPA, Health Canada, OEHHA) based on external dose; past regulatory risk assessments also based on epidemiology
- Metabolites vary greatly in propensity (e.g., potency and species differences) to react with cellular macromolecules
- DNA and Hb adduct biomarker data are available in rodents and humans to estimate internal dose; species differences can be quantified using Hb biomarker and in vitro TK data (parallelogram approach)
- Idealized assessment of cancer potency based on multiple datasets would converge if consider TK and TD differences in mode of action, otherwise there is a wide range of potency estimates
- NTP bioassay data in rats and mice; large species differences in metabolic activity are well documented. EPA (2002) identified that none of the existing PBK models are sufficient to describe the complexity of the metabolic pathways. EPA (2014) concluded that biomarker data used to derive a data-derive extrapolation factor (DDEF) better suited than PBK modeling to describe species differences for quantitative risk assessment.
- Propose a DDEF approach that uses biomarker data to estimate AUC and to estimate relative potency to calculate a Genotoxicity Index based on sum of metabolite biomarker data. The DDEF will be the ratio of the AUC and GI.
- Genotoxicity data are available for DNA damage, mutations, and micronuclei- should these endpoints be weighted equally?
- BMD_{10} extra risk was the default approach using human equivalent concentrations (HEC) based on DDEF using only the multistage model (up to 5^{th} degree); the linear term drives the predicted response rate for most tumors; the unit risk values across all sites were summed in additive manner using Monte Carlo. Results: Based on external dose, ~1150x-fold difference in risk estimates compared to 60x-fold range if based on internal dose.
- Sensitive subpopulations and age-dependent adjustment factors; interspecies TK differences anticipated to be 2-3x, TD: some populations have DNA repair deficiencies, but this contribution is anticipated to be small; conclusion- age-dependent adjustment factor may not be necessary for BD.
- Mouse lymphoma data behaved a bit differently (e.g., CxT) compared to other tumors in mice and the tumors in rats.

- A brief overview of the epidemiology dataset, which have been updated multiple times in the past decade, was provided, including some strengths and limitations.
- Cox Proportional Hazards Model was used to estimate cancer risk; supplemental analyses were also conducted to consider other factors, such as time (ten Berge) and aggregate endpoint (leukemia and bladder and urinary tumors) along with their advantages and disadvantages.
- Epidemiology dataset potency estimates based on aggregate endpoint fall in the middle of the rodent-based unit risk factors.
- Conclusion: This is a great dataset to work with and gets better over time in the past 20 years and as such should be used to derive a unit risk for BD. We hope to soon publish this work.

Discussion and Comments from Panelists and Observers

Panelist 7: Table 6- For the extrapolation factor, do your interspecies toxicokinetics ratios need to be flipped?

Christopher Kirman response: Because the assumption is linear kinetics at the point of departure, the numbers work out either way. Linear kinetics is the simplifying assumption at the point of departure. I can make this clarification in the updated case study.

Panelist 4: EPA applies an age-dependent adjustment factor for mutagenic carcinogens, not just genotoxic compounds. It is applied at the Risk Characterization stage (e.g., adjusted slope factor) and since this is an occupational/industrial compound, there won't be neonatal exposures.

Christopher Kirman response: I appreciate the distinction between mutagenic versus genotoxic. Second-hand smoke from tobacco use might result in early life exposure.

Panelist 10: Since potency has also been incorporated in the DDEF, it is also encompassing toxicodynamics and not just toxicokinetics. This consideration should be made more explicit.

Christopher Kirman response: Agree that with mutation and micronuclei, that some aspect of the toxicodynamics are also embedded in the DDEF, but I am not sure if all residual differences in toxicodynamics have been considered.

Panelist 5: If genotoxicity potency is based on *in vivo* genotoxicity data, would the ratio and/or potency be the same?

Christopher Kirman response: It would be difficult to ascertain, because it is a mixture exposure and not a single metabolite and therefore we cannot distinguish relative potency. We have not controlled for what it's contributing.

Panelist 3: Great job! I would note that at a past CIIT meeting, various different BD modelers could not agree on these aspects. There was more concordance demonstrated here versus at that CIIT meeting. Were all of these studies conducted by inhalation exposures? I am wondering because I was struck by the appearance of forestomach tumors.

Christopher Kirman response: Yes, they were all inhalation exposures for the cancer bioassays. It would be difficult to exposure by any other route for BD.

Panelist 7: Was there any double dipping in the tumor counts when combining the tumor types for analysis?

Response by Christopher Kirman: There is a remote chance that a worker may have both tumor types. The tumor rates in general were very low. We will look into this and confirm.

Panelist 11: I know we don't have smoking data, but how much of the bladder cancer incidence can be confounded by smoking? Was there any calculation or adjustment for smoking?

Response by Christopher Kirman: There is no way to directly answer this. An indirect approach was to assess association with BD exposure and COPD that is well known to be related to smoking to look for an association. The results are mixed in male workers, there was an association with COPD, in women there was lung cancer that did not correlate with BD exposure. There are differences in smoking behavior (e.g., hourly versus salary), but it is uncertain whether it contributes.

Panelist 11: With respect to conflict of interest, declared that he is retired but was previously employed by Exxon Mobil which manufactured BD.

Panelist 7: I have a clarification question on the Unit Risk Probability Density Function; how was the cumulative distribution estimated? How does this compare to other previously derived BD estimates by U.S. EPA and others?

Christopher Kirman response: The BMD software estimated the cumulative distribution and unit risk. The earlier regulatory estimates were much higher, based on mouse external dose or epidemiology data.

Panelist 11: Is there a potency difference in the rates for leukemia and bladder tumors? They look comparable. In terms of combining the two, there may not be a good scientific, epidemiological reason to combine them. They have different characteristics (blood versus solid tumor with different latency, risk factors, and etiology. There may be a policy-related reason to combine.

Christopher Kirman response: They are fairly similar in potency, the leukemia may be slightly higher but of similar magnitude.

Panelist 10: Congratulations on a very well written case study. The combined estimate seems appropriate, but evidence for causality of bladder cancer is not as strong as the causality for leukemia. Given the mode of action and multiple reactive metabolites, it is reasonable to include the combined dataset analysis, which was one of EPA's concerns. We have looked at the evidence for causality of leukemia and considered it strong- we were satisfied.

Christopher Kirman response: We looked at combining them from a worst case scenario, but agree that the leukemia route alone is sufficient.

Observer: Family history (household) of smoking has contributed to bladder cancer risk. Are there family history data available?

Christopher Kirman response: It would be very difficult to obtain family or household smoking history.

Panelist 4: Smoking is such a common confounder, and it is presented as a supporting analysis and the limitations have been recognized, so it is reasonable.

Panelist 11: There is an exposure response trend for BD and bladder cancer that can't be explained by smoking alone; it is appropriate to include bladder cancer. Other smoking-related diseases were not elevated in that cohort.

Panelist 3: For the age-dependent adjustment factor (ADAF) (the Hines database), was glutathione transferase (GST) ontogeny included, e.g., were differences in activation and detoxification capacities for different ages included? GST has been implicated in the age and species differences. Just a note, Ron Hines' ontogeny data are being updated and incorporated into Dustin Kapraun's life-stage model by EPA.

Christopher Kirman response: It is something we need to explore further and agree that we should consider both activation and detoxification. NHANES may have some early life data and there may be some age-related differences in biomarkers.

Panelist 11: I do not have a strong opinion on the ADAF. The cancer bioassay is unique, and an argument could be made to not include an ADAF.

Panelist 4: EPA has specified ADAF for a mutagenic mode of action, but other authorities consider the concept more broadly and apply it be for a "genotoxic" mode of action. Regardless, you need to demonstrate a mode of action. The case study noted that "mutagenicity is assumed". Neither IPCS nor EPA guidance say that a mode of action can be assumed: rather it must be demonstrated. EPA has default procedures to be applied in the absence of a mode of action. Perhaps it is useful to construct a weight of evidence for mutagenicity in an adverse outcome pathway/mode of action framework, especially since tumors are being combined for the analysis.

Panelist 10: When the epidemiological data are strong and when there is a reactive metabolite that is mutagenic, it is a reasonable assumption that BD has a mutagenic MOA. We need to look at the mode of action framework concordance. It comes down to science policy- apply ADAF if data support it, as opposed to applying it as a default. The science basis that you presented today doesn't look sufficient to apply an ADAF, and previous Panelist comments are also notable.

Sabine Lange Panelist: For the acute cancer bioassay, were the rodents treated with a promoter agent after initiation of the exposure? Two-stage cancer models have shown that an initiator alone won't cause a tumor unless there is a promoter as well. That high of an exposure may have killed off the initiated cells.

***Christopher Kirman response*:** No, there was no promoter. They were very high exposure concentrations.

Panelist 7: How old were the animals at commencement of exposure. What age does the metabolic activation become active?

Panelist 3: It depends on the enzyme.

***Christopher Kirman response*:** It is likely a standard protocol where the animals were 6-8 weeks old or adolescents at the beginning of treatment. The metabolic activation is active at this age but not sure regarding the detoxification.

Panelist 7: There are EPA assigned life stages potency factors (e.g., 3x or 10x), depending on age.

Panelist 9: Info that 'could' be included might be population health impact models, such as disability adjusted life years lost and possibly extend the DALY to a burden of disease estimate. E.g., a 10^{-6} risk would be associated with DALY burden of a specific disease. All of the possible cancers that might arise and how they might contribute to DALY or burden of disease.

Panelist 6: Can you look at concordance in endpoints based on individual metabolites between animals and humans?

Christopher Kirman response: We did look at concordance in metabolites. We did not see any greater concordance for one versus another metabolite. It might be a different set of assumptions for mice versus rats.

Panelist 4: More weight should be given to mutation in a standard assay that meets contemporary standards. Interpretation of what constitutes a positive response and a standard study has evolved. Do not throw out data, but instead could consider doing some quantitative weighting.

Christopher Kirman response: Anything that makes EB more potent pushes potency estimates to the right but by not more than ~30%. Using only mutation would have a small effect on potency.

Panelist 7: If only use mutations, the ratio and probability function would be different, would you be closer to EPA and TCEQ assessments?

Panelist 10: I'd suggest to select the endpoint closest to the adverse outcome. Could consider using the best estimate endpoint of mutation and bounding it with estimates for the other endpoints (key events).

Panelist 10: Hemoglobin adduct is more appropriate than external dose as the dose metric. Other panelists agreed.

Panelist 7: A marker of exposure is always preferred compared to an external dose.

Panelist 5: Blood dosimetry is the most relevant.

Panelist 5: This is an excellent and creative case study.

Panelist 1: This is a great case study and nice presentation.

Panelist 4: Agree, excellent case study and presentation.

Panelist 2: Good case study and explanation.

Panelist 10: Very nice and clear presentation with multiple supplemental analyses; If you can't bound the quantitative metric, can describe what is the preferred option and why in order to transparently convey greater confidence in the preferred option.

Panelist 9: Great case study. The pie chart demonstrated a compelling significant change. The demonstration of the four PDFs that humans were overlaying about the median was also compelling.

Panelist 11: I echo previous comments about this comprehensive case study that was well written. Final PDF illustration is a very powerful graphic.

Panelist 3: Agree, the case study is comprehensive and elegant, and I am enthusiastic about the fantastic visuals demonstrating how the approach improves concordance among data.

Panelist 7: I echo all the sentiments. It was mentioned that a different way to look at lifetime cancer risk, via disability-adjusted life years (DALY), may be useful and as such might make a good future case study. There was consensus to update the case study write up to consider or reflect panelist feedback for inclusion on the ARA website.

Summary of the Workshop

Michael Dourson: we appreciate all the open dialogue in person and online and look forward to future workshops where we can all meet in person. The keynote and three of the ongoing activity talks were on NAMs, *in vivo* and in *vitro* models, and their novel or progressive insights. Ongoing activities could consider whether or how they could be expanded for presentation as full case studies at future ARA workshops. A brief overview of the four case studies was reiterated. Happy one-year anniversary to IAFSN.

Overall Thoughts:

Panelist 5: ToxCalc should include common regulatory exposure assumptions and equations, in addition to the resultant dose conversions.

Panelist 2: The workshop was a much-appreciated learning opportunity in risk assessment.

Panelist 4: The intellectual stimulation was wonderfully appreciated as was the discourse. It was also fun to be in person after such a long hiatus. Thank you to Christen at TERA and Steve at IAFSN for wonderful IT assistance.

Panelist 6: Thank you all. Scientists can have a tendency to work in silos and this workshop was enlightening because it was an opportunity to take the biology and show how it is used for regulatory decisions. The two sectors, public and private, should communicate more often. Congratulations.

Panelist 1: Compliments to the presenters. This forum helps translate the science to the regulatory community, which helps make better research and policies.

Panelist 5: The workshop provided a great perspective of proper problem formulation and how it helps bring clarity for a path forward. TERA and ARA now have an excellent compendium of case studies that need more publicity.

Panelist 11: Thank you to the organizers for cutting edge learning. There was a nice mix of participants and a multistakeholder approach with an emphasis on methods that reflect relevant biology, advance 3Rs, etc. The case studies have met that mark- it was quite well done.

Panelist 9: I have similar impressions to those already expressed. There were high quality case studies across the board.

Panelist 3: Thank you to organizers and to Christen. The biological insights and technological advances that were discussed, such as BBDR models, IATA, QIVIVE, and similar approaches that foster quantitative mechanistic modeling are the way forward.

Panelist 10: The case studies get better and better. The methods are evolving to incorporate more biological information. It might be helpful to request feedback from the presenters, such as the template/outline for presenting the case study information- how it can be improved.

Michael Dourson: clarified the next steps. Virunya Bhat will develop draft meeting report that temporarily includes Panelist and Observer names that will ultimately be redacted. The names that will be retained in the report will be the case study sponsors and presenters. The presentations and recordings will be available on the ARA website soon.

Wendelyn Jones: Thank you everyone online for hanging in there. Thank you to everyone here on sight. IAFNS shares commonality with ARA to bring together multi-sector approaches that lead with science. Today is IAFNS's one-year anniversary! Thank you to Courtney, Steve, Neil from IAFNS for logistical support.

Biographical Sketches of Workshop Hosts, Co-Chairs, Speakers, Presenters, and Science Panelists

Dr. Michael Aschner, Albert Einstein College of Medicine, focuses on the interaction between genetics and the environment in triggering disease both during central nervous system (CNS) development and senescence. We are addressing metal uptake across the blood-brain barrier (BBB) and distribution in the brain (neurons and glia), specifically with methylmercury (MeHg) and manganese (Mn), as well as their cellular and molecular mechanisms of neurotoxicity. Our studies address mechanisms of transport and neurodegeneration in various experimental models (C. elegans, tissue cultures and rodents), as well as follow-up on the sequalae of heavy metal deposition in the brains of human neonates by means of magnetic resonance imaging (MRI).

Hypotheses presently tested include the following: (1) Modulation of C. elegans genes (aat, skn-1, daf-16) that are homologous to mammalian regulators of MeHg uptake and cellular resistance will modify dopaminergic neurodegeneration in response to MeHg exposure. (2) Under conditions of MeHg-induced oxidative stress, Nrf2 (a master regulator of antioxidant responses) coordinates the upregulation of cytoprotective genes that combat MeHg-induced oxidative injury, and that genetic and biochemical changes that negatively impact upon Nrf2 function increase MeHg's neurotoxicity. (3) PARK2, a strong PD genetic risk factor, alters neuronal vulnerability to modifiers of cellular Mn status, particularly at the level of mitochondrial dysfunction and oxidative stress.

Our studies are ultimately designed to (1) shed novel mechanistic insight into metal-induced neurodegeneration; (2) provide novel targets for genetic or pharmacologic modulation of neurodegenerative disorders; (3) increase knowledge of the pathway involved in oxidative stress, a common etiologic factor in neurodegenerative disorders; (4) develop improved research models for human disease using knowledge of environmental sciences.

Dr. Maria Baltazar, Unilever, is a Safety Science Leader at Unilever's Safety & Environmental Assurance Centre where she leads the Inhalation Team working on alternatives for animal testing for inhalation toxicity and risk assessment. Dr Baltazar also provides strategic science leadership for the application of advance cell models, such as microphysiological systems in the context of cosmetic next generation risk assessments (NGRA). She is also one of the company's representing in the Long-Range Science Strategy programme of Cosmetics Europe and co-leads the Unilever NGRA case studies. Dr Baltazar has received the award for Best published paper the application of risk assessment from the Risk Assessment Specialty Section (RASS) of the SOT in 2021 for the landmark paper "A Next-Generation Risk Assessment Case Study for Coumarin in Cosmetic Products". Dr Baltazar has been a Toxicologist in the Fast-Moving Consumer Goods Industry for the past 8 years and she is a European Registered Toxicologist, member of both EUROTOX and BTS Societies. Prior to Unilever, Dr Baltazar worked as risk assessor in Imperial Brands where she conducted toxicological risk assessments of ingredients and materials proposed for use in, or in contact with tobacco and other nicotine products. She obtained her Integrated Master's degree in Pharmaceutical Sciences and her PhD in Toxicology at the Faculty of Pharmacy, University of Porto. During her PhD, Dr Baltazar was also a lecturer in Food Toxicology Analysis at the Advanced Institute of Health Sciences (Portugal).

Dr. Christopher Bates, Cardno ChemRisk, is a Senior Health Scientist with Cardno ChemRisk with over five years of consulting experience in toxicology and human health risk assessment. His primary training and areas of expertise include developmental and reproductive toxicology, regulatory toxicology, and heavy metal neurotoxicology. Dr. Bates has provided toxicological support in health hazard evaluations and risk

assessment in addition to safety data compilations and evaluations using existing scientific literature and data development to support NDIN, GRAS determinations, and food contact notifications to FDA, as well as FIFRA submissions to the EPA. Experienced in the writing and analysis of toxicological profiles of pre-market, food-related products, chemical risk assessments of extractables and leachables for regulatory submissions of food-related and consumer products, and health hazard evaluations in response to food and environmental contamination events. Dr. Bates has a B.S. in Biochemistry and a Ph.D. in Integrative Neuroscience from Purdue University. Dr. Bates is a Diplomate of the American Board of Toxicology (DABT) in 2019. Dr. Bates is also a member of the Society of Toxicology (SOT) and the American College of Toxicology (ACT).

Virunya Bhat, Visiting Scientist, TERA, is a board-certified toxicologist with over 20 years of international experience in the public and private sectors assessing potential health risks of industrial and environmental chemicals across all major commercial sectors encompassing food/nutrition, drinking water, crop protection, pharmaceuticals, cosmetics, personal care and consumer products. Her extensive experience developing national standards and health-based exposure limits includes chemical additives, excipients, contaminants, residues, degradants and impurities. Dr. Bhat has authored over 100 peer-reviewed publications and technical reports and her research has been recognized by the Society of Toxicology as being among the best abstracts and the best papers advancing the science of risk assessment. She has served as an invited expert for the World Health Organization, Health Canada, and California Environmental Protection Agency and has coordinated international expert working groups, including serving as rapporteur for peer review meetings on food safety, genotoxicity, dose-response modeling, drinking water quality, and fit-for-purpose and problem-driven risk assessment methods. She has also provided risk assessment training in the U.S. and internationally to toxicologists and public health experts in all sectors and career stages.

Kenneth Bogen, Exponent, is a consulting toxicologist and has nationally recognized expertise in environmental health risk assessment and in related exposure, pharmacokinetic, dose-response, statistical, and uncertainty analysis. Before joining Exponent in 2007, he led experimental, epidemiological, and mathematical modeling research on health risks posed by environmental exposures to chemicals and ionizing radiation, as a University of California environmental scientist for 20 years at Lawrence Livermore National Laboratory. Dr. Bogen consults as an expert on quantitative exposure and health-risk assessments addressing dose response, carcinogen risk and mode-of-action, dermal absorption of organic chemicals, toxicokinetics, and mathematical (including physiologically based pharmacokinetic [PBPK], biologically based, toxicodynamic, and statistical) modeling and data analysis, and related occupational, consumer-product, Proposition 65, and groundwater issues. He authored or co-authored more than 100 peer-reviewed scientific journal publications on these topics, and provides related expert, litigation-support, regulatory evaluation, and technical-analysis services. His publications and research have addressed agents including: asbestos, allergic contact sensitizers, arsenic, benzene, cesium-137, chromium, 1,3dichloropropanol, diethanolamine, dibenzo[a,l]pyrene, formaldehyde, hydrogen sulfide, lead, malathion, 3methylchloropropanol, 4-methylimidazole, methyl isobutyl ketone (MIBK), methyl tert-butyl ether (MTBE), musks, β-myrcene, naphthalene, nickel, paperboard chemicals, PhIP, phthalates, radionuclides, radon, transuranic elements (uranium, plutonium), and VOCs. Dr. Bogen served as a Member of the National Academy of Sciences/National Research Council (NRC) committees that issued Science and Judgment in Risk Assessment (1994) and Review of the Army's Technical Guides on Assessing and Managing Chemical Hazards to Deployed Personnel (2004); chaired the U.S. Consumer Product Safety Commission's Chronic Hazards Advisory Panel on Diisononyl Phthalate (DINP) (2000-2001); chaired the Metabolism and Mode of Action Panel, Naphthalene State of the Science Symposium (NS3), Monterey, CA (2006); and served as

expert panelist at the NRC Standing Committee on Risk Analysis Issues and Reviews, Workshop on Uncertainty in Cancer Risk Based on Bioassay Data (2007). Dr. Bogen served as President (1995) and Councilor (2004-2006) of the Northern California Chapter of the Society for Risk Analysis (SRA), and currently is Chair-Elect of the SRA Dose Response Specialty Group (2016).

Dr. James Bus, Exponent, has over 40 years of toxicology experience focused on research and evidence-based literature analyses informing potential health risks associated with chemical and pesticide exposures. He offers chemical specific and strategic toxicology expertise addressing development, stewardship, and regulatory needs to individual industry clients and business consortia and government and non-governmental agencies. Dr. Bus provides expertise in design, implementation, and interpretation of toxicity tests and mode of action and dose response/exposure evaluations furthering translation of toxicology findings to risk assessment. His expertise includes target-organ and endpoint-specific modes of action, and specific toxicity of chemicals including chlorinated organics, ethylene glycol and glycol ethers, aromatic derivatives benzene, styrene, aniline and others, and pesticides such as 2,4-D and glyphosate.

Dr. Christine Chaisson, The Lifeline Group. For almost four decades Dr. Chaisson led the design of multiple assessment models addressing aggregate and cumulative risk concepts, exposure based chemical prioritization, dietary and activity profiling for unique communities (including tribal and ethnically defined communities) and post-disaster chemical hazard profiling for communities. Dr. Chaisson began her career in risk assessment in the U.S. Environmental Protection Agency in the Office of Pesticides and Toxic Substances. At EPA Dr. Chaisson designed a probabilistic dietary exposure assessment model to aid pesticide regulatory decision-making. She was also the liaison to international regulatory agencies such as AID and WHO. In 1985, Dr. Chaisson co-founded Technical Assessment Systems (TAS), which became a premier exposure/risk assessment consulting firm internationally. Dr. Chaisson co-founded The LifeLine Group in 2000, a not-for-profit organization developing publicly available state-of -the-art exposure and risk assessment models with applications to contemporary issues. Dr. Chaisson led research for Smithsonian curators at the Museum of the American Indian to assess potential exposures resulting from their work with artifacts made with toxic paints or stored in containers with very toxic pesticides. Dr. Chaisson has been a Councilor in the International Society of Exposure Assessment, a member of Society of Risk Assessment [past President of its DC chapter], the Toxicology Forum, The United Agribusiness League and the Institute of Food Technologists. She also served on the National Council for Arts and Sciences of the George Washington University and the Dean's Advisory Board for the GWU Graduate School of Political Management. Dr. Chaisson serves as a member of the External Advisory Board of the Center for Indigenous Environmental Health Research at the Zuckerman College of Public Health / University of Arizona. She is an advisor to Food Quality magazine. She has published extensively in the fields of exposure and risk assessment. In 2011 Dr. Chaisson was the invited Co-Chair of the Milan ISES/SETAC special conference on exposure science challenges presented by global legislative initiatives on consumer products and chemicals in trade. Career activity in additional organizations include Society of Environmental Toxicology and Chemistry, International Society of Regulatory Toxicology and Pharmacology, and the Institute of Food Technology. Dr. Chaisson mentored several doctoral students' thesis projects (including Boston University, Johns Hopkins University, University of California, University of Arizona). Currently Dr. Chaisson is a Director and Senior Scientist of The LifeLine Group, directing research including serving as Co-PI on projects funded by NIEHS.

Dr. Rebecca Clewell, 21st Century Tox Consulting, is an internationally recognized expert on the development of in vitro and computational tools to support chemical safety decisions. Bringing together 20

years of experience in quantitative biology and chemical dose-response, Dr. Clewell conducts research and designs testing strategies that maximize the use of New Approach Methods in determining chemical mode of action and dose-response to support quantitative risk-based decisions. She has worked with industry, government, and academic thought leaders around the globe to identify tools and technologies that are needed to build confidence in this new era of toxicology and to develop a framework for chemical testing using primarily *in vitro* and *in silico* approaches. This framework, which uses high-throughput screening approaches for chemical triage and biologically complex *in vitro* systems and computational pathway models to evaluate chemical dose-response, supports chemical decisions from early screening and prioritization to in-depth evaluation of dose-response and margins of exposure. Dr. Clewell has worked with industry and government to provide practical examples of how these strategies may be used for decision making. As a consultant, Dr. Clewell supports clients in the application of New Approach Methods (NAMs) to chemical safety decisions.

Dr. Rory Conolly, **Ramboll U.S. Consulting, Inc.** Rory Conolly's research interests include (1) biological mechanisms of the dose-response and time-course behaviors that determine how exposures to toxicants result in adverse health effects, (2) the use of biologically based computational modeling to study these mechanisms and, (3) the application of these models to quantitative dose-response assessment. Dr. Conolly has extensive experience in physiologically based pharmacokinetic (PBPK) modeling and in computational modeling of multistage carcinogenesis. An important ancillary interest is the identification of experimental designs that efficiently support development of computational models of toxicological mechanisms. He has about 140 peer-reviewed publications.

Dr. Conolly received the U.S. Society of Toxicology's (SOT) Lehman Award for lifetime achievement in risk assessment in 2005. He was a member of the National Academy of Sciences Board on Environmental Studies and Toxicology (2004 – 2005), President of the SOT Biological Modeling Specialty Section (2000 – 2001), President of the SOT Risk Assessment Specialty Section (1997 - 1998), a member of the SOT Risk Assessment Task Force (1998 - 2000) and a Councilor with the Risk Assessment Specialty Section (2010 – 2011). He has held adjunct appointments at North Carolina State University and Colorado State University and is currently Adjunct Professor of Toxicology at Michigan State University. He has three times received awards from the SOT Risk Assessment Specialty Section (1991, 1999, 2004), twice from the SOT Biological Modeling Specialty Section (2011, 2014), once from the SOT Exposure Specialty Section (2020) and once from the SOT Mixtures Specialty Section (2022). Dr. Conolly maintains an active interest in teaching, having recently participated in 3-day courses on PBPK modeling and on computational systems biology at Michigan State University, as well as lectured at North Carolina Central University and North Carolina State University. In addition to the SOT, he is a member of the American Association for the Advancement of Science. He became a diplomate of the American Board of Toxicology in 1980.

Dr. Conolly was born in London, England and raised in Canada and the United States. He received a bachelor's degree in biology from Harvard College in 1972, a doctorate in physiology/toxicology from the Harvard School of Public Health in 1978 and spent a post-doctoral year at the Central Toxicology Laboratory of Imperial Chemical Industries in Cheshire, England. He was a member of the Toxicology Faculty at The University of Michigan School of Public Health from 1979 through 1986 and worked with the U.S. Air Force Toxic Hazards Research Division, Wright-Patterson Air Force Base, Ohio from 1986 until 1989. In 1989 Dr. Conolly joined the Chemical Industry Institute of Toxicology (CIIT) and worked there until 2005, when he joined the U.S. EPA. Dr. Conolly retired from the EPA in 2020 and then joined Ramboll U.S. Consulting as a part-time Senior Management Consultant.

Dr. Michael Dourson, Toxicology Excellence for Risk Assessment (TERA), has a PhD in toxicology from the University of Cincinnati, College of Medicine, and is a board-certified toxicologist (i.e., DABT) serving as the Director of Science at the 501c3 nonprofit organization Toxicology Excellence for Risk Assessment (TERA). Prior to this, he was Senior Advisor in the Office of the Administrator at the U.S. EPA. Before this, he was a Professor in the Risk Science Center at the University of Cincinnati, College of Medicine and also worked at TERA and U.S. EPA. He has been awarded the Arnold J. Lehman award from the Society of Toxicology, the International Achievement Award by the International Society of Regulatory Toxicology and Pharmacology, and 4 bronze medals from the U.S. Environmental Protection Agency. He has been elected as a Fellow of the Academy of Toxicological Sciences (i.e., FATS) and as a Fellow for the Society for Risk Analysis (i.e., FSRA). He has co-published more than 150 papers on risk assessment methods or chemical-specific analyses, and co-authored well over 100 government risk assessment documents, many of them risk assessment guidance texts. He has made over 150 invited presentations to a variety of organizations and has chaired over 150 sessions at scientific meetings and independent peer reviews. He has been elected to multiple officer positions in the American Board of Toxicology (including its President), the Society of Toxicology (including the presidency of 3 specialty sections), the Society for Risk Analysis (including its Secretary) and is currently the President of the Toxicology Education Foundation, a nonprofit organization with a vision to help our public understand the essentials of toxicology. In addition to numerous appointments on government panels, such as EPA's Science Advisory Board, he is a current member on the editorial board of Regulatory Toxicology and Pharmacology.

Dr. Neeraja K. Erraguntla (Neera), American Chemistry Council, is a Director at the Chemical Products and Technology division at the American Chemistry Council (ACC). Dr. Erraguntla is responsible for managing and directing ACC's 1,3-Butadiene TSCA Risk Evaluation Consortium and the Center for Advancing Risk Assessment and Science policy under ACC's Center for Chemical Safety. In addition, she also manages four other industrial chemical groups that endeavor for the development and application of up-to-date, scientifically sound methods for conducting chemical assessments. Dr. Erraguntla directs complex projects involving systematic reviews, mode-of-action, exposure characterization, and endocrine disruption.

Prior to ACC, Dr. Erraguntla was a senior regulatory toxicologist at the Texas Commission on Environmental Quality (TCEQ) from 2005 to 2015. At TCEQ, she was a team lead to review available tools for conducting systematic reviews and evidence integration and to develop a position paper on how TCEQ conducts systematic reviews and evidence integration. Neera also determined inhalation toxicity factors of arsenic compounds and hexavalent chromium compounds and used threshold of concern to determine acute toxicity for chemicals with limited toxicity information. Neera played a major role in understanding and addressing community concerns about increased asthma rates in children and adults and prepared several science-based regulatory evaluations.

Dr. Erraguntla is a diplomate of American Board of Toxicology (DABT) and has a Ph.D. from Louisiana State University. She volunteers with SOT Risk Assessment Specialty Section and has also volunteered and served on the committee for SOT Exposure Specialty Section. Dr. Erraguntla was nominated as a Council Member for the International Society of Regulatory Toxicology & Pharmacology (ISRTP). In 2016, she served as a reviewer for the Government's Accountability Office and was a peer reviewer of the National Academies report, Acute Exposure Guideline Levels for Selected Airborne Chemicals, Volume 20, from the Board on Environmental Studies and Toxicology. Previously, Dr. Erraguntla also served as a Science Advisory Board (SAB) for U.S. EPA's Environmental Justice Technical Guidance Panel and has been on the

National Academy of Sciences Acute Exposure Guidelines Committee. Previously, she served as an Adjunct Assistant Professor at Texas A&M School of Public Health.

Dr. Lorna Ewart, Emulate, is the Chief Scientific Officer, where she provides strategic guidance and oversight for the company's scientific vision, partners with government agencies in an effort to achieve regulatory acceptance, and expands collaborations with key customers to advance the field of organ-on-a-chip technology. A classically trained pharmacologist, Dr. Ewart previously spent 20 years at AstraZeneca, where she successfully established and led the Microphysiological Systems Centre of Excellence within the R&D Biopharmaceuticals Unit. Throughout her tenure at AstraZeneca, Dr. Ewart held additional roles of increasing responsibility, including Director of Toxicology Projects (Respiratory, Inflammation and Autoimmune). She obtained her honors degree at the University of Aberdeen and her PhD at the William Harvey Research Institute in London. She is a frequent author on publications showcasing the applicability of organ-on-a-chip technology, a sought-after keynote speaker at conferences surrounding the advancement of alternative methods, and a fellow of the Royal Society of Biology and British Pharmacological Society.

Dr. Suzanne Fitzpatrick, **U.S. Food and Drug Administration**, is Senior Science Advisor for Toxicology in FDA's Center for Food Safety and Applied Nutrition. A board-certified toxicologist in the U.S. and in Europe, Dr. Fitzpatrick is the FDA lead for the federal collaboration among FDA, EPA, and NIH, Toxicology Te sting in the 21st Century (Tox 21), which looks to develop alternatives to animal testing as well as chair of the FDA Predictive Toxicology Roadmap Committee. Dr. Fitzpatrick played a pivotal role in helping launch the organsonFDA. achip tool, a revolutionar y testing technology being evaluated by She is also an Adjunct Professor at Johns Hopkins University, the FDA representative to the Johns Hopkins Center for Alternatives to Animal Testing Board, and past president of the American College of Toxicolog y and of the Nation's Capital Chapter of the Society of Toxicology. Dr. Fitzpatrick received her B.A. from the University of California at San Diego and her Ph.D. from Georgetown University.

Dr. Bernard Gadagbui, Toxicology Excellence for Risk Assessment (TERA), joined TERA since 2004 and is currently a Senior Toxicologist at TERA, with extensive experience in toxicology and human health risk assessment. Dr. Gadagbui received a BSc in Biochemistry with Chemistry from the University of Ghana, Legon, Ghana, and MSc in Biochemistry and PhD in Environmental Health from the University of Bergen, Norway. He has sound understanding of toxicology/risk assessment principles/practices, scientific basis for toxicity testing guidelines and application of science-based risk assessment methodologies. His extensive evaluation of clinical and non-clinical data and use of read across approaches has resulted in derivation of numerous high quality toxicologically-based risk values including reference doses/concentrations, occupational exposure limits, acceptable daily exposures, and permitted daily exposures for data-rich and data-poor chemicals, including industrial chemicals, manufacturing reagents, pesticides, cosmetic and personal care ingredients and products, botanicals and botanical preparations, petroleum hydrocarbons, and active and inactive pharmaceutical ingredients. Dr. Gadagbui is certified as a Diplomate of the American Board of Toxicology (DABT) and is also a European Registered Toxicologist (ERT). He has held leadership positions in the Toxicologists of African Origin (TAO), a Specialty Interest Group of the Society of Toxicology (SOT), African Society of Toxicological Sciences (ASTS), Ohio Valley Chapter of SOT, Ohio Chapter of Society for Risk Analysis (SRA) and currently one of the three Advisors of the recently formed African Chapter of SRA (SRA-Africa).

Ms. Annie M. Jarabek, U.S. Environmental Protection Agency, currently serves as a Senior Science Advisor in the immediate office of the Center for Public Health and Environmental Assessment (CPHEA) at

its Health and Environmental Effects Assessment Division (HEEAD) in the Research Triangle Park, within the U.S. Environmental Protection Agency's Office of Research and Development (ORD), following recent service as the Deputy Director of the Human Health Risk Assessment (HHRA) national research program in ORD. Ms. Jarabek has significant experience and training in inhalation toxicology in both laboratory and clinical environments, dosimetry modeling, risk assessment, and decision analysis. She was principal author of the Agency's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry*. Ms. Jarabek has worked on risk assessments, dosimetry models or analysis methods across all media and routes of exposure. She was the lead for the Agency's risk assessment of ingested perchlorate and some of her other work addressed several priority interdisciplinary Agency assessments including: inhaled particulate matter, vinyl acetate, manganese, and Libby amphibole asbestos. Her current research efforts focus on multi-scale dosimetry modeling, including approaches for *in vitro* to *in vivo* extrapolation (IVIVE) of inhalation exposures to advance the application of emerging methods for translation and evidence integration across various experimental platforms. A manuscript on her collaborative IVIVE work received an honorable mention as the best 2018 paper from the Biological Modeling Specialty Section (BMSS) at the 2019 annual Society of Toxicology (SOT) meeting. Ms. Jarabek has received three awards for best manuscript in risk assessment application from the Risk Assessment Specialty Section (RASS) of the SOT, along with several best abstract presentation awards. She has also received a Lifetime Achievement Award from the University of Massachusetts, the Risk Practitioner of the Year award from the Society of Risk Analysis (SRA), the Superfund National Notable Achievement Award, and several award medals (gold, silver and bronze) and technical or special service awards from the Agency. She was awarded the Lehman award for risk assessment by the SOT in 2020.

Dr. Mark S. Johnson, U.S. Army Public Health Center, currently serves as the Director of Toxicology, U.S. Army Public Health Center at Aberdeen Proving Ground, MD where he is responsible for the operational and technical arm of the Army Surgeon General and the Assistant Secretary of the Army for toxicological matters. He has worked extensively in the evaluation of the toxicity of military unique compounds and development and evaluation of a phased approach to the gathering of toxicity data for new compounds under development. He has authored over 100 peer-reviewed publications, book chapters, and technical reports and serves on several NATO and EPA panels. He has been a member of Society of Environmental Toxicology and Chemistry (SETAC) since 1997 and is a past Steering Group Member of the Wildlife Toxicology World Interest Group, past chair of Ecological Risk Assessment World Interest Group, and a member of the World Science Committee for SETAC and SETAC North America. Dr. Johnson is also a member of the International Board of Environmental Risk Assessors (IBERA). He has been a member of the Society of Toxicology since 2009.

Dr. Johnson is a fellow of the Academy of Toxicological Sciences, Chair of the Tri-Service Toxicology Consortium (TSTC), past Steering Committee Chair of the Joint Army-Navy-NASA-Air Force (JANNAF) Propulsion Committee, Subcommittee on Safety and Environmental Protection, the past president of the American Board of Toxicology (ABT).

Dr. Wendelyn Jones, Institute for the Advancement of Food and Nutrition Sciences, is the CEO/Executive Director at IAFNS, a non-profit, science research organization. She builds interdisciplinary teams to advance food safety and nutrition and has a strong regulatory/technical and commercial background across all sectors of food/agriculture/chemical industry. Her experience in industry, government, and trade associations, uniquely allows the leveraging of technical skill with business acumen. Her global experience includes working and living in the EU, Japan, Korea, Taiwan, Brazil, Chile, Argentina, and Australia. She is

skilled in managing multi-location, large international teams, by fostering productive, engaged environments that achieve organizational goals. Her extensive experience includes Fortune 500 companies in agribusiness. She has been quoted in top media, including Wall Street Journal and Forbes and she has been an invited presenter and speaker on innovation in the food and agrochemicals space.

Mr. Christopher Kirman, Summit Toxicology, is a Principal at Summit Toxicology, LLP, located in Orange Village, Ohio. Mr. Kirman is a toxicologist with more than 20 years of experience in regulatory toxicology, dose-response modeling, exposure assessment, and human health risk assessment. Mr. Kirman has published extensively in the areas of quantitative risk and safety assessment and has developed a number of chemical-specific reference doses (RfDs), reference concentrations (RfCs), cancer slope factors (CSFs), and biomonitoring equivalents (BEs) using techniques such as benchmark dose modeling, physiologically based pharmacokinetic modeling, and pooling of data sets. Mr. Kirman has used Monte Carlo methods to gain understanding of the impact of uncertainty and variation in exposure and dose-response assessments. Additionally, Mr. Kirman has extensive experience conducting weight of evidence and mode of action evaluations. He earned his B.A. in Chemistry from Case Western Reserve University (CWRU) and his M.S. degree in Toxicology and Nutrition from CWRU.

Dr. Sabine Lange, Texas Commission on Environmental Quality (TCEQ), is the section manager for the Toxicology Division at the TCEQ. Dr. Lange's responsibilities include overseeing health effects risk assessments of air permit applications, ambient air monitoring projects, and hazardous waste sites; overseeing the development of chemical toxicity factors; and conducting and overseeing systematic reviews and independent analyses of risk assessments. Dr. Lange serves as a technical resource for the State and citizens of Texas for human health and environmental risk assessment, especially related to air and water quality. Dr. Lange's research interests include the toxicology of criteria air pollutants, and risk assessment methods used for derivation of toxicity factors. Dr. Lange received a bachelor's degree from the University of Western Ontario in Canada and completed a Ph.D. and post-doctoral training in biochemistry and molecular carcinogenesis at the University of Texas at Houston and MD Anderson Cancer Center. Dr. Lange is a Diplomate of the American Board of Toxicology.

Dr. Jeff Lewis, Exxon Mobil (retired), is currently an independent consultant in epidemiology and human health risk assessment. He was a Distinguished Scientific Associate in the Epidemiology, Health Surveillance and Quality Assurance Section of ExxonMobil Biomedical Sciences, Inc (EMBSI) from 1990 to 2021. In this role, he supported ExxonMobil scientific programs related to naphthalene, 1,3-butadiene, human health risk assessment, regulatory affairs and regulatory impact analysis. He also served as Chair of EMBSI's Senior Technical Council. Dr Lewis has more than 30 years of experience and numerous scientific publications in occupational epidemiology and human health risk assessment. He has served on a number of industry trade association scientific committees (e.g., American Chemistry Council), external science advisory boards (e.g., the Alliance for Risk Assessment Expert Science Panel, U.S. Environmental Protection Agency Science Advisory Board for 1,3-butadiene) and was a member of the American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Value (TLV) Committee. Dr. Lewis also served as Treasurer for both the Society for Risk Analysis and the Society for Benefit-Cost Analysis. Dr. Lewis received his Bachelor of Science degree in biology from the University of Kansas and M.S. and Ph.D. degrees in Epidemiology from the University of Texas School of Public Health. In addition, he earned a Master of Business Administration degree from Rutgers University.

Dr. Kun Lu, University of North Carolina. The overarching goal of Dr. Lu's laboratory is to better understand health effects of environmental exposure and individual response by integrating the microbiome, exposome, omics profiling, and biomarker development. Dr. Lu's laboratory is working on a number of important environmental chemicals ranging from heavy metals to pesticides, as well as others with significant public health concerns. The current emphasis is being placed on microbiome research and exposome mapping. Dr. Lu's laboratory aims at answering how gut microbiome interacts with environmental exposure, how gut microbiome affects disease susceptibility, and how host factors crosstalk with microbiome to influence its response. Another focus of Dr. Lu's laboratory is to map exposome for human disease, with the goals of characterizing all exposures over the lifespan via high-resolution mass spectrometry, understanding the health impact of the exposome, and designing strategies to reduce exposure-associated adverse effects. Dr. Lu's laboratory combines both systems-level and targeted approaches in research. In particular, Dr. Lu's laboratory utilizes highly integrated system-level approaches including DNA sequencing, metabolomics, proteomics and lipidomics, coupled with the application of diverse cell, animal and disease models to interrogate the pathogenesis of human disease.

Dr. Andrew Maier, Cardno ChemRisk, has over 25 years of professional work experience in the areas of environmental health, occupational hygiene, and toxicology. He currently serves as a Principal Science Advisor with Cardno ChemRisk. Prior to joining Cardno he served as an Associate Professor of Environmental and Industrial Hygiene at the University of Cincinnati (UC) College of Medicine leading a research program in occupational exposure assessment, toxicology and risk assessment. Prior to joining UC, he served as the Director for the non-profit organization Toxicology Excellence for Risk Assessment (TERA). In his capacity as an industrial hygienist, toxicologist and risk assessor, he has led numerous projects and has co-authored toxicological reviews, EPA and NIOSH technical reports and human health risk assessment documents for several hundred individual substances. Dr. Maier has an established history in occupational toxicology and industrial hygiene. He completed his B.S. in natural resources from Ball State University and M.S. in industrial health from the University of Michigan. He is certified in comprehensive industrial hygiene practice by the American Board of Industrial Hygiene (CIH). Dr. Maier completed his Ph.D. in toxicology from the University of Cincinnati and is board certified in toxicology (DABT). He continues to be actively engaged in teaching and developing research to improve risk assessment approaches through the integration of basic biology and risk assessment science. He is a lead instructor for risk assessment professional development courses offered through various non-profit organizations. He has served as a Toxicology Fellow at NIOSH in support of exposure limit methods development and is the Publications Coordinator and past-Chair of the Workplace Environmental Exposure Levels (WEEL) Committee.

Dr. Bette Meek, University of Ottawa, is the Associate Director of Chemical Risk Assessment at the McLaughlin Centre for Risk Science, Faculty of Medicine, University of Ottawa. Previously, she contributed to and managed several chemical risk assessment programs within Health Canada. With colleagues internationally, she has contributed to or led initiatives in developing methodology in chemical risk assessment, including mode of action, chemical specific adjustment factors, physiologically-based pharmacokinetic modeling, combined exposures and predictive modeling. These initiatives have involved collaborations with a range of international organizations and national Agencies, including the World Health Organization International Programme on Chemical Safety, the Organization for Economic Cooperation and Development, the U.S. Environmental Protection Agency, the European Joint Research Centre and the Agency for Food, Environmental and Occupational Health and Safety of France (ANSES). She has authored approximately 200 publications in this area and received several awards for contribution in this domain.

Dr. Adriana Oller, NiPERA, is a Toxicologist Emeritus and past Executive Director at NiPERA. She joined NiPERA in 1994 following post-doctoral research at both the Lineberger Cancer Research Center and the National Institute of Environmental Health Sciences (NIEHS) in North Carolina. Originally from Argentina, Adriana earned a Master's degree in Biochemistry from Buenos Aires University and a Ph.D. in Genetic Toxicology from the Massachusetts Institute of Technology (MIT). She is a Diplomat of the American Board of Toxicology and is a fellow of the Royal Society of Chemistry. She has authored or co-authored over 30 peer reviewed papers on nickel and her expertise on nickel toxicology is recognized at a European, US and international level.

Dr. Greg Paoli, Risk Sciences International (RSI). As RSI's Principal Risk Scientist and COO, Greg Paoli is currently working with a broad spectrum of clients in the public and private sectors, with the greatest emphasis on the development of new approaches to transform regulatory systems to be more "risk-based" at multiple levels: how senior decision-makers process risk information and make the "tough choices" in regulating health and safety, how an organization can optimize its resources across a diverse mandate to maximize total "portfolio-level" risk reduction, and how to operationalize key resources like the scheduling of inspections when faced with thousands of inspection targets, but not enough resources to inspect all of them.

While food safety and chemical safety continue to be an important part of his work, he has also been fortunate to gain a deep understanding of a very wide variety of risks borne by the public or the public interest. He has also shifted from focusing on specific risk issues on behalf of his clients to addressing the organization-wide capacity to conduct risk assessment and developing enabling technologies to allow for more robust risk assessment work within the client organizations. He was particularly fortunate to work with Public Safety Canada and the Defence Research and Development Canada (DRDC) in the development and application of the All-Hazards Risk Assessment Methodology for Whole-of-Government application of Emergency Management. During the same period, he was working with the Canadian Conservation Institute in applying risk management methods to the preservation of cultural property with hazards ranging from theft, to sunlight and humidity ruining paintings, to toxic ink destroying the very documents that record history.

One example of an organization-enabling technological achievement has been the development of FDA-iRISK (with RSI colleagues Todd Ruthman, Hong Duan and Emma Hartnett, and colleagues Yuhuan Chen, Regis Pouillot, Jane van Doren and Sherri Dennis from the US Food and Drug Administration). This technology allows food safety professionals from all over the world to develop robust, rapid risk assessments ranging from simple models to highly complex models including quantitative characterization of natural variability and epistemic uncertainty (applying so-called 2-Dimensional Monte Carlo simulation). In addition, this tool has the capacity to span microbiological, chemical, allergenic and nutritional aspects of food safety. This tool was chosen as one of six finalists for the HHSInnovates awards within the Department of Health and Human Services in the US.

The most recent phase of his work is to engage with diverse organizations in the journey toward the concept of "risk-based decision-making". The concept of being "risk-based" within organizations is somewhat pandemic in its spread throughout regulatory organizations around the world. It has been described in the literature as a "badge of legitimacy." Despite the pandemic nature, it is rarely ever defined and there is no roadmap. This presents a problem and an opportunity. One particularly satisfying engagement was, in

working with RSI colleagues and colleagues from the Alliance of Blood Operators (the suppliers of blood products in most highly developed countries), to develop a Framework for Risk Based Decision-making for Blood Safety. He was also pleased to contribute to the University of Pennsylvania Law School's "Best-in-Class Regulator Project, where he was tasked to define "The Analytical Capabilities of a Best-in-Class Regulator." (link) This was followed by a series of similar engagements (some spanning many years) to develop Frameworks (and the methodologies within them) in the areas of the safety of Engineered Devices (elevators, escalators, boilers, pressure vessels, fuel distribution and pipelines), across four modes of transportation (Transport Canada) and across a diverse Environmental Enforcement mandate ranging from greenhouse gases, toxic emissions, to smuggled endangered species (Environment and Climate Change Canada).

Dr. Reena Sandhu, SafeDose. Four score and seven years ago (or so it seems), Reena started her career as a biologist and ethologist, studying aggressive behaviours in farrowing sows. Finding this career choice mildly restrictive and slightly terrifying, Reena reinvented herself as a health scientist, working in drug discovery and development for biotech firms. In version 2.0, Reena became a toxicologist and risk assessor, setting safe dose limits for chemicals in the air, water, food, soil, consumer products, pharmaceuticals, and other media. Now in version 3.0, Reena is having loads of fun enlisting technology to serve her passion for developing software tools that will improve the efficiency and quality of human health risk assessments.

Dr. Rita Schoeny, U.S. Environmental Protection Agency (retired), is a consultant in risk assessment and science policy, who retired after 30 years from the United States Environmental Protection Agency. Her Ph.D. in microbiology is from the School of Medicine of the University of Cincinnati, and she was assistant professor of Environmental Health there. Dr. Schoeny has held adjunct appointments in several graduate schools; she continues to give lectures, trainings and courses on risk assessment, science policy and toxicology in many areas of the world. At the U.S.EPA, Dr. Schoeny held senior scientist and management positions in the Offices of Research Development and Water. She was responsible for major assessments and programs in support of EPA's legislative mandates including Safe Drinking Water Act, Clean Water Act, Clean Air Act, and Food Quality Protection Act. She publishes in the areas of toxicity of PCBs and polycyclic aromatic hydrocarbons; assessment of complex environmental mixtures; health and ecological effects of mercury; drinking water contaminants; and principles and practice of human health risk assessment, most recently focused on mode of action and adverse outcome pathways. Dr. Schoeny is the recipient of numerous awards from the Federal government and scientific societies. She is a Fellow of the Society for Risk Analysis.

Dr. James Sherman, Celanese, is a Science Fellow, with over 35 years of professional experience as a toxicologist. He has been both a Diplomat American Board of Toxicology and full member of the Society of Toxicology for over 25 years. Prior to joining Celanese, Dr. Sherman worked for Monsanto, Solutia, BASF, and the USEPA. He has considerable world-wide regulatory experience in risk assessment, industrial chemicals management, pesticide registration, air permitting, hazardous waste management, site remediation, and has planned and directed hundreds of guideline-compliant and original mode-of-action toxicology studies. Dr. Sherman has served as the lead scientist on numerous trade association panels, representing and building scientific consensus amongst diverse sectors of the industrial chemical and agrochemical industries. Jim holds a B.S. degree in Genetics from University of Illinois and a Ph.D. in toxicology from University of Cincinnati College of Medicine.

Dr. Michael Taylor, NiPERA, is responsible for overseeing research to fill data gaps in the risk assessment and human health effects associated with exposure to nickel and nickel compounds. Prior to joining NiPERA in 2013, Mike was senior toxicologist for Afton Chemical Corporation, where he was responsible for identifying potential regulatory and risk assessment gaps regarding the human health effects of inorganic manganese. Mike holds a Bachelor's degree in Biology and a PhD in Toxicology, both from West Virginia University. He also conducted post-doctoral research on the health effects of metal-containing dusts and fumes for the National Research Council. He has been a Diplomat of the American Board of Toxicology since 2009 and is based in Durham.

Dr. Russell Thomas, U.S. Environmental Protection Agency, is the director of the Center for Computational Toxicology and Exposure (CCTE). Dr. Thomas' area of expertise is in the application of high-throughput and high data content approaches to chemical toxicity testing. He has a broad, multidisciplinary background and experience but his formal academic training was in chemistry, radiation health physics, and toxicology. He then received postdoctoral training in molecular biology and genomics. Following his academic training, Dr. Thomas performed bioinformatics and genomics research in the biotechnology sector and gained experience in high-throughput screening and in vitro assay development in the biopharma sector. Prior to coming to the U.S. EPA, Dr. Thomas worked as an investigator and the director of the Institute for Chemical Safety Sciences at The Hamner Institutes for Health Sciences.

Dr. Nitin Verma, Chitkara University, is a Professor and Principal at School of Pharmacy, Chitkara University, Himachal Pradesh, India. He is responsible for setting academic rules and policies, developing academic programs and performing other administrative functions, coordinating student activities, participating in activities of faculty committees, advising authorities on academic matters and providing academic counseling or advising to students and faculty, improving school programs and monitor policies, while making sure all accreditation, State, and Council criterion concerning academics are strictly followed. His area of interest is in Natural Products, Toxicology, Risk Assessment of Chemicals and their impact on Environment and Human Health.

Dr. Pamela Williams, E Risk Sciences, LLP, is a Principal at E Risk Sciences, LLP, an independent scientific consulting firm that provides sound analyses and tools to support risk-based decision-making related to human health and the environment. She previously served as a Clinical Assistant Professor in the Department of Environmental and Occupational Health at the Colorado School of Public Health. She is currently a Fellow with Toxicology Excellence for Risk Assessment (TERA). Dr. Williams specializes in assessing human exposures and health risks in environmental, community, and occupational settings. Her particular areas of expertise include human health risk assessment, exposure science, industrial hygiene, exposure modeling, and uncertainty analysis. She has published over 100 papers, book chapters, and presentation abstracts on various exposure and risk-related topics. She has also lectured in undergraduate, graduate-level, and continuing education courses related to industrial hygiene and exposure and risk assessment at the Colorado School of Public Health, Colorado School of Pharmacy, Harvard School of Public Health, Clarkson University, the Society of Toxicology, and the American Industrial Hygiene Association (AIHA). She routinely serves as a technical peer-reviewer for a number of scientific journals, peer review panels, and government agencies. Dr. Williams is past President of the Society for Risk Analysis (SRA) and past Chair of AIHA's Risk Committee. She has received several awards for her contributions to the fields of risk analysis, exposure science, and industrial hygiene. Dr. Williams has a B.A. in Sociology with a certificate in Applied Social Research from San Diego State University, M.S. in Health

and Social Behavior from Harvard University, and ScD in Environmental Health and Health Policy and Management from Harvard University. She is also a certified industrial hygienist (CIH).

Risk Assessment Advisory Committee (*attended virtually)

- James Bus, Exponent
- Michael Dourson, TERA
- Neeraja Erraguntia, ACC*
- Suzanne Fitzpatrick, U.S. FDA*
- Mark Johnson, U.S. ARMY
- Sabine Lange, TCEQ*
- Pam Williams, E Risk Sciences, LLP*

Members of the Science Panel for Workshop XIII (*attended virtually)

- Michael Ascher, Albert Einstein College of Medicine
- James Bus, Exponent
- Chris Chaisson, The Lifeline Group*
- Michael Dourson, TERA
- Annie Jarabek, U.S. EPA*
- Jeff Lewis, Exxon Mobil (retired)*
- Bette Meek, University of Ottawa*
- Greg Paoli, Risk Sciences International*
- Rita Schoeny, U.S. EPA (retired)
- Nitin Verma, Chitkara University, School of Pharmacy
- Christine Whittaker, NIOSH (unable to attend)