U.S. Environmental Protection Agency Board of Scientific Counselors

Chemical Safety for Sustainability and Health and Environmental Risk Assessment Subcommittee

Virtual Meeting Minutes

February 2-5, February 25, March 11, 2021

Dates and Times: February 2, 2021, 12:00 to 5:00 p.m.; February 3, 2021, 12:00 to 5:30 p.m.; February 4, 2021, 12:00 to 5:00 p.m.; February 5, 2021, 12:00 to 5:00 p.m.; February 25, 2021, 2:00 to 5:00 p.m.; March 11, 2021, 2:00 to 5:00 p.m. Eastern Time

Location: Virtual

Meeting Minutes

Provided below is a list of the presentations and discussions that took place during the meeting with hyperlinked page numbers. The minutes follow. The agenda is provided in Appendix A, the participants are listed in Appendix B, and the charge questions are provided in Appendix C.

Tuesday, February 2, 2021

The meeting generally followed the issues and timing as presented in the agenda provided in Appendix A of this meeting summary.

Meeting Kick Off, Federal Advisory Committee Act Rules, Expectations, and Logistics

Tom Tracy, Designated Federal Officer, Office of Science Advisor, Policy, and Engagement

The meeting convened at approximately 12:00 p.m., Eastern Time. Mr. Tom Tracy, Designated Federal Official (DFO) for the Board of Scientific Counselors (BOSC) opened the meeting by welcoming the BOSC Subcommittee members. He informed BOSC Subcommittee members that the meeting materials were posted to EPA's public website, and he noted meeting minutes would be posted following the meeting. Mr. Tracy stated that all BOSC members had completed their ethics training and no conflicts of interest were identified. Finally, he discussed Federal Advisory Committee Act (FACA) stipulations governing the meeting, which require the meeting is open to the public and with time reserved for public comments.

Office of Research and Development Welcome

Jennifer Orme-Zavaleta, Principal Deputy Assistant Administrator for Science, Office of Research and Development

Dr. Jennifer Orme-Zavaleta thanked participants for joining the virtual meeting. She then explained that there were new EPA staff and BOSC members with the change of administration. She briefly introduced Dr. Chris Frey and provided him the opportunity to introduce himself in more detail. Dr. Frey spoke to the Subcommittee members and EPA staff and noted his work experience on multiple advisory boards for the Agency over the last few years. He then thanked the Subcommittee for their work to support ORD and EPA in its entirety. He also expressed his welcome of the Subcommittee's feedback. Speaking on behalf of the Biden/Harris Administration, Dr. Frey assured members that the Administration is committed to science and appreciates the BOSC subcommittees' efforts.

Dr. Orme-Zavaleta shared that ORD is pleased to welcome Dr. Frey to the ORD leadership team and looks forward to working with him. Dr. Orme-Zavaleta also shared the goals of the Chemical Safety for Sustainability/Health and Environmental Risk Assessment (CSS/HERA) Subcommittee meeting, which was to determine if the Agency's current scientific efforts accomplish the strategic goals in the CSS/HERA programs Strategic Research Action Plans (StrAPs). She explained how there would be discussions around per- and polyfluoroalkyl substances (PFAS) research in the future, but this meeting would exemplify research activities that have been implemented by both the CSS and HERA programs. She also assured Subcommittee members that while the research discussed is exclusive to those programs, there is clear overlap between the two programs, as well as a link to other programs within EPA.

Dr. Orme-Zavaleta outlined research areas that have successfully been addressed by the CSS and HERA programs. The disruptive nature of the Coronavirus Disease 2019 (COVID-19) pandemic has delayed EPA's ability to conduct and complete research. Dr. Orme-Zavaleta expressed her optimism that research conditions will improve as the pandemic complications subside, and it

will be more feasible to initiate new research projects. She closed by highlighting Dr. Frey's comments thanking the CSS/HERA subcommittee for their work.

- **James Stevens:** We recently discussed two recommendations recently, the first being studies that are well conducted but do not protect patient privacy, the second being the replacement of animal testing. There was a concern that replacement of animal testing could hamper risk-based decisions. Could you reiterate what was discussed for the subcommittees?
    - **Jennifer Orme-Zavaleta**: The rules around patient privacy will not go into effect as it was struck down. To the second recommendation, the goals of new approach methods (NAMs) will help make science-based decisions using new methods. Once the new EPA Administrator is confirmed, the Biden-Harris Administration will discuss if they still support the current NAMs plans.
    - **Chris Foley:** I have not yet had the opportunity to meet with the new Administrator, but we will need to understand and discuss as an Agency what is a reasonable timeline for the implementation of NAMs.
- **Ponisseril Somasundaran:** Regarding the climate crisis, the best way to help solve this problem is to establish space colonies. The United States is falling behind other countries.

After addressing subcommittee members' questions, Dr. Orme-Zavaleta introduced Dr. Katrina Waters as the next speaker.

Subcommittee Chair Opening Remarks and Introductions

Katrina Waters, Chair, Chemical Safety for Sustainability and Health and Environmental Risk Assessment Subcommittee

Dr. Waters stated that she was looking forward to the subcommittees' discussion on execution plans and how the BOSC subcommittees can best advise the CSS and HERA programs. She noted that this will be a marathon meeting and how she appreciates everyone's time and flexibility throughout the meeting.

Dr. Waters introduced herself, reviewed the agenda, and started a round of introductions for the BOSC subcommittee members. The following members introduced themselves: James (Jim) Stevens, Anthony Bahinski, Rick Becker, Juan Colberg, Richard Di Giulio, Chris Gennings, Dale Johnson, Daland Juberg, Juleen Lam, Timothy Malloy, Jane Rose, Ponisseril Somasundaran, Gina Solomon, and Donna Vorhees.

Dr. Waters explained that the CSS/HERA subcommittee members would review each charge question, and separate breakout sessions would correspond directly to the charge questions. She also reviewed logistics for the virtual meetings. She then opened the floor for questions from the CSS/HERA subcommittee about the agenda. Hearing none, Dr. Waters introduced Dr. Jeff Frithsen as the first presenter.

Chemical Safety for Sustainability New Approach Methodologies Research and Development Portfolio: Connecting the Dots to Relevance and Acceptance

Jeff Frithsen, National Program Director, Chemical Safety for Sustainability Research Program

Dr. Jeff Frithsen explained how previous BOSC meetings have focused on StRAP development, whereas the focus of this meeting would be on the implementation of the StRAP. He then specified that the CSS program overview would focus on NAMs examples and tools and the HERA program overview would focus on applying NAMs in assessments and include other systematic review tools Dr. Frithsen then reviewed the definition of NAMs.

Dr. Frithsen reinforced that the focus of this meeting was on NAMs, and that future CSS/HERA subcommittee meetings will review other areas of the CSS/HERA StRAP. Research planning and research implementation are two sides shared by the ORD matrix, and he explained how resources were allocated by research area level. Dr. Frithsen reviewed the process going from planning to delivering products.

Dr. Frithsen explained EPA's interests in NAMs research, and he shared how the CSS Research Program has focused on NAMs research for over a decade. He discussed how EPA scientists created CompTox, and its goal being to have the best science to evaluate chemicals. The CSS program has a critical long-term vision of having access to the best information to inform Agency decisions about chemicals. He explained that having more access to the best science can inform chemical decision-making by accelerating the pace of chemical assessment, reducing animal testing, and providing scientific innovation and leadership to transform chemical screening and assessment.

Dr. Frithsen then reviewed the Toxic Substances Control Act (TSCA) StRAP Pan for NAMs. He described how TSCA is not the only partner served by CSS for NAMs development, and he listed the other groups inside and outside of ORD. Dr. Frithsen also discussed the CSS sessions, respective goals, and charge questions. He pointed out that any research in progress should not be cited or quoted, whereas any works already published can be cited or quoted.

- **Katrina Waters:** I noticed in the materials that there was not a list of NAMs. It would be helpful for the CSS/HERA Subcommittee to have a list of NAMs to better address the charge questions to address gaps and possible advancements. If this could be posted to the BOSC SharePoint, that would be helpful.
    - **Jeff Frithsen:** There is a list of NAMs in the TSCA Roadmap, and publications are also listed here. We can post the TSCA list, but not every CSS program NAM is on there, and please note that some of the NAMs are not from the CSS program.
- **James Stevens:** If it says publication, does this indicate an existing publication or an anticipated publication? I have the same question for models.
    - **Jeff Frithsen:** There are two lists in the background materials. One is the publication list, which includes all publications from 2019 and 2020. The other is the list of products developed as a part of the 2019-2020 StRAP. These lists

differentiate the planning and strategic phase from the implementation phase. These are completed products and have been delivered to the Agency's partners and might represent one or more publications.

- **Kathie Dioniso:** I would also note the grey highlighted products are completed.

Health and Environmental Risk Assessment Advancing the Science and Practice of Assessments

Samantha Jones, National Program Director, Health and Environmental Risk Assessment Research Program

Dr. Samantha Jones introduced herself and welcomed participants to the implementation part of these discussions. Dr. Jones discussed the HERA program, including the centers, and noted how the partners are shared with other ORD programs. Dr. Jones reminded the CSS/HERA Subcommittee of their discussion in May 2020 about the HERA program's focus on developing and translating assessments to advance science and the practice. Dr. Jones then presented a small subset of products developed under HERA since 2019, including ISAs, Integrated Risk Information System (IRIS) values, and Provisional Peer-Reviewed Toxicity Values (PPRTVs).

Dr. Jones directed the CSS/HERA Subcommittee to review the appendices and the HERA program's subtopics. She discussed ORD's roles in NAMs development and HERA's portfolio of NAMs. She also shared how establishing and maintaining a research platform, including tool libraries and trainings, will be the focus future meetings.

- **Dale Johnson:** You mentioned working partners, who are the partners?
  - **Samantha Jones:** Given the HERA program's focus on assessments, primary partners include EPA regions, EPA's Office of Air and Radiation, EPA's Office of Water, OLEM, and OSCPP.

Translating Strategy into Action: Research Implementation Plans in Office of Research and Development

Jill Franzosa, Assistant Center Director, Center for Computational Toxicology and Exposure

Dr. Jill Franzosa described the implementation process of the StRAPs, including ORD investigators' research. She explained the purpose, roles, and process of the Research Area Coordination Teams (RACTs). She then outlined how research area descriptions are created and delivered (i.e., needs, output and product), and she provided examples of the CSS program translating partner needs to outputs, specifically the developmental neurotoxicity (DNT) partner needs. She highlighted the CSS program's metrics for developed outputs and products by topic and research area.

- **Jennifer McPartland.** Why is April to September the timeframe for RACTs? Are RACTs still occurring?
  - **Jill Franzosa:** Program offices approved a set of outputs April. We began the product portfolio to prepare for the fiscal year, which ended in September. Yes, RACTs are still meeting, but the frequency and level of meetings has varied.

- **Anuradha Mudipalli:** What stage is the decision-making process at for the validation and use of zebra fish data as a NAMs?
    - **Jill Franzosa:** Rusty Thomas will address the NAMs, as for the DNT specifics Dr. Shaeffer will detail this in his presentation.
- **Richard Becker**: Are only EPA members part of the RACTs?
    - **Jill Franzosa:** Yes, that is correct.
- **Donna Vorhees:** Were you surprised with any need requests from the RACT meetings?
    - **Jill Franzosa**: We did not have of additional need surprises, but we learned more about the current needs from participants.

Evolution of New Approach Methodologies in EPA: From Research to Application

Rusty Thomas, Center Director, Center for Computational Toxicology and Exposure

Dr. Rusty Thomas discussed the evolution of NAMs at the Agency and the challenges in evaluating human and environmental risks from chemicals. Dr. Thomas noted that decision-making depends on the amount and reliability of the available data.

Dr. Thomas explained ORD's history with NAMs research and how ORD has addressed these challenges, including the Strategic Plan for the CompTox research program. He described the integrated strategy and overlapping elements of the Agency's NAMs research strategies with the CSS and HERA programs to develop research products. Dr. Thomas highlighted research areas that address high throughput and computation modeling, including hazard evaluation, exposure assessment, chemical characterization, toxicokinetic, and *in vitro* dispositions.

Dr. Thomas discussed EPA's collaboration with federal, state, and international collaborators to develop, evaluate, and apply NAMs. He discussed the Accelerating the Pace of Chemical Risk Assessment (APCRA) inter-governmental workshop, bi- and tri-lateral engagements and cross-federal collaborations with the U.S. Geological Survey (USGS), and other agencies. Lastly, he emphasized the need for outreach and training to build confidence and disseminate knowledge within ORD.

- **Juan Colberg:** To reach the confidence level of data moving from standard to NAMs, what outcome do you expect for chemical groups to not be accessed under these different methods?
    - **Rusty Thomas:** Different methods have different applicability. We may need to explore the development of different methods. The level of confidence varies depending on the decision you make and the context of use. This confidence level must fit the context it is being applied in, such as the characterization under TSCA. We are evaluating the confidence, variably, and uncertainties of the traditional models in comparison to NAMs.

BOSC Subcommittee Discussion and Questions and Answers

Katrina Waters, Chair

Dr. Waters took questions from the CSS/HERA Subcommittee members.

- **Ponisseril Somasundaran:** Can you elaborate on the relationship with of Minnesota Department of Health (MDH)?
    - **Rusty Thomas:** This is a state initiative to identify contaminants in Minnesota. They are working to apply computational and experimental tools to leverage their application.
- **Jane Rose:** Acknowledging EPA has only hit the tip of the iceberg with NAMs and understanding the time limitation, should we only focus on those NAMs specifically presented as we address CSS Charge Question 1? In addition, how were the presentations chosen?
    - **Jeff Frithsen:** These serve as examples on how we are moving forward. CSS Charge Question 1 is to discuss what we are seeing and identify if there is something we may be missing. We chose to highlight these projects as they demonstrate the depth and breadth of what we are doing.
- **Richard Becker:** Regarding confidence in new models for intended purposes and ideas for creating a general NAMs framework, how does the framework fit into the CSS StRAP?
    - **Rusty Thomas**: There is a deliverable to develop the scientific context framework, due in Quarter 3 of fiscal year 2022, and it is associated with the StRAP under the NAMs workplan. The June 2020 EPA NAMs Work Plan is Appendix C of the background materials, which is posted on the BOSC SharePoint site.
- **Richard Becker:** Would it be helpful to distribute the June 2020 strategy, so we could analyze the data that would be harder to evaluate than other types of NAMs?
    - **Rusty Thomas**: The elements of the scientific context still need to be filled in to provide a broader, more generic confidence framework.
- **Katrina Waters:** If the shading of publications list indicates completion, are all products in the list anticipated in 2022 or is there short-term prioritization needs? Are these lists updated annually? Perhaps they could include an anticipate delivery date.
    - **Jeff Frithsen:** We are on track with delivery for most products, but some will be pushed to fiscal year 2023 and may appear in the next CSS StRAP. We can update the lists for fall 2021.
    - **Samantha Jones:** This is true for the HERA program as well. Most products are on track and expected to be completed in fiscal year 2022.
- **James Stevens:** For the products list, should some be considered more of a milestone towards a work product? For instance, are publications a milestone to a product or is an assay a validation to a product?
    - **Jeff Frithsen:** The products are listed and defined as a deliverable to the partners. For example, an assay is the delivery of the products, tools and papers are also milestones.
- **James Stevens:** To follow up to Richard Becker's question, how do you address those varying degrees of validation? Do you prioritize TSCA or pesticide registration?

    - **Rusty Thomas:** Under TSCA's proof of concept and prioritization of chemicals, some were developed in case studies with program partners but not a formal validation. Partners were comfortable applying to that context, but if applying more broadly, then they would apply the Organization for Economic Co-operation and Development (OECD) ring-trail validation.
- **Donna Vorhees:** On the discussion of work products and stages, I found an EPA central website listing all NAMs. Will all products end up on this website? Will this be the central repository to check progress?
    - **Jeff Frithen:** The TSCA NAMs list you mentioned, alternative_testing_nams_list_first_update_final.pdf (epa.gov) serves as list, but does not to track CSS progress.
    - **Monica Linnenbrink:** That is a great suggestion to add a direct link to the list of NAMs published per TSCA Section 4(h)(2)(C). You can search the website to get to the link via the link to the Alternatives to Animal Testing Strategy (https://www.epa.gov/research/epa-new-approach-methods-efforts-reduce-use-animals-chemical-testing).

New Approach Methodologies Research Introduction with Charge Question

Jeff Frithsen, National Program Director, Chemical Safety for Sustainability Research Program

Dr. Frithsen discussed CSS Charge Question 1, and he addressed clarification questions on the differences between CSS Charge Question 1 and CSS Charge Questions 2. He explained how CSS Charge Question 1 focused on the science, development, and testing, while CSS Charge Question 2 outlined science application. The Subcommittee and presenters were placed into one of four sessions within breakout rooms to continue the discussion. The sessions included: Session A: Emerging Approaches to Hazard Testing, Session B: NAMs for Exposure, Session C: NAMs for ECOTOXicology Knowledgebase (ECOTOX) Applications, and Session D: System-specific Models and Approaches.

- **Katrina Waters:** Can you expand on the difference between CSS Charge Questions 1 and 2?
    - **Jeff Frithsen:** CSS Charge Question 1 deals with the science, development, and testing, while CSS Charge Question 2 looks at the application of the science.
- **James Stevens**: Under which charge question would any feedback on validation fit?
    - **Jeff Frithsen**: I see that under more CSS Charge Question 1, but parts of the conversation might be more applicable in CSS Charge Question 2.

Session A: Emerging Approaches to Hazard Testing

High Throughput Phenotypic Profiling

Joshua, Harrill, Toxicologist, Center for Computational Toxicology and Exposure

Joshua Harrill presented on high throughput phenotypic profiling, and he answered the CSS/HERA Subcommittee members' questions.

- **Jennifer McPartland:** What cell types were used in the and high-throughput phenotypic profiling (HTPP) assay set? Can you speak about differential responses across different cell types in this assay? Are any of these cell lines metabolically competent?
    - **Joshua Harrill:** The work started with the cell line used by MIT development with the intention to deploy it across multiple diverse cell lines. We have been using computation algorithms to find which cell lines are most complimentary to each other. It is too early to make definite conclusions. Some of the potencies vary across cell lines, and that varies from chemical to chemical. You do see chemical similarity and mitochondrial morphology in some cell lines. The cell lines used are not metabolically competent, but the last talk in this session is talking about integrating that capability.
- **James Stevens:** Over 90 percent of ribonucleic acid (RNA) in cells is ribosomal followed by transfer ribonucleic acid (tRNA). When you visualize RNA, what are you visualizing?
    - **Joshua Harrill:** RNA that has a greater affinity for single strand. We are using this to visualize the nucleolus.
- **James Stevens**: Are you looking at messenger RNA, not ribosomal or tRNA? Are there restrictions on cell size?
    - **Joshua Harrill**: I would suspect that we are looking at all types. Comparative studies were run at low and high densities and there was good reproducibility of potency estimates at both densities. Problems start when cells stack on top of each other. There is a critical point when cells get too dense to segment properly.

High Throughput Transcriptomics

Logan Everett, Bioinformatics Scientist, Center for Computational Toxicology and Exposure

Logan Everett presented on high throughput transcriptomics, and he answered the CSS/HERA Subcommittee members' questions.

- **James Stevens:** Are points of departure with Gene Set Enrichment Analysis (GSEA) scores better than points of departure with fold scores?
    - **Logan Everett:** Yes, it is based on a method described as benchmark dose (BMD) express.
    - **James Stevens:** It was published previously that based on enrichment scores, there was no difference between a fold change point of departure derived benchmark dose and benchmark dose based on enrichment scores. If you are suggesting GSEA scores improve, I think it is important to get into the literature.
    - **Logan Everett:** Whether the two methods show a difference depends on a few things. Maybe the signal-to-noise is good enough that the probe level point of departure is as good as the signature scores. MCF-7 cells tend to provide weaker effect sizes in responses. We observed that in our data aside from a few

chemicals. Most chemicals in MCF-7 cells are giving a more subtle response in the individual probe level.

- **James Stevens:** Was the Minimum Ignition Energy (MIE) output based on a classification of an unknown with known molecules? Or more linked to a prediction based on a linkage to a phenotypic response?
    - **Logan Everett:** MIE's are more fine scale than that. Activation of estrogen receptor alpha or inhibition of estrogen receptor alpha, those tags come from RefDB as individual binary classifiers. For each MIE we could find examples of, we trained a separate binary classifier for that MIE.
    - **James Stevens:** It is a gene set acting as a signature MIE derived from existing data?
    - **Logan Everett**: We are feeding in individual profiles from the L1000 data and training the classifiers based on those. It was tested on individual or probe level changes and there were no changes observed. The changes happen in the machine learning context.

Metabolic Augmentation in in vitro Systems

Chad Deisenroth, Cell Biologist, Center for Computational Toxicology and Exposure

Chad Deisenroth presented on metabolic augmentation in *in vitro* systems, and he answered the CSS/HERA Subcommittee members' questions.

- **Jennifer McPartland:** Is the idea that to put through all compounds that are being evaluated through high-throughput or *in vitro* assays through metabolic system, intracellular and extracellular?
    - **Chad Deisenroth:** The goal is not to repeat ToxCast 2.0 with metabolism, rather focus on a future-forward approach. There are a few options on where to integrate metabolism, one is to explore a set of test chemicals in High Throughput Transcriptomics (HTTr), HTPP, and the second criteria performs a method transfer study. Metabolism will likely be integrated into endocrine toxicology as well.
    - **Jennifer McPartland:** Are you are suggesting that this work would be integrated into newer assays with some assays that have been run in the past?
    - **Chad Deisenroth:** It will be a more targeted approach. There is a current project with National Center for Advancing Translational Sciences (NCATS) to evaluate the role of metabolism in a P53 reporter assay. Assuming that a method is identified, it is not unreasonable to think they will run some of the Tox21 assays.
- **James Stevens:** Metabolism will play a role in categorizing and phenotyping any sort of hazard. When do you identify the metabolism component? You are moving in parallel if the data has been collected, and at what point do you decide whether to use a metabolic system? How does this strategy come together?
    - **Chad Deisenroth:** This has been in development for four to five years. We currently have a method we feel comfortable with and there is space to explore

integration of metabolism. In terms of deciding when to look at metabolism across all activities, I do not have a direct answer. Those questions will come up in the spring 2021 Strategic Plan planning cycle. We have a second set of 768 chemicals that were screened, and we are in the process of writing that manuscript. We need to separate hazard from prioritization. We will address integrating metabolism, and hopefully develop a cohesive strategy in the next planning cycle.

- **Joshua Harrill:** We do not want to hold one thing up to make sure everything is synchronized. We want to get methods established in each data stream.

Session B: NAMs for Exposure

High Throughput Exposure Models (SEEM)

John Wambaugh, Physical Scientist, Center for Computational Toxicology and Exposure

John Wambaugh presented on high throughput exposure models and how to use them with the systematic empirical evaluation of models (SEEM) framework. He answered the CSS/HERA Subcommittee members' questions.

- **Daland Juberg:** One of the vexing areas is how to integrate animal toxicology studies with epidemiological evidence. There has been guidance created to address this, but it is still a vexing area. Have you all done any work on how to apply NAMS to ecologic and epidemiologic studies?
  - **John Wambaugh:** We have not had the opportunity to do that kind of work. You need a way to correlate a known exposure and know effect with the epidemiology. I have a collaborator at the National Institute of Environmental Health Sciences (NIEHS) that is attempting to look at variations at the county level in the United States. I view that as a geospatial analytics challenge right now to get the epidemiological and exposure data on that granularity. For water, we are trying to do this nationally. We plan to start having exposure models that can do those types of linkages. We are starting to get the toxicokinetics. The tools are being built and I am speaking with people who want to do this work.
- **Donna Vorhees:** Can you elaborate how you think about uncertainty in the ensemble predictions process and in putting together your meta-model?
  - **John Wambaugh:** We have a set of evaluation chemicals and check how CDC models do on average for these 100-plus chemicals. We take this average performance and attempt to propagate that to other chemicals. We are trying to reproduce the type of error you would get with our crude technique.
- **Donna Vorhees:** You also showed a slide of the consensus modeling of the medium chemical intake. Are you getting questions about the upward bound intake? Is EPA interested in that level of exposure?
  - **John Wambaugh:** We get that question all the time. If you are uncomfortable about the level uncertainty for that medium estimate, our level of uncertainty for that upward population is a lot higher. Right now, we tend to simulate highly

sensitive exposure. We do have additional occupational data, so we can calibrate. It is mostly a statistical problem. It is an area of interest and exploration, but it is tricky.

- **Donna Vorhees:** In what context is your work being used? Is your work being used to make decisions?
    - **John Wambaugh:** That works was originally developed for the endocrine disrupter screening program for EPA to develop priorities for testing and some considerations to incorporate it into TSCA. Those are two areas it is being looked at the most. It has also been used by ORD itself to determine the drivers of greatest uncertainties.
    - **Donna Vorhees:** Are any of you state partners using your work?
    - **John Wambaugh:** Dr. Kristin Isaacs will present work conducted in collaboration with the state of Minnesota that uses all of these tools.

High Throughput Toxicokinetic Models and In Vitro to In Vivo Extrapolation

Barbara Wetmore, Toxicologist, Center for Computational Toxicology and Exposure

Barbara Wetmore presented on ORD research efforts on the area of toxicokinetics, and she answered the CSS/HERA Subcommittee members' questions.

- **Daland Juberg:** Could this work handle or have began to consider mixtures?
    - **Barbara Wetmore:** We have. It is going to be a process. I know you are well aware of the amount of work Paul Price has put into performing cumulative assessments. We have had discussions with him on how to bring in different chemicals together. We could put forth some of those learnings into the *in vitro* to *in vivo* extrapolation (IVIVE) process.

Non-Targeted Analysis

Jon Sobus, Physical Scientist, Center for Computational Toxicology and Exposure

Jon Sobus presented on non-targeted analysis research being conducted by the Chemical Safety for Sustainability National Research Program and he answered the CSS/HERA Subcommittee members' questions.

- **Dana:** Are you working with anyone from HERA or any risk-analysist to understand the utility of these predictions to date? Are there going to be meaningful results coming out of these analyses now or sometime in the perceivable future?
    - **Jon Sobus:** We will at some point in the foreseeable future. When you do analytical chemistry, you take your sample of interest and prepare it. A fraction of that gets spike onto the mass spectrometer. When you do semi-quantitative experiments, you first estimate the upward bound concentration in the prepared solution and then you must go back to the original sample medium. The much more challenging process is going back to the sample medium. Generating those upward bound semiquantitative experiments in the prepared solution is something

we are doing now but extrapolating that to solution upward bound to the original solution at a medium concentration is a challenge.

- **Katrina Waters:** I think it a great part of what you are doing to create a set of standards for publishing. I would ask you for the basis of working with this consortium, what are you learning about best practices in that space, whether computational or technological?
    - **Jon Sobus**: It is both, your selection of hardware platform will influence what you will do downstream in terms of software and workflow. It is not one thing that allows you to have confidence in your structural identification, it is several things. The combination of that is influenced by the hardware platforms you select. The challenge is the more hardware you use, the more software required that needs to be optimized, the more performance testing of those optimization and the more need to compare to others work. It snowballs quickly. Coming up with community standards to evaluate performance would allow these methods to be more easily compared.
    - **Katrina Waters:** Appreciating that every combination of technology requires some combination of informatics approaches to extract that information is also made more complicated by the matrix that they are analyzing and the sensitivity of these chemicals. It is a challenging problem, but it is important.
    - **Jon Sobus:** I think it is up to the folks at EPA and other federal institution to play a pivotal role to create these benchmarks.
- **Daland Juberg:** Where do you see the greatest opportunity for application of NTA, broadly?
    - **Jon Sobus:** I was writing a paper on this now on who using these different technologies for different application. What was striking was the number of NTA publications for food applications far outweigh environmental applications. In food safety evaluations, they do not care about the specific chemicals they are finding. They mostly look at the raw NTA data as a fingerprint to determine if food follows safety criteria. There are dozens of applications for NTA. Anyone that has extractable problems related to ecological, human, and public health, I think NTA can be brought to bear to come up with solutions.
- **Katrina Waters:** The partnership and consortium model are great. How are you internalizing this for into EPA now for how would you apply it for ORD?
    - **Jon Sobus:** We are working with the community to create guidelines, run experiments, and running intact to assess performance. We take lessons learned from BP guidance and intact to do our web application. What is unique to the regulatory groups is the semi-quantitative work. It is limited and an area where a lot of critical thinking was done. That is the approach we are using. I was not able to go into the implementation of these methods to look at chemicals in various media.

Session C: NAMs for Ecotoxicological Applications

Approaches and Models for Species Extrapolation

Carlie LaLone, Bioinformatics Scientist, Center for Computational Toxicology and Exposure

Carlie LaLone presented on approaches and models for species extrapolation, and she answered the CSS/HERA Subcommittee members' questions.

- **Richard Di Giulio:** You mentioned the key target protein, is it possible some will have a totally different protein?
    - **Carlie LaLone:** Extrapolation can come from species and the tool can be used in multiple of different ways. We used a different target as a query species.
- **Richard Becker:** How do you address non-specific binding? Can you identify the relative potency and relative effect?
    - **Carlie LaLone:** That is a limitation of the SeqAPASS tool if we do not understand the binding of the chemical. You are identifying the challenges as we move this tool forward.
- **Chris Jennings**: In framework of experimental design of negative and positive controls, how do you validate in a laboratory? If the dose level is a hazard assessment for a single chemical, is the evaluation of mixtures beyond the scope?
    - **Carlie LaLone:** In this example, it tests the level 3 prediction and makes the query species more like a different species. Currently it tests for a "yes" or "no," but the next step is how to probe questions and limitations.
- **Timothy Malloy**: Where do you see this tool being used for decision making? Will it be a screening or problem formulation tool? Who is using it and for what purpose?
    - **Carlie LaLone:** We are working closely with the Endocrine Disruptor Research Program and testing the extrapolation from *in vitro* assays using additional lines of evidence. The program offices added visualization options to identify endangered species. We continue to evolve the tool based on needs from program offices.

Novel in vitro Methods for Ecological Species

Brett Blackwell, Environmental Toxicologist, Center for Computational Toxicology and Exposure

Dr. Brett Blackwell presented on novel *in vitro* methods for ecological species, and he answered the CSS/HERA Subcommittee members' questions.

- **Chris Jennings:** Referencing the slide with water samples, did you characterize the water?
    - **Brett Blackwell:** It was surface water impacted by treated wastewater, environmental waters. We screened for 150 chemicals in that sample. The initial figure included only human receptors for the secondary assay. The human receptor was protective in showing we are not missing activity.
- **Richard Di Giulio:** For those receptors, humans are the best sentinel and the most protective. Is there any *in vivo* work ongoing?

- **Brett Blackwell:** This is empirically testing if the human species is protective of all species. Humans might be adequate to represent other species**.** There are not many *in vivo* studies, although we did some fish exposures *in vitro*.

High Throughput Transcriptomics: A Multi-Species Approach

Kevin Flynn, ORD Biologist, Center for Computational Toxicology and Exposure

Dr. Kevin Flynn presented on high throughput transcriptomics, and he answered the CSS/HERA Subcommittee members' questions.

- **Chris Jennings:** Looking at one chemical at a time, can you look at dose response with mixtures at a ratio of chemical relevant to human exposure? Could you look at values of PFAS relative to other chemicals?
  - **Kevin Flynn:** From an exposure side, we can run mixtures. We could look at this data and utilize this strategy to tease apart the data. Currently, as a whole mixture we consider tire crumb and collect road run off to look at all the components.
- **Richard Becker:** Regarding the discussion about how to look at and integrate benchmark dose, can you elaborate on that and where that research falls? Can you clarify some of the challenges?
  - **Kevin Flynn**: This is an active part of CSS research. There are potentially hundreds of differentially expressed genes (DEGs) and the transcriptomic-based point-of-departure (tPOD) is coming up with a way to integrate the benchmark dose (BMDs) into one value. This can have a substantial impact on the tPOD. Environment Canada looked at estrogenic chemicals in fathead minnows and interpreted a valid number of a tPOD. For instance, only 10 datasets are needed to start making analysis.
  - **Jill. Franzosa:** The CSS program has a funded request for application (RfA) for assessing mixtures on EPA' website.
- **Richard Di Giulio:** How are you measuring behavior in the fish and how did it work as an apical endpoint?
  - **Kevin Flynn:** We are using simple observations but working with artificial intelligence video and using a DanoVision system to look at in the future.
- **Chris Jennings**: What do you mean by behavior, how is that relevant to neurotoxicology?
  - **Kevin Flynn:** We use particle tracking and time spent in water column.

Session D: System-specific Models and Approaches

Respiratory tract models

Shaun McCullough, Principal Investigator, Center for Public Health and Environmental Assessment

Dr. Shaun McCullough presented on *in vitro* respiratory tract models, emphasizing a few points: The current bronchial epithelial cell models provided valuable information but may not

be representative of more complex tissue; a fit-for-purpose multi-cellular models are necessary for accurate and reliable inhalation toxicity testing; human data from environmental inhaled materials is invaluable in lung model development; the bronchial epithelial/stromal co-culture model indicates that trans-epithelial exposure effects on the stroma may exceed direct effects; the outcomes in the ACRE-Mark 1 model align with *in vivo* human data; the ACRE-Mark 2 model is in progress as well as the incorporation of immune cells into the models. Last, he noted that the protocols and methods being developed are designed to be accessible, cost-effective, and compatible with high throughput assays and will be publicly available.

- **Ponisseril Somasundaran:** Does this take into effect lung surfactant? I have worked with surfactant and it was a huge consideration factor in this area. I can send you a paper that allows you to help copy the expanding effect.
    - **Shaun McCullough:** This is one of the limitations and it is not quite there yet. We cannot let perfect be the enemy of the good where this model does work well even without it.
- **Dale Johnson**: Can you elaborate on how you dose the cells? Do you use animal data?
    - **Shaun McCullough:** It depends on the medium, for example you can dose the liquid medium. We have not used animal data yet, as we have controlled human data. We do work with other groups to evaluate this data as well.
- **Jane Rose:** You are probably aware of other lung tissue models. I am curious if you have compared your results to other systems (EpiAirway, etc)?
    - **Shaun McCullough:** The full thickness EpiAirway would be closest to ours, one thing that the models cannot do that our model can do is the dissociation of specific systems, where theirs you would have to lyse all cells, but ours does not have cells in direct contact could be a downside. We noticed that the separation of the cells does not seem to negatively impact the cell systems.

Inhalation models

Mark Higuchi, Inhalation Toxicology Facilities Branch Chief, Center for Public Health and Environmental Assessment

Dr. Mark Higuchi presented the inhalation models and noted that this novel exposure approach transects traditional *in vitro* submerged dosing and *in vivo* inhalation exposures. The work supports EPA program offices' risk assessors by providing NAMs to directly test chemicals of interest in a similar way to *in vivo* inhalation exposure. This data is used by ToxCast and helps develop NAMs for analytical dosimetry in cell cultures to translate to *in vivo* inhalation studies.

- **Ponisseril Somasundaran:** You have tested many chemicals, but I did not see benzopyrene as tested when that is the biggest part of smoke.
    - **Mark Higuchi:** It was not included in this list as a priority.
- **Jane Rose:** Looking at the slide where you compared *in vitro* to *in vivo* exposures, what would be the exposure directly at the cell versus what would be an air concentration external to the lung? What do you think about those differences?

  - **Mark Higuchi:** We do eventually want to expose the cells that Shaun McCullough showed in our system, and we currently have a limited well format and want to increase it later. We keep each cell in a slightly positive condition. If we want to simulate a breathing condition, we could do that. When we use the primary cells and they do have goblet cells and secrete mucus, we must wash the cells before exposure as they secrete so much mucus that it can change our results.
  - **Jane Rose:** The idea would be to translate the *in vitro* air exposure to in the vivo air concentration that would best simulate what that cell would see in a real-life scenario?
  - **Mark Higuchi:** We could set up the same system to set up animal dosing and cell dosing at the same time to assess the difference.
- **Anthony Bahinski:** Looking at the conditions on the cultures, I know in commercial cells there is extrapolation in blank cells. Are you looking into what is deposited onto the cells?
  - **Mark Higuchi:** Aerosolized chemicals are the ones that we are starting with, as vapors and gases are more difficult to work with, we can easily assess how much is deposited onto the cell and we can calculate how much is taken up into the cell. We have 4 replicates and run them across three days, so we have 12 cell wells at each concentration so hopefully we would have the power to see the differences.

Neurovascular Unit Modeling and Blood Brain Barrier Function

Tom Knudsen, Biologist, Center for Computational Toxicology and Exposure

Dr. Tom Knudsen presented how neurovascular units (NVUs) are composed of multiple cell types and over 400 genes, at least 86 of which play important roles in blood-brain barrier (BBB) development and function as well as that the BBB becomes functional soon after it forms during organogenesis (6 to 14 weeks in human gestation). He also noted that development and function is perturbed by multiple pathophysiological conditions and may underlie neurodevelopmental disorders linked to chemical exposure during pregnancy. Lastly, he noted that the dynamics of the system can be modeled *in silico* and *in vitro*, focusing on microglial sensing as potential roles in neurodevelopmental toxicity linked to the activation.

- **Ponisseril Somasundaran:** I am interested in using nanomotion to develop drugs through the blood brain barrier using plasonics when droplets become bubbles and the idea is when the expansion occurs you can move through in the blood. Can your system be used to study that?
  - **Tom Knudsen:** As far as I know we have not considered nanoparticles or nanodroplets in an interaction with the system, but in theory we could be able to. At least in the *in* silico modelling we can make almost anything happen, but we can discuss a potential collaboration with Sid Hunter.

Questions not specific to a certain presentation.

- **Dale Johnson:** How do transporters for the BBB and their development fit into the model?
    - **Tom Knudsen:** From a perspective of phylogeny, when we look at the BBB looking at evolution from octopus, where it first appears, to primates. Some of the first genes you see are transporters, so the first genes I would expect to be involved in the development of the barrier are molecular transporters. As we look at our networks in the evolution of the BBB, then you start seeing a cooptation of signaling types. Much remains unknown. Zebra fish studies have looked at some of these issues, but I can investigate this more.
- **Dale Johnson:** To Shaun McCullough and Mark Higuchi, the analytical measurement of variability between those systems and what is going to happen from an *in vivo* standpoint.
    - **Shaun McCullough:** On our end, what we have done so far is with ozone as an example in a new study. Part of the discussion that we are going to write up is how the data that can be used and how it is incorporated in future areas. We do see different degrees of individual variability, but fortunately we have access to primary bronchial epithelial cells to get different primary cell types from various sources. It gives us the opportunity to do larger studies.
    - **Mark Higuchi:** From the animal side we do know that the biological variability is going to be higher than in our cell lines, so in the end it is going to be what is that actual variability and figuring out what the best replicate set is going to be especially when we get down to measuring doses down at the cellular level.
- **Jane Rose:** For Shaun McCullough and Mark Higuchi, one of the challenges in inhalation toxicology is that all *in vivo* data is in rodent models. We know that there are many differences between rodent and human anatomy and how that impacts toxicology. As you look at verifying or validating your models, are you thinking beyond how to look at animal data and making more human relevant *in vitro* models? I see this as a strength in moving toward *in vitro* models.
    - **Mark Higuchi:** From the animal side, this is where TSCA chemicals are tested. We have data, but we do not know what the doses are for the animal studies. We know what was applied and for how long, but we do not know the uptake.
    - **Shaun McCullough:** We have the ability in our facility to do a direct *in vitro* and *in vivo* comparison in different modalities. We would like your input if you believed it is worth the time and effort making these sorts of studies.
- **Jane Rose:** Looking at the historical data would help before embarking on a potentially expensive clinical study. Also, around looking at and understanding what the *in vivo* animal exposure historical data is saying could be helpful.
    - **Anthony Bahinski:** We run into this in the pharma industry. We could use clinical data or historical data to see how some of these reference chemicals will respond. We know that using some of these animals as a benchmark will not always be helpful or perfectly predictive.

BOSC Subcommittee Discussion and Questions and Answers

Katrina Waters, Chair

- **Katrina Waters:** I would like to start with the committee members that are assigned to CSS Charge Question 1, Anthony Bahinski and Richard Di Giulio. Do you have any questions for the group?
- **Richard Di Giulio:** One theme that kept coming up in our discussions was mixtures. That seems to be an important issue in the ecotoxicology realm, so that would be an important goal for NAMs is to effectively deal with mixtures.
- **Anthony Bahinski:** In discussing NAMs translation and validation, what is the appropriate benchmark, existing human, or animal data? How do we best compare them?
- **Katrina Waters:** What about a holistic approach with your work?
    - **Jeff Frithsen:** A lot of the work that you are seeing is being done on single chemicals. We have a little work on mixtures, but I wanted to take this opportunity to say that we have posted a notice of intent to fund an RfA on looking at the toxicity of chemical mixtures. We recognize that the BOSC is interested in working with mixtures and that we have a regulatory community that looked at single chemicals in the past due to the way legislature is written, but we would like to start moving that way. There are parts of our portfolio that are applicable to mixtures, but we recognize that we could be doing more.
- **Katrina Waters:** Regarding Tony Williams's question on benchmarks for traditional models, what approach are you taking there?
    - **Jeff Frithsen:** We have many approaches and learned from *in vivo* approaches that those approaches are not the gold standard.
    - **Scott Jenkins:** This question was asked in the context of *in vitro* models against *in vivo* models in the inhalation world. We also have controlled human trials.
    - **Rusty Thomas:** The benchmark depends on the endpoint and decision contexts.
- **Rick Becker:** I have a question about confidence and the domain of applicability. In terms of not just chemical structure but potency, there is a tendency when developing these assays to use highly potent substances that are not widely applicable in terms of their targets. We learned that there is a great deal of variability in terms of potency. If we look at industrial chemicals, they are not designed for biological activity, as opposed to pharmaceutical compounds. We have seen that there is activity for the lower potency substances, how is that being considered in the development and testing of NAMs?
    - **Rick Becker:** I will think about writing that into a recommendation then.
    - **Rusty Thomas:** There have been many efforts in ORD to begin to unpack this question. Work by Richard Judsen and looking at the burst phenomenon occurs across different NAMs. What that means physiologically we are still exploring but we certainly see the non-specific effect for these industrial chemicals whereas with pharmaceuticals you tend to see a leftward shift for targets in those same assays.
- **Anthony Bahinski:** Most NAMs are about acute exposure, is there a focus to look at more chronic exposure and toxicity testing?

  - **Jeff Frithsen:** We are not only considering assays, but we are also integrating our virtual tissue work, which can be at a longer period of time. We are using the adverse outcome pathway (AOP) construct to put together a sequence that better puts together the pathways and how a short-term dose leads to a long-term response at an apical endpoint.
  - **Shaun McCullough:** On the inhalation side we are looking at developing assays for acute exposure and looking ahead on how to build out these systems for longer testing in the future, being able to build open the systems we have.
- **James Stevens:** Something that concerns me about NAMs is that we assume human cells in systems will be more predictive of human biological outcomes instead of traditional animal data. We tested this a few years ago using transcriptomic data trying to see what looks more like a rat liver – a mouse liver or rat hepatocytes in culture, and it was the mouse liver. As we move forward into the decision context of not just prioritizing TSCA chemicals but feeding AOPs that would be used for more formal risk assessments, and all the presentations we saw in session A were talking about feeding an AOP as part of the scheme. How do we address the question of is the biology of the system appropriate to decide risks?
  - **Rusty Thomas:** This is certainly a research question that is being actively explored. Looking at concordance between animal and human models in pharmaceuticals, a positive predictive value is difficulty in many cases, a negative predictive value is easier to see. Until you know more of the mechanistic information it is harder. From a human risk assessment, where do you see the lack of a response? Using that to determine a mechanism of action for an AOP that the genomic data could identify.
- **James Stevens:** The negative predictive value can be more useful as a system but may be overly conservative and be triggered more easily. Being negative is more confident. Is this a strategy being used for more formal risk assessments?
  - **Rusty Thomas:** I agree that using the absence of an effect is a more confident answer. For many industrial chemicals they are nonspecific and trying to do this for those chemicals will be more difficult. Pursuing this from a practical standpoint is difficult. Some specific chemicals may be easier to start with.
- **Dale Johnson:** The concept that some people that are more susceptible to certain toxicities comes up in cell line studies. Can you do a prediction on who would be more susceptible to certain toxicities? Is there a way to look at groups that could be more susceptible in *in vitro* models?
  - **John Wambaugh:** For example, we do not eat grapefruit with certain medications as the enzymes cause issues, so something similar could be at play here. I know Barbara Wetmore has investigated this in toxicokinetics.
  - **Barbara Wetmore:** What we were able to do in simulations was to incorporate lifestage-specific levels in the systems and overlay lifestage-specific information and how it works into the effects.

  - **Tom Knudsen:** We have some of these factors in our *in silico* models and through them we can look at interactions with other polymorphisms and pathways that are involved.
  - **Josh Harrill:** We have a Tox21 project underway investigating a difference of cell lines in the study.
  - **Shaun McCullough:** With our larger donor group we have looked at the relationship between polymorphisms, we do have a limitation in the allelic frequency in the human population sample size. Also, specifically histone modifications.
  - **Ponisseril Somasundaran:** Mixtures effects depend on the sequence as well.
- **Chris Gennings:** Do you think about how you can prove that what we have done is actually correct with respect to NAMs? I would like to see the EPA get more involved in the Human Health Exposure Analysis Resource (HHEAR) consortium and do studies on real human samples and link it to specific outcomes.
  - **Jon Sobus:** We spoke with groups like HHEAR and are aware of this work and this data. We are also aware of the lack of collaboration between these similar groups. We could improve the collaboration and inclusion.
  - **Jeff Frithsen:** This is part of the feedback on the areas that we are looking into. Some of the groups that have been brought up have also looked at trying to link exposure data to medical data. Another way of bringing things together and something that we are learning from the assays and modeling approaches.

Dr. Katrina Waters and Dr. Jeff Frithsen thanked everyone for their time and having a productive meeting. They both shared how learning about everyone's work was engaging and look forward continuing the disuccion.

Adjourn

The meeting adjourned at 5:00 p.m., Eastern Time.

Wednesday, February 3, 2021

Welcome – Day 2

The meeting reconvened at approximately 12:00 p.m., Eastern Time.

Public Comments

Tom Tracy, Designated Federal Officer, Office of Science Advisor, Policy, and Engagement

No public comment

BOSC Subcommittee Chair Opening Remarks

Katrina Waters, Chair

Dr. Katrina Waters said the focus of today's meetings is CSS Sessions 2 and 3 with corresponding Charge Question 2 and 3. No public comment. Some committee members are out

due to their teaching commitments. There is not a lot of work time today and committee members can comment on any question and should not feel constrained to any one question.

New Approach Methodologies Applications Introduction with Charge Question

Jeff Frithsen, National Program Director, Chemical Safety for Sustainability Research Program

Dr. Jeff Frithsen explained how the focus of Session 2 is on four models that follow the model where partner needs drive partner needs and those drive what products drive along the way. They show case studies of developed work – work that's done for various partners and stakeholders.

Dr. Frithsen asked for clarification on terms (partners and stakeholders) in CSS they use partners for those within the Agency often co-authors on publications. Stakeholders are those that EPA serves (states and tribal communities) some of those entities sometimes become partners. In active communication with tribal communities to ensure EPA ORD's work and research are relevant.

Office of Chemical Safety and Pollution Prevention-Toxic Substances Control Act Inventory: Prioritization Proof of Concept

Richard Judson, Bioinformatician, Center for Computational Toxicology and Exposure

Dr. Frtihsen introduced Dr. Richard Judson to discuss the CSS program's work on proof-of-concepts for chemical prioritization. Dr. Richard Judson explained how many organizations, stakeholders, and partners share the problem of having tens of thousands of chemicals, which then need to be prioritized. He discussed prioritization and pre-prioritization of chemicals for detailed assessments. He then described how under the Lautenberg Act, 2016 Amendment to TSCA, EPA must establish a risk-based process to determine which chemicals to prioritize for assessment, identifying them as "high" or low" priority substances.

Dr. Richard discussed how the Proteomic Identification of Cleavage Site Specificity (PICS) approach was developed to better understand the landscape of publicly available information for large numbers of chemical substances. It combines results from domain-specific workflows that reflect the overall degree of potential concern related to human health and the environment with the amount of relevant information. It is intended to focus expert review on substances that may have a greater potential for selection as high- or low-priority candidates. The proof-of-concept case study demonstrated that the PICS approach generally resulted in higher metrics for the high-priority candidates as compared to the low-priority candidates and identified areas for potential information gathering.

- **Katrina Waters:** What does it mean that there is a report coming out and reviewer comments? Is that your team?
    - **Richard Judson:** There is also an external peer review process going on.
    - **Jeff Frithsen:** This was a contractor coordinated peer reviewer. When EPA does these things, we provide a list of needed expertise to the contractor who will then look at those potential peer reviewers. Others could make suggestions, but ultimately it is the contractor who does these peer reviews.

- **Jennifer McPartland:** How broadly is EPA going out into the literature?
    - **Richard Judson:** The contractor is gathering literature. In general, we are restricting our search and not doing an open literature search. The exception would be per- and polyfluoroalkyl substances (PFAS), for which we are doing a full chemical search.
- **Chris Gennings:** When you create an overall scientific domain metric, would you consider mixture assessment map when prioritizing chemicals?
    - **Richard Judson:** We will consider doing so.

Developmental Neurotoxicity *in vitro* Battery as an Alternative to Developmental Neurotoxicity *in vivo* Guideline Studies Used by Office of Pesticide Programs

Tim Shafer, Research Toxicologist, Center for Computational Toxicology and Exposure

Dr. Tim Shafter presented EPA's work with developmental neurotoxicity *in vitro* battery as an alternative to developmental neurotoxicity *in vivo* guideline studies, and he answered CSS/HERA Subcommittee members' questions.

- **James Stevens:** I noticed mention of network formation assay on slide 19. There seems to be a shift to the left of increased sensitivity. Do you have any preliminary information on the endpoints given that it is an emerging issue with biological systems?
    - **Tim Shafer:** The processes for what is being evaluated in these assays is not what is being assessed in Toxcast.
- **Jennifer McPartland:** Can you please speak to metabolic components of this assay?
    - **Tim Shafer:** We do not know what metabolic capabilities these cells have. We are looking at making more refined assays to provide more information.

Chemicals of Emerging Concern: A Prioritization Case Study with Minnesota Department of Health

Kristin Isaacs, Research Physical Scientist, Center for Computational Toxicology and Exposure

Dr. Kristin Isaacs discussed CCTE's collaboration with the MDH to use new chemical data generated from scientific approaches to prioritize chemicals for further evaluation and inform risk assessment. Dr. Isaacs described how CCTE and MDH finalized a formal Cooperative Research and Development Agreement (CRADA) in 2019, which has a goal of addressing up to five MDH chemical evaluation activities. Dr. Isaacs explained how MDH works with partners and the public to identify contaminants of interest in drinking water through its Contaminants of Emerging Concern (CEC) initiative. This workflow allowed MDH health scientists to accelerate exposure screening evaluations, freeing resources to complete the more complex aspects of exposure assessment.

Dr. Isaacs described how large libraries of chemicals relevant to MDH can be rapidly screened to identify and prioritize new potential nominees. The implemented workflow has formed a basis for exposure screening under another MDH regulatory program, the Toxic Free Kids Initiative.

Finally, Dr. Isaacs discussed how MDH is concurrently developing screening algorithms in collaboration with ORD.

- **James Steven:** Can you expand on how you plan to incorporate your work with exposure potentials? How much overlap do you expect that there would be with scientific domains? Would those processes be complimentary or separate?
    - **Kristin Isaacs:** We call it exposure because we cannot quantify this, but we are taking the numbers provided by the consensus models for the average numbers of milligrams combined with *in vivo* or *in vitro* exposure.
- **Donna Vorhees:** What is the quality of data sets assessed in selecting what to incorporate into models?
    - **Kristin Isaacs:** We have not had conversations about that with other states. We consider screening level data quality that could be tiered. I believe there are public and private data sources.

Application of New Approach Methodologies and Adverse Outcome Pathways to Surface Water Surveillance and Monitoring in the Great Lakes (EPA Region 5) and a Western River (EPA Region 8)

Dan Villeneuve, Research Toxicologist, Center for Computational Toxicology and Exposure

Dr. Dan Villeneuve discussed the application of NAMs and AOPs and EPA Region 5 and the Western River.

- **James Steven:** When do you use AOP scores? How do AOPs have an impact on risk assessment decisions?
    - **Dan Villeneuve:** We consider the actual prioritization and then adjust for the relative potency of chemicals. The AOP part at this point is largely qualitative, and the AOP helps connect to potential effects.
- **Chris Gennings:** How close was EPA to a value of concern? Are you getting push back from the community?
    - **Dan Villeneuve:** In many cases, we do not have a benchmark or a reference chemical.
- **James Stevens:** What was the reason for selecting the 67 chemicals?
    - **Dan Villeneuve:** The 67 chemicals aligned with the chemical schedule that would fit with the schedule and budget and monitoring activity. USGS has a wastewater list. It was cost effective enough to perform on over 700 chemicals.

BOSC Subcommittee Discussion and Questions and Answers

Katrina Waters, Chair

- **Rick Becker:** With the AOP concept, where does the Agency go next?
    - **Dan Villeneuve:** If you have well identified referenced chemicals and dose response pathways, you can use that information to establish a chemical agnostic

response. The chemicals act additively along that pathway and have similar shapes of dose response curves.
    - **Rick Becker:** How long does it take to get from one key event to the next key event? You do not have to start with the MIE, and the challenge is the middle key events.
- **James Stevens:** Regarding quantitative AOPs, what is a variable that one could use to conduct a quantitative assessment? I am going to examine a collection of genes.
    - **Dan Villeneuve**: There is synergy and potential transcripdomic with measures of apical outcomes to find the space where those are predictive.
- **James Stevens:** What is the biology that determines the point of departure?
    - **Richard Judson:** We find not just points of departure, but what is the biology that determines the point of departure.
    - **James Stevens:** Regarding preservation of evolutionary biology, data from *in vitro* studies we believe are preserved in *in vivo* and human data.
    - **Richard Judson:** We have a large zebra fish data set, and we are looking at the AOPs that are triggered. Many developmental pathways are conserved in zebra fish and humans. If a chemical is very potent there, it will be potent in the zebra fish assay. We have approximately 800 chemicals on both sides.
    - **James Stevens:** I have concerns over calling qualitative data versus quantitative data.
- **Katrina Waters:** It was unclear how the case study was now being used by OCSPP. How are you dealing with the 'messiness' of data within ORD? Do you have an internal repository to clean up and save datasets?
    - **Richard Judson**: The reason why this question cannot be addressed at this time and is not being taken up is complicated.
- **Donna Vorhees:** There was discussion earlier about the scoring and confidence. My question is where could one go to read more about the confidence scoring parameters?
    - **Richard Judson:** Currently is an internal report, that EPA hopes to make public in 2022. It will go public.
    - **Jeff Frithsen:** the engagement with the office has been intense and it has lagged because their primary focus is on. It is one high priority chemical completed and another gets put onto the list. They are typically working on approximately 20 chemicals, and 50 percent of those chemicals comes from the list.
    - **James Stevens:** The presentations touched on the mixture issues we discussed in previous reviews. Is there any hope to come up with standardized mixtures to design those mixtures to allow some of the NAMs to be shifted from standardized?
    - **Dan Villeneuve:** We have characterized mixtures across the NAMs approaches.
- **Donna Vorhees:** What about the confidence in data?

- **Richard Judson:** We are within an order of magnitude.We could imagine cutting this up into nine boxes. How confident are you that Chemical X is in box nine?
- **James Stevens:** Could we think about shifting the boxes?
- **Richard Judson:** We took several quantitative levels, and it was a policy decision.
- **John Wambaugh:** We can be uncertain about the exact dose of uncertainty.
- **James Stevens:** As an example, what is your confidence of being right or wrong in a particular bin of chemicals?

New Approach Methodologies Tools Demo Intro with Charge Question

Jeff Frithsen, National Program Director, Chemical Safety for Sustainability Research Program

Dr. Frithsen focused on the efforts of translation and delivery, including how the CSS program reserves data for others to use. He explained how it is important to match tools to partners' needs and EPA's needs. The fall 2021 BOSC CSS/HERA Subcommittee meeting will highlight the tools, synthesis, and informatics. He emphasized that these tools are representative and not the complete set. Dr. Frithsen emphasized the goal of CSS Charge Question 3, which is to address how to improve these products.

CompTox Chemicals Dashboard

Tony Williams, Chemist, Center for Computational Toxicology and Exposure

Dr. Tony Williams provided an overview of the CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard), which is a publicly available tool containing over 883,000 chemical substances. He discussed the curation efforts to work collaboratively with other agencies' websites and public data resources. He highlighted the integration of multiple EPA databases and other sources. Dr. Williams demonstrated the CompTox Chemicals Dashboard, including the executive summary, associated literature, and a summary of available toxicity and safety data. He highlighted how the integration of the data sources compiles all the data from throughout the Agency.

Dr. Williams discussed mapping and filtering the relationships to similar chemicals, and he demonstrated how to evaluate related data and where the chemical resides in a mixture. He showcased the list mode, which is the active inventory for various lists, demonstrating the complexities of substances of unknown or variable composition, complex reaction products, or biological materials (UVCBs) and how most do not have structures. He explained the need for constant curation, including the new TSCA inventory will require the addition of 130 new chemicals.

Lastly, Dr. Williams demonstrated the Dashboard search function by Product and Use Categories, and the ability to download the data. He emphasized that data aggregation for all data sources continues abated, and search capabilities continue to expand release-to-release. The current application is being rearchitected to also develop a public Application Programming Interface (API).

- **Katrina Waters:** I appreciate the constant curation and recognized need to re-architecture. The public APIs will help others to continue to link to the data.
    - **Tony Williams:** We are hoping for alpha-release in September 2021. We are committed to have a public API to harvest data.
- **Jane Rose:** What percentage of the 883,00 chemicals have been curated? As we think about enormity of data and the number of customers as the new CompTox Chemical Dashboard launches, how will you communicate and disseminate this tool to customers?
    - **Tony Williams:** There is a way to see the curation levels via the link to a publication on the CompTox Chemical Dashboard. We have a pesticide list with 4,000 chemicals, but this took over one year to assess a few hundred chemicals. In preparation for the major revamp, we will have a full manual, video set of the sites, and training materials. For further details regarding how we curate and the history of the DSSTox database, visit: https://www.sciencedirect.com/science/article/pii/S2468111319300234.

Sequence Alignment to Predict Across Species Susceptibility

Carlie LaLone, Bioinformaticist, Center for Computational Toxicology and Exposure

Dr. LaLone discussed the Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool and its components. She described how the tool compiles the knowledge of a known sensitive species and the chemical protein interaction. Dr. Lalone described the tool's evolution and the addition of interoperability and data visualization. She demonstrated the tool's user guides, information tabs, and query function, which requires entering a protein of interest. She showcased the reports' function and ability to link to the National Center for Biotechnology Information (NCBI) and the ECOTOX databases. She discussed the susceptibility similarity and ortholog candidates. She also described the endangered species feature. Lastly, Dr. Lalone demonstrated the customization of data visualization and ability to include plots in the downloaded summary report.

Factotum: Curation of Exposure-Relevant Public Data

Kristin Isaacs, Research Physical Scientist, Center for Computational Toxicology and Exposure

Dr. Isaacs demonstrated Factotum, factorum.epa.gov, an internal data management system to support exposure assessment. She explained the statistics for the studying of consumer products, and ran through a demonstration on the chemical, formaldehyde.

Dr. Isaacs demonstrated the tools for the consumer personal care products and discussed the data providence and the audit log features. She explained that Factotum can be used to look other chemicals in the personal care category. She discussed the challenge of curation of individual products and provided an example of paint category.

Dr. Isaacs discussed the refinement to the models, curation of data in differential exposure, and integration of exposure data streams and chemical release information to implement the search

and report capabilities. She explained how internal partners can use the data, but the tool is not publicly available.

- **Katrina Waters:** Will Factotum be publicly available or pushed through the Dashboard for public release? Will it be available to the partners but maybe not stakeholders?
    - **Kristin Isaacs:** It was developed as an outward facing, but that requires substantial effort. The public APIs will allow for data to be pulled through public API.
    - **Jeff Frithsen:** When we are confident of data quality it will be pushed out publicly; for now, it serves for internal use only.
- **Jane Rose:** The material safety data sheet shown for formaldehyde is from 2007. Are there plans to update the historical data? I can share a database with updated information.
    - **Kristin Isaacs**: We have active curators and can filter on the dates. We have considered using the historical data to examine the longitudinal progression of how chemical formulations have changed.
- **Katrina Waters:** What is the volume of data to make Factotum comprehensive? How and when will you get there?
    - **Kristin Isaacs:** We are currently focusing on the needs of the stakeholders, which is now occupational exposure. It becomes a matter of priorities.
    - **Jeff Frithsen:** EPA is responsive to what industry is doing. Perhaps you can let us know how EPA could respond to industry to make these products better.
    - **Jane Rose:** There are other places where this information already exists. PNG uses Smart Label to divulge ingredients in our products. Now industry is required to provide this information. EPA could benefit from access to this data.

BOSC Subcommittee Discussion and Questions and Answers

Katrina Waters, Chair

- **James Stevens:** General questions to all presenters with respect to the collection of user information: What do the users access? What information do they use? How often do they use it?
    - **Carlie LaLone:** For SeqAPASS, we use Google Analytics and can get that information from the users. We can obtain the number of users and what type of evaluations they are using. We also have a collection of data from the user when they sign up to use the tool.
    - **Tony Williams:** We also use Google Analytics, but can improve our collection of metrics. We do collect statistics but difficult to know where they are coming from. We also have a feedback form and address the comments received.
- **James Stevens:** A barrier to complex systems is how to train users. What are some models to get better uptake?

  - **Tony Williams:** Lots of training and engagement. We are trying to move to train the trainer and have key people at each engagement. The planning for the re-architecture includes making applications more user focused. The Dashboard will expand complexity but will make it more user-friendly.
  - **Carlie LaLone:** We value the edification of champions. We are collaborating with partners to provide case examples to bring in other groups. The feedback is valuable to integrate the components the users would prefer to see.
  - **Tony Williams:** We have built-in inherent flexibility so we can add the requests identified at meetings. These actions have increased the number of users by fixing and changing components.
  - **Jeff Frithsen:** We serve such a variety of partners, it is ongoing. Sometimes we react to what partners are requesting, sometimes we are teaching our partners what we have developed. For example, for SeqAPASS there was an office that was so interested that the partner provided a post-doc and funding to learn the tool.
  - **James Stevens:** The process is often more challenging that the development of the technical piece since there is more focus on small number of key people. We can highlight this in the report.
- **Chris Gennings:** Is it possible to link to use patterns for a risk assessment? What about co-exposure with a set of products?
  - **Kristin Isaacs:** Yes, high throughput and mid-tier exposure models are directly linked to the data. The product use category links to the data on the consumer use patterns and inhalation algorithms. From the Chemical and Products Database (CPDat), we know what products the chemical is in, and we make the data available to link to other ontologies. This tool also allows co-exposures with product sets.
- **Katrina Waters:** With recent discussions involving government and export control, are there internal conversations about publicly available data and sharing of the information as well as benefits of dataset integration?
  - **Jeff Frithsen:** There are conversation with the European Chemicals Agency (ECHA), the European Food Safety Authority (EFSA), and Health Canada to exchange information. There are private and publicly available data involving confidential business information (CBI) laws and navigating the process. It is a question of what we can learn from sharing public data and if there more to learn from sharing CBI data.
  - **Tony Williams:** We discussed the internal and external side of the tool. The interoperability must be protected. We also discussed integration of CBI data.
- **Juan Colberg:** International cooperation is increasing the value of models. Is there global coordination, such as with the International Council for Harmonization of

Technical Requirements for Pharmaceuticals for Human Use (ICH) for drugs, and an effort to create the form for data for foreign languages? How can we use global integration on reporting data moving forward?

  - **Jeff Frithsen:** That is a good comment, and the CSS program welcomes the CSS/HERA Subcommittee's comments. OECD considers data sharing for specific data. These data tools are time and resource intensive. The amount of effort to compile and curate this amount of data should not be underestimated.

- **Katrina Waters**: What is the security protocol for these databases?
  - **Jeff Frithsen:** We deal with the EPA security construct and the review the process, external and internal to ORD.
  - **Tony Williams:** EPA conducts a thorough process of the entire software scan to identify vulnerabilities and appropriate firewalls. This is appropriate but can slow down the cycle.

Adjourn

Mr. Tracy reminded everyone to utilize the track changes feature when working with the documents. The meeting adjourned at 5:40 p.m., Eastern Time.

Thursday, February 4, 2021

Welcome – Day 3

The meeting reconvened at approximately 12:00 p.m., Eastern Time.

BOSC Subcommittee Chair Opening Remarks

Katrina Waters, Chair

Dr. Waters opened the meeting and noted that committee members can address any of the questions. She asked members to please go into the template and provide feedback for the sessions they participated in. She reminded members that presentations would be followed by question and answer sessions.

Connecting Assessment Needs to Health and Environmental Risk Assessment Research

Samantha Jones, National Program Director, Health and Environmental Risk Assessment Research Program

Dr. Jones explained how the HERA program staff are excited to present their work and research to the CSS/HERA Subcommittee. She then introduced Dr. Wayne Cascio.

Office of Research and Development (ORD) Implementation

Wayne Cascio, Center Director, Center for Public Health and Environmental Assessment

Dr. Wayne Cascio provided an overview of the HERA program and reminded participants that the HERA StRAP had been posted for fiscal years 2019-2022. Dr. Cascio communicated to those

in attendance that ORD conducts research that informs the Agency's priorities. When considering the priorities of the Agency, ORD not only addresses the problems and concerns of today, but also looks forward and conducts anticipatory research.

Dr. Casio further discussed how ORD has 4 research centers: CPHEA, CCTE, CEMM, and CESER. CSS and HERA serve many similar clients but use different approaches and support these research centers in different ways. He emphasized that it is important that the groups work in a coordinated effort. CPHEA provides administrative and personnel support for HERA, with CPAD and HEEAD being the two divisions they most closely support. These staff primarily conduct ISAs, IRIS assessments, and maintain the HERO database. Dr. Cascio closed by noting that they look forward to presenting their work.

Connecting Assessment Needs to Health and Environmental Risk Assessment Research

Beth Owens, Title, PANPD, Health and Environmental Risk Assessment (HERA)

Dr. Beth Owens presented on connecting assessment needs to HERO Research. HERA is split into two topic areas: Science Assessments and Translation and Advancing Science and Practice of Risk Assessment. Dr. Owens focused on discussion of the second topic area. The two areas are closely related and influence each other. Referring to the HERA StRAP, Dr. Owens stated they are looking for ways to implement the goals, needs and priorities of the program. To deliver the high-quality assessments, Dr. Owens suggested the need to continue addressing data gaps and identifying new tools. She emphasized that training and tools directly feed back into the assessments.

Dr. Owens stated that specific projects were aligned with desired strategic outputs. She mentioned that the provided appendix was updated to reflect what products have been collected and delivered to their stakeholders. Dr. Owens continued by noting a large and ambitious list of products to be created to meet the outputs. She highlighted ongoing partner engagement within the HERA program and discussed how product level partnerships have been critical to the implementation of the plans to meet the identified needs. This makes sure that the products are meeting quality, usability, and timeliness standards.

Focus areas for these presentations will be in 3.1 (NAMs), 3.4 (Systematic Review), 3.5 (Dose Response), and 4.1 (Suite of Software Tools)

- **Rick Becker:** What about improvements to the MMPD model? Are there specific areas that will be highlighted?
    - **Beth Owens:** Annie Jarabek will be presenting on that topic tomorrow.

BOSC Subcommittee Discussion and Questions and Answers

Katrina Waters, Chair

Dr. Waters began by reviewing the HERA charge questions and offering time for introductory questions about these charge questions.

- **Donna Vorhees:** On HERA Charge Question 1, I am interpreting this as advice on how to advance the science but also the use of these methodologies in HERA assessments, is that correct?
    - **Samantha Jones:** Yes, that is correct.
    - **Beth Owens:** I agree, it was to understand how we best bring these NAMs data streams and approaches into our portfolio to use them to inform our assessments.
- **Chris Gennings:** Based on your descriptions of the program, how did you come up with the charge questions? They are very specific about the program and is that what you want as opposed to a more general look at human health risk assessment and how you are approaching it?
    - **Beth Owens:** We wanted to focus this BOSC meeting on specific areas of our program but in the fall meeting we intend on addressing other areas and to focus more on our training portfolio.
    - **Samantha Jones:** When we were planning for this meeting, we looked at discussions that were had when we were developing the StRAP. We considered how to group the topics and coordinate with CSS to match topics. The nature of the HERA program is that it is almost "split." The program was a feedback mechanism where we put forward the assessments that go through many review bodies, which results in a very complex group of topics. We felt it was most natural and helpful to focus on here.
    - **Chris Gennings:** I am gathering that some of the general comments would be reflected in more general parts of the document as opposed to specific questions.
    - **Samantha Jones**: Anything more general that does not fit in a charge question can go in the more general notes portions.
- **Rick Becker:** The Research Area Coordinating Teams (RACT), do each of the projects that you are presenting have a specific team, or maybe one team with several projects? Is that hardwired into all projects or is that more dependent on the specific projects?
    - **Beth Owens:** There are two RACTs, one that focuses on Area 3 and one for Area 4. We recognize that this is a diverse area of research in Area 3, so that RACT split into smaller teams to get into the specifics of some projects. They come back to the larger group to report back.
- **Rick Becker:** I am intrigued by that and one of our previous comments of how to connect with the end users. It seems like this is a way to get at that. Thinking ahead, are there other research areas that could benefit from an implementation of this team approach?
    - **Beth Owens:** We did not make one for Area 1 as that already has such a structured relationship with partners. We actively engage with them throughout the assessment. In Area 2, since those requests come directly from another partner, it is inherent that this collaboration will happen.

  - **Samantha Jones:** I agree that the RACT's intentions are already in place in Research Area 1 and Research Area 2. While it is not named, these same procedures are in fact in place.
  - **Bruce Rodan:** From a higher level, we have similar groups across ORD and these vehicles are meant to be a crucible for discussion and connection between the program offices and regions and the ORD. These are just two specific examples and they have continued to develop and will continue to be used into the implementation phase and future planning phases.
- **Rick Becker:** These are a great addition to the program and addresses comments that the BOSC has made in the past to connect with the "users" of the work. Are the RACTs are comprised of internal EPA partners?
  - **Bruce Rodan**: There are many state representatives. Both sides of the ORD matrix and more are involved in the state pilots.
  - **Rick Becker:** We heard a program about the CRETAs yesterday. Are these the same?
  - **Bruce Rodan:** No, the CRETAs are different. This gets more into discussing sensitive topics that are kept behind the "firewall" of EPA. Each state pilot had to get approved by their elected representative so they could be brought in under the legal umbrella.
- **Dale Johnson:** Are mixtures risk assessments going to be addressed in these questions?
  - **Samantha Jones:** There will be some discussion in the first presentation as one presentation does touch on mixtures, but mixtures will be covered more in detail in later meetings. Future StRAP cycles will also discuss mixtures.

Applying New Approach Methodologies to Inform Health and Environmental Risk Assessment Research Program Assessments with Charge Question

Luci Lizarraga, Chemist, Center for Public Health and Environmental Assessment

Dr. Luci Lizarraga started her presentation by providing an overview of how to advance, translate and build confidence in the application of NAMs and data in risk assessment. Dr. Lizarraga explained that a fit-for-purpose approach is being proposed to integrate NAMs into HERA Assessments. Chemicals with limited data ("data-poor chemicals") would use NAMs to drive data, and data-rich chemicals would use NAMs to fill specific gaps. Specific case studies would be developed to demonstrate their use and increase confidence and reliability in NAMs.

Dr. Lizarraga highlighted an example of using NAMs to inform Hazard Conclusions – ToxCast/Tox21 mechanistic data from assays used in an AOP setting to determine potential health effects. The proposed research products fill gaps in toxicokinetic and toxicodynamic data. Broadly, these projects will show where the product is and discuss the future utility and progress to be conducted.

Dr. Lizarraga then spoke about an integrated approach to human health assessments. The approach begins by identifying a chemical of interest and continues with a thorough and independent systematic review to determine if there is enough *in vivo* data to decide. If there is not enough data, NAMs could be used to fill the gaps. Any outputs are evaluated, and end user feedback is captured.

Dr. Lizarraga concluded by noting that the output objectives and proposed products are consistent with broader NAM efforts to reduce mammalian use in health assessments, that the research is tethered to assessment products and technical support efforts within HERA, and that the research coordinates with other National Research programs and constantly seeks partner engagement.

- **Gina Solomon:** That presentation was very helpful, and this research is exciting. I noticed that you talked about using gene expression changes and that stuck out to me as it seems that is a little slower to develop than on the CSS side. Could you talk about any data there and the work or examples?
    - **Luci Lizarraga:** This builds upon previous work that showed concordance between gene expression changes and apical endpoints. For those efforts *in vivo* data was used, but for this work we are bringing in *in vitro* transcriptomics and more as well and how to use this information to inform mixtures risk assessments.
- **Chris Gennings:** When you had the slide of linking PFAS with liver toxicity, was that a representative chemical used there or was it multiple PFAS chemicals?
    - **Luci Lizarraga:** The HERA program is reviewing five high priority PFAS chemicals. Some of that work it looked across that group, but in the assessment, the data for specific chemicals is summarized in the supporting materials.
- **Gina Solomon:** The PFAS case study was great, are there more case studies in the upcoming presentations?
    - **Luci Lizarraga:** There are other case studies throughout the presentations.
- **Ponisseril Somasundaran:** I appreciate the difficulty of data poor and data rich chemical concerns, especially when a chemical is present in a mixture. I know it is difficult, but how do you determine when something is data rich or poor when mixtures are present, especially regarding neurotoxicity?
    - **Samantha Jones:** It is a relative classification. As we move into NAMs, how can you build up the data for a chemical?

Advancing Read-Across in the Health and Environmental Risk Assessment Research Program

Luci Lizarraga, Toxicologist, Center for Public Health and Environmental Assessment

Dr. Luci Lizarraga presented on Review Provisional Peer-Reviewed Toxicity Values (PPRTVs) and read-across methodology. PPRTVs evaluate data related to sub-chronic and chronic exposure for a chemical of interest through oral and inhalation routes, for both cancer and non-

cancer health endpoints. Over 400 chemicals have been assessed in the PPRTV program, covering a large range of chemicals and a large spectrum of available data. Read-across methodologies are used to fill data gaps for a target chemical by using analogues that are considered similar by scientific justification.

Dr. Lizarraga explained that the structural similarity metrics currently used to identify and rank analogues have inherent limitations. Therefore, additional software tools and expert judgement are need during the analogue search process. Case studies demonstrate the need to identify structural, toxicokinetic, and toxicodynamic similarities to identify and evaluate the suitability of analogues for quantitative read-across. Dr. Lizarraga emphasized that NAM data streams such as *in vitro* or *in silico* metabolism predictions are necessary to fill in knowledge gaps.

Dr. Lizarraga continued with a case study on n-heptanol, a published example of the application of read-across methodologies. Three structure analogues were identified that had the inhalation values of interest and shared a similar modality. The analogues were evaluated for similarities in metabolism and excretion pathways. Effects were similar across the analogues, but potencies varied. Therefore, toxicokinetic data was excluded. Dr. Lizarraga then discussed limitations in more detail, focusing on that a combination of software and expert interpretation is necessary to determine proper analogues.

Dr. Lizarraga concluded by noting that read-across is routinely used for hazard assessment and deriving toxicity values within the PPRTV program, that a revised methodology is proposed based on practical experience and advances in the fields of NAMs and particularly read-across, and that there is an opportunity to expand the scope of read across applications to support HERA-related products.

- **Gina Solomon:** It is great to see the progress in using NAMs for PPRTVs as this group made that recommendation a few years ago. In slide 11 you noted how NAMs could be brought in, but what about bringing in the data from other NAMs for more than metabolic predictions? Is that a logical next step?
    - **Luci Lizarraga:** The framework has a lot of flexibility to incorporate different NAMs based on the needs of that chemical assessment. I noted how structure is not the best way to do this. We noted using some biological properties of the chemicals to compare the information.
    - **Gina Solomon:** Is there an easy mechanism to go back to CSS to get more data or try different tools? If so, did it work?
    - **Luci Lizarraga:** In some case studies we realized we did not have enough data and generated *in vitro* data to help augment the data we had. It did work in some cases, but some are noted for later decisions.
- **Rick Becker:** You mentioned the approach for using read across for the potential to identify chemicals with carcinogenic action. Oncologic is a system that does this, is that what was used here?

  - **Luci Lizarraga:** The workflow presented was to outline some databases that we wanted to tap into to identify analogues.
  - **Grace Patlewicz:** The tool is being updated and it could be integrated later.
- **Donna Vorhees:** In terms of the needs for further tools, are you saying ones that you need to develop or ones you need to incorporate?
  - **Luci Lizarraga:** Both. We need to develop some more tools and incorporate more tools. We do not have a specific need, but we do always look for more information.

Filling Metabolism Data Gaps in Read-Across

Grace Patlewicz, Chemist,Center for Computational Toxicology and Exposure

Dr. Grace Patlewicz reviewed backgrounds of read across, outlined the ongoing issues of read-across, and identified uncertainties while still identifying ways to reduce that uncertainty. She explained how this project is conducted to investigate the concordance of *in vivo*, *in vitro*, and *in silico* information and how can it be used to assess metabolic similarity for read-across. These tools are freely and commercially available but there are not many studies that have compared the tools. The goal was to take 37 proof-of-concept substances from ToxCast library, generate *in silico* values for these substances, and extract metabolites for these substances.

Dr. Patlewicz reviewed the selected tools and other procedures conducted during this project including that a literature review was conducted. Identified all known metabolites of the 37 chemicals, and then registering the metabolites in EPA's DSSTox to assign specific register numbers. Then had to account for structural isomer as there were 20 isomers reported in the literature.

Dr. Patlewicz then reviewed coverage referring to how well model A matches model B predictions as well as sensitivity and precision. Looking at the relative coverage of the models. It showed that there was significant overlap between ToolBox and TIMEs models and to ensure the proper coverage, we need to use a battery of different tools. It is important to note that no one tool was providing the best information or all information. Comparing the performance relative to the literature data, need to review the tools to see what the best options are. The values are pretty low but that is not a surprise as they were probably looking at that metabolite not just any transformation of a substance.

Dr. Patlewicz concluded by noting that metabolic similarity is an important component in evaluating analogue suitability within read-across and that approaches to characterize and quantify metabolic similarity is needed. She also described how a proof-of-concept study compares different metabolism information sources to evaluate their utility for read-across. She also noted that specific *in vitro* data has been generated and that predictions have been generated using a selection of *in silico* tools and that experimental data is extracted from literature.

- **Jennifer McPartland:** Do these models account for genetic polymorphisms across the population?

    - **Grace Patlewicz:** No, they are not that specific. You may get some sort of confidence score that tells you how likely that transformation is going to happen but no it does not extend further than that.
- **Jennifer McPartland:** The *in vitro* data you were referring to for *in vitro* metabolites, is that data that CSS is developing?
    - **Grace Patlewicz:** Yes, that is part of the effort that John Wambaugh works on.
- **James Stevens:** Since this is under the HERA session, what is the most important output of the metabolism prediction NAM methodology? What are you thinking you need most from this sort of approach?
    - **Grace Patlewicz:** From my work under HERA and CSS is that I am looking for better ways for how we can determine similarities that drive and inform predictions of toxicity and how do we determine relevant analogues.
    - **Luci Lizarraga:** From the HERA program's perspective there are two things we really want to get out of this project, evaluating metabolism of chemicals and how that affects toxicity and understanding how the different tools can be used for incorporating into chemical assessments.

Adverse Outcome Pathway Footprinting for Mixtures

Jason Lambert, Supervisory Toxicologist, Center for Computational Toxicology and Exposure

Mr. Jason Lambert presented on the adverse outcome pathway (AOP) "footprinting" concept. He introduced the background of this work, stating that there are many of chemicals that do not have enough hazard and dose response data. In response, the Agency is looking to leverage NAMs data to advance mixtures research in risk assessment.

Mr. Lambert suggested that NAMs should always be a part of the toolbox in problem formulation. For mixtures assessments, it would be a part of an integrated testing and assessment approaches (IATA) for mixtures. Employing data mining techniques for hazard and diose response data. The major topic here is the AOP footprinting idea. Trying to make sense of the increasing information within the AOP research realm, and more importantly how to leverage quantitative AOPs for a mixtures risk assessment. Is there enough weight of evidence (WOE) or information to identify AOPs for chemicals of interest in a mixture? I use the terminology interchangeably but an AOP I the same as an index chemical.

Mr. Lambert explained that current AOP theory focuses on AOP networks that result in nodules. Key events are the functional unit of observations and represent what happened in terms of an observable change in biological state. The key event depends on the level that is being reviewed (specific systems or a whole organism). Relationships between key events are the functional unit of quantitative inference within an AOP. Some state of a directionality or magnitude of a response has a relationship to one or more key events, or the adverse outcome itself. AOPs are supported by sufficient information to biological plausibility and the weight of evidence.

In contrast to AOP theory which posits a chemical agnostic description of the MIE to AOP, the footprinting approach first requires identification of well-characterized (hazard and dose-response) chemical(s) as the "anchor" or "index" for each toxicologically operative AOP. He described AOP footprinting as a stepwise profiling and comparison of AOPs at the level of key events moving backward from the most downstream key event to the molecular initiating event. The goal is to identify the key event(s) within each AOP suspected of contributing to a given adverse outcome, at which similarity between mixture chemicals can confidently be identified. These key events are identified as the "footprint" for a given AOP. Mixture categories are then assigned to the appropriate footprint category. Key event dose-response relationships (KEW) for each chemical within a category are then used to evaluate mixture additivity.

The further down an AOP one gets, the more biologically relevant information one may be able to discern as it relates to mixtures. Mr. Lambert admitted that in a perfect world, they would only be talking about BMDs; however, if a BMD cannot be identified then effect levels are needed, such as lowest observable adverse effect levels (LOAELs).

Mr. Lambert concluded by noting that AOP footprinting leverages elements of both the AOP and the mode of action (MOA) approach and that identifying AOP anchor chemicals is key. He noted that this offers opportunities to integrate NAMs into both quantitative and qualitative assessments but cautioned about the default assumptions of additivity within an AOP or across an AOP network. He finished by noting that a situation could arise where mixture component chemicals may not have sufficient WOE for quantitative evaluation using NAM data, but decisions on AOP footprint membership could still inform potential for additivity in the mixture.

- **Dale Johnson:** Is it correct to assume the AOPs theory is largely quantitative?
    - **Jason Lambert:** It is not a fully biologically based dose response model for an AOP, rather leveraging individual nodal response data across an entire AOP.
    - **Dale Johnson:** It would allow you to be able to pick a correct NAM essentially?
    - **Jason Lambert:** Yes.
- **Daland Juberg:** We saw great use of NAMs in use by CSS during these presentations, but with the advent of NAMs, I am assuming that there is real opportunity for collaboration and communication between HERA and CSS as HERA needs more NAMs and that the relationships are already in place?
    - **Samantha Jones:** That is something that we have been focused on over the last few years, examples from Grace Patlewicz and Luci Lizarraga, but one of the things that we have to focus on in HERA is that the assessments we produce are based on agency needs and priorities, so we use some of these connections as we need to and trying to identify areas where we can improve these mechanisms. Gina Solomon had noted how we increase our mechanisms for feedback and working together and that is something we are looking for in our future plans in how we can best make our production efficient. It is important to note that CSS

and HERA have inherent differences in goals but that we are looking to help each other and make sure that we are working together.
    - **Daland Juberg:** I will note that we had modelers in other areas of the company that could have helped in our assessments, so I understand the difficulties there.
    - **Samantha Jones:** I will note that when ORD reorganized this was a common theme that we were looking to implement so it has been a recurring theme.
- **Gina Solomon:** Can you describe some more on a day-to-day level, what is the process for HERA to reach out to CSS and connect on what data is needed? Is it something at the staff level where one person calls another or is there a committee or management level or a form or something? How does it happen?
    - **Samantha Jones:** It is a combination of a lot of things. A lot of it comes out at the ground level as we are going through projects and we identify gaps that we have, and we can reach out and talk about needs. One of our struggles is when we plan out research for four years across ORD it is hard to incorporate those small things on a short notice. At the management level that is where we really start trying to make sure that we are identifying everything that we can and being flexible when these needs come up. We make sure to balance these things.
- **Donna Vorhees:** I am curious about the interaction of HERA and CSS and the other Agency partners and external partners. Are they picked up immediately, do you get questioned about things and must reassess, and in a bigger picture, when do you say that these NAMs are good enough and will there be a protocol for that eventually?
    - **Samantha Jones:** We have been incorporating those approaches in PPRTVs for over 10 years. As the familiarity is increasing and more work is being done to fill gaps and the confidence is increasing. From HERA we are needing the ability to draw conclusions about a chemical about hazard or dose response, so the way that we see and the way that we build on that is that our assessments have to be high quality, transparent and scientifically defendable. We have been lucky to use the PPRTVs to work on these methods and have been able to work closely with OLEM.
    - **Donna Vorhees:** I was just interested in seeing how the PPRTVs were taken up and if they were proving to be able to stand up to scrutiny.
    - **Samantha Jones:** I would say yes in a short answer that they have stood up and are successful.
- **James Stevens:** Jason Lambert, you and I had looked at agnostic POD and biologically relevant POD and you noted that this one is where a biologically relevant POD is important. So how do we understand how toxicogenomics weaves through workflows. How good of an overlap is there between hallmark gene sets and the MIEs and KEs in AOPs and between MIEs. Can you zero in on gene sets that give information that could be

rolled up into a quantitative AOP approach using these more hallmark chemicals for dose equivalency estimates?

  - **Jason Lambert**: I agree, I know across the community that papers are being written on hallmarks of many areas of interest. For any bioassay type, I think that this will be dictated by the context of use, scoping, full assessments, filling data gaps, and then how do we link the less than apical effect concentration to an apical outcome. This is holding us up and understanding how much of a perturbation at some key event node that equates to another change.
  - **James Stevens:** It will be important to understand if *in vivo* AOPs that are identified are being seen in the *in vitro* models.

- **Chris Gennings:** If you think about how a system could be used for a mixture, I think that there is a good set of tools moving forward that could be used for mixtures. I would recommend reviewing these tools and thinking how they can be combined and work together to advance this area.

BOSC Subcommittee Discussion and Questions and Answers

Katrina Waters, Chair

- **James Stevens:** Does the AOP based on non-clinical data operate in the *in vitro* model? How do you tackle the specific translation problem from *in vitro* to *in vivo*? How do you know the human *in vivo* is linked in the same way? Can I use the same key event and how will I know that?
  - **Jason Lambert:** Great questions. We are assuming it is relevant until we find it is not.
- **Chris Gennings**: This is good choice of chemicals. The chemicals could be chosen and based on real human exposures and then go back to Factotum and use those chemicals.
  - **Katrina Waters:** There is a lot of data coming out of the Superfund program coming out of NIEHS and could derive some mixtures or known co-exposure.
  - **Chris Gennings**: Yes, how do we get that into the report?
  - **Jason Lambert:** It would be interesting to do something with PFAS.
  - **James Stevens:** The challenge is the overlap between the CSS and HERA programs because they are so interconnected.
  - **Katrina Waters:** We can be specific to the program office.

Advancing Systematic Review Methods and Tools Introduction with Charge Question

Kris Thayer, Director, Center for Public Health and Environmental Assessment

Dr. Kris Thayer discussed the advancement of systematic review methods and tools. She discussed the release of systematic review resources including the Assessment Methods in the IRIS Program and ORD Staff Handbook for Developing IRIS Assessments ("IRIS Handbook"), released in November 2020 for public comment. She showcased the activities that occur during

the systematic review method development, and the application to data curation and *in vitro* and absorption, distribution, metabolism, and excretion (ADME) literature.

Dr. Thayer discussed the publicly available resources including the IRIS Handbook and assessment-material templates, the draft template for "fit for purpose" systematic evidence map (SEM), and the publicly accessible Health Assessment Workspace Collaborative (HAWC) project to share targeted resources, and other specialized software tools. She also described the engagement with groups, within and outside EPA.

Organizing and Evaluating Mechanistic Evidence

Catherine Gibbons, Genetic Toxicologist, Center for Public Health and Environmental Assessment

Dr. Gibbons described mechanistic evidence and the importance of human health assessments. She outlined the mechanistic study identification process and the population, exposure, comparator, and outcome (PECO) statement and mechanistic study inventories. She provided an example of how HERA shares the information and use of Distiller. She provided some examples of focused key science issues for evaluation and discussed the rationale for prioritizing mechanistic outcomes for more in-depth analysis. She described the pilot testing of *in vitro* studies evaluation domains.

Automated/Machine Learning Approaches

Michele Taylor, Toxicologist, Center for Public Health and Environmental Assessment

Dr. Taylor discussed the development of a systematic method that allows for semi-automated extraction of data to increase efficiency and more easily integrate with other data management platforms. She highlighted the progress made to develop algorithms that extract common entities (chemical, dose, species) from animal toxicology studies.

Dr. Taylor discussed the next steps with include the dissemination of guidance on data extraction which ties into ontology. She described the collaboration across disciplines to develop algorithms, training sets and quality controls checks. She acknowledged the work will be expanded into other disciplines (ECOTOX) and includes extensive collaboration across CSS and HERA as well as other agencies/offices conducting assessments.

- **Dale Johnson:** Do you have deal with copyright issues?
    - **Michele Taylor:** No, we are working with publicly available data.

Semantic Ontology Mapping

Michelle Angrish, Toxicologist, Center for Public Health and Environmental Assessment

Dr. Angrish described the approach for sematic ontology mapping to apply ontologies to increase the efficiency of information retrieval and prioritization and expand controlled vocabulary to normalize information extracted using systematic review methodology.

Dr. Angrish highlighted the information retrieval challenge and the semantic factor and conceptual factor. She discussed the application of ontologies for information retrieval by

discussion the work done in collaboration with the Endocrine Screening program in EPA/OCSPP. She provided an example of practical application and how the controlled vocabularies effect data management and interoperability. Lastly, Dr. Angrish demonstrated the semantic ontology mapping for automated workflow.

- **Dale Johnson:** Can you use information submitted by a company?
    - **Michele Angrish:** The barrier would be making it available on the public side of the tool. The semantic ontology mapping work will be presented at an upcoming Environmental Health Vocabulary Initiative workshop with government, non-government, academic and industry partners.
- **Timothy Malloy:** Is this mostly internal use? Who are the customers?
    - **Michele Angrish**: We are partnering with The Australian National Genomic Information Service and OECD to incorporate the work into the adverse outcome pathway key event. We have had conversations with other groups who were developing IATA. We are trying to utilize the open-source tools and hope to make available through the Chemistry Dashboard.
    - **Kris Thayer:** We focus on two levels of users, those that do assessment work and those consumers that query the dashboard for assessment information.

PFAS 150 Systematic Evidence Maps

Laura Carlson, Toxicologist, Center for Public Health and Environmental Assessment

Dr. Carlson described the use of Systematic Evidence Maps (SEM) a pre-decisional analysis that uses systematic review methods to compile and summarize evidence but does not reach assessment hazard or toxicity value conclusions.

She described the EPA PFAS Action Plan involves the use of new approach methods to help fill information gaps. This ongoing work involves tiered toxicity testing of a structurally diverse landscape of PFAS using a suite of *in vitro* toxicity and toxicokinetic assays. She highlighted the status of the PFAS 150 Systematic Evidence Map (SEM).

Dr. Carlson described the methods and searches, using machine-learning to screen at the title and abstract level. She described examples of epidemiology data heat maps the PFAS 150 SEM literature inventory. She discussed how the linkage of SEMs to the EPA CompTox Chemical Dashboard is maximizing interoperability.

BOSC Subcommittee Discussion and Questions and Answers

Katrina Waters, Chair

- **Timothy Malloy:** Are you looking for recommendation on all the outputs or just what the presentations covered?
    - **Samantha Jones:** The output is associated with HERA Charge Question 1. The charge question is more specific to the building up and the use of the approach and less about the SEM. We have updated the background materials with shading.

It might be helpful to see the published SEMs in Research Area 3 and Research Area 4.

- **Ponisseril Somasundaran:** Many PFAS are not captured, can you expand on your focus?
    - **Laura Carlson:** There are several PFAS not included because the goals was a scoping exercise to capture data on the less known PFAS.
- **Katrina Waters**: Are journals going to a way so that they are standardized to assist in the process the systematic review methods?
    - **Kris Thayer**: For several journals it is required, some journals are disseminating templates and helping to evolve the community.
- **Dale Johnson:** I noticed some of the data is from chemical companies, why is that?
    - **Kris Thayer**: A lot of the information is from ECHA. Typically, this organization produces a full report and it publicly available.
- **James Stevens:** Have you thought about how to triangulate studies that are based on a key event, ontology, AOPs, and the use of mechanistic information? In addition, have you considered the gene ontologies that are tightly coupled?
    - **Michele Angrish:** We are working on developing a method for improving the ontologies in words to track the AOPs, include new test methods, such as the use of Go programming language. Additionally, there is no one ontology to cover the entire domain and map them together. The use of Go and other programming languages might inform others.

Dr. Waters reviewed the remaining agenda and asked for questions or suggestions from the board. Mr. Tracy made closing remarks.

Adjourn

The meeting adjourned at 4:45 p.m., Eastern Time.

Friday, February 5, 2021

Welcome – Day 4

The meeting reconvened at approximately [Time, a.m. or p.m., Eastern Time]

Meeting Kick Off, Federal Advisory Committee Act Rules, Expectations, and Logistics

Tom Tracy, Designated Federal Officer, Office of Science Advisor, Policy, and Engagement

The meeting convened at approximately 12:00 p.m. Eastern Time. Mr. Tom Tracy, DFO, thanked the members for their attendance. He made brief announcements regarding virtual meeting reminders.

BOSC Subcommittee Chair Opening Remarks

Katrina Waters, Chair

Dr. Waters gave a brief overview of the agenda and breakout groups. She requested that the groups have a full draft with strengths and recommendations by the February 25, 2021 meeting. Dr. Waters described in detail the process of creating a final draft.

Advancing Dose-Response Introduction with Charge Question

John Vandenberg, National Program Director, Human Health Risk Assessment Program

Dr. John Vandenberg gave a background of advancing dose-response and analysis tools. Characterizing dose-response relationships is fundamental to health risk assessment. Dr. Vandenberg discussed the Integrated Exposure Uptake Biokinetic (IEUBK) model. There have been advancements in measuring blood lead levels that are essential to the work EPA is doing in this area. Characterizing dose is of key importance but is very challenging for inhalation exposures. Dr. Vandenberg gave a brief overview of the different research areas and what they would be covering in later presentations today.

Multi-Path Particle Dosimetry Model

Annie Jarabek, Senior Science Advisor, Center for Public Health and Environmental Assessment

Dr. Annie Jarabek presented the EPA Multi-path Particle Dosimetry (MPPD) Model 2021. Particle dosimetry modeling has matured through additional algorithms and the move from empirical modeling to mechanistic description of deposition, and clearance to predict retained dose. With mechanistic modeling, the range of particle size used has been extended. The Food and Drug Administration (FDA) is also using MPPD to study vaping and particle interactions, and we see that as an opportunity for MPPD being used as a base model. MPPD provides clearance to predict retained dose, which is effective in looking at chronic studies. She mentioned that the three-fold product was created to assist EPA and external partners have consistent knowledge of usage for the MPPD model.

Dr. Jarabek addressed development and application, starting with a systematic review of the literature, followed by a mechanistic refinement. In this case, mechanistic data was physical chemical properties, as well as potential health effects. The mechanistic query of data helped identify novel approach of methods. Dosimetry models, notably MPPD, feature interspecies extrapolation and human exposure parameters to include the benchmark for inclusion or exclusion in this polymer category.

Dr. Jarabek described risk assessment as a two-fold process including interspecies extrapolation and improved characterization of target human scenario. MPPD now allows users to entertain specific size distribution density and replace default parameters normally used in risk assessment. She defined particle overload and how novel deployment of MPPD demonstrates overload occurrence.

Dr. Jarabek discussed the next steps and timeline. Dosimetry modeling is a critical link to translate exposure to internal dose for response analysis.

- **Daland Juberg:** In your work, you are thinking of kinetically derived maximum doses, relative to understanding the non-linear kinetic range. Looking at kinetics with dose-response, and if it is out of the range, you take it into consideration.
    - **Annie Jarabek:** Overload is a perfect example of this. Now that we can employ MPPD to verify when overload occurs, I think this is an advancement.
- **Jane Rose:** You mentioned computational fluid dynamics (CFD) models that can also be used for dosimetry but that they do not cover the pulmonary region well. Is there opportunity to merge MPPD and CFD? Can you comment on CFD performing better than MPPD?
    - **Annie Jarabek:** CFD can provide localized estimates of dose. CFD cannot go into pulmonary regions because the imaging technology does not provide high enough resolution. Ventilation in MPPD is inhalation and exhalation. That breathing is important to develop the airflow field. The next generation of this modeling will be a fusion of CFD and MPPD.
    - **Jane Rose:** You will be involved with the integration of MPPD into dosimetry, correct?
    - **Annie Jarabek:** Correct. We chose MPPD because it is resource intensive.
- **Chris Gennings:** Can you say more about nanoparticles? Do you think about blood-brain and placental barriers?
    - **Annie Jarabek**: MPPD can scribe mechanisms of particle and transport. NIOSH extended into the nano size range, which is the reason for MPPD having a larger size range as of now. One concern is that clearance pathways for direct upper respiratory are not currently formulated. We asked ICRP experts to be part of the peer-review so we can formulate how to address that pathway. Placental transfer is about portal of entry and is not explicitly part of the model structure.
- **Ponisseril Somasundaran**: In addition to size, the shape is very important, particularly nanoparticles. In addition to particles, for example, smoke, those particles are droplets. Composite particles are polymers with nanoparticles attached to it.
    - **Annie Jarabek:** I am pleased both of those issues are raised as future research directions for potential extension.
- **Jennifer McPartland:** You identified opportunities for future research. Is there a document for what ideally you would like to have developed for ways the MPPD could be augmented?
    - **Annie Jarabek:** I do not have a road map, but there is an entire chapter devoted to research needs. For example, the model we currently use covers a certain set of species that is not covered by the MPPD. Geometries in MPPD are based on males. MPPD allows us to look at different age. Data covers 3 months to adult, but the data are sparse. Nobody wants to fund the foundational data.
    - **Jennifer McPartland**: Do you think the EPA Strategic Plan, as currently written, account/acknowledges that research?

  - **Annie Jarabek:** I do not know if it is linked, but we are aware that EPA is mandated to cover the population. There was a conference 2 years ago where the anatomical limitations were a repeated theme. We could use more resources to extend fundamental resources.
- **James Stevens:** What are pitfalls of normalization to body surface area with deposition?
  - **Annie Jarabek:** If I said body surface I mis-spoke. Regional surface is correct.

Bayesian Model Averaging and Benchmark Dose Software 3.2

Jeff Gift, Title?, Center for Public Health and Environmental Assessment

Dr. Jeff Gift discussed Benchmark Dose Software (BMDS) as the primary EPA tool to estimate reference doses and cancer slope factors for risk assessment. He then described Bayesian Model Averaging and noted it would be available online in April 2021. When fitting a model to a dataset, models are selected based on statistical fit and rigorous and complex guidance is needed for a reproducible result.

Dr. Gift explained issues around model uncertainty, and he noted how multiple approaches have been developed for addressing and characterizing model uncertainty. Semi or non-parametric models have been studied and were found to be hyper flexible and completely data driven. Model averaging is a method by which the results of a suite of individual models are averaged together. BMDS Bayesian Model Averaging is an EPA/NIOSH approach using informed priors. A Laplace approximation of posterior density is used with favorable and reproducible results.

Dr. Gift discussed the benefits and issues of using a focused prior. He noted that EPA and NIOSH conducted research that tested against uninformed priors, selection criteria, and flexible non-parametric models.

- **Chris Gennings:** You mentioned optimizing for the BMD. Are users provided guidance in terms of study design?
  - **Jeff Gift:** It is not our arena to write that type of guidance. We have worked with NTP in the past and are emphasizing the importance of more dose groups and fewer animals per dose group.
- **Juleen Lam:** As more advanced options for users are being developed, how much is being integrated into guidance on applying these methods? Could you talk about options for batch processing data in BMDS software?
  - **Jeff Gift:** EPA is working on a Python version that will allow batch processing which will be available soon. Guidance for model averaging is in process, and it is expected to be from a user endpoint perspective.
- **Gina Solomon:** I am having a hard time not feeling uncomfortable about the priors. It seems to be a difficult focal point. Relative to the guidance, how would you communicate to a community group the choice of the priors and how that effects the ultimate results of the modeling?

- **Jeff Gift**: I think that may be the reason adopting the approach has been slow. There is still an outlying question about the priors. We are doing research in that, and when we feel comfortable with it, the process will move forward. We want to make sure that everyone is comfortable with the priors.

Approximate Probabilistic Analysis

Todd Blessinger, Title?, Center for Public Health and Environmental Assessment

Dr. Todd Blessinger discussed quantitative uncertainty analysis and APROBA. Quantitative uncertainty analysis is recommended by National Research Council to increase transparency and flexibility for reference value derivation. He reviewed the reference values formulas. He stated the APROBA method developed by the World Health Organization (WHO) is an approximate probabilistic analysis.

Dr. Blessinger addressed risk-specific concentration. More data must be continually collected to update distributions. Input from users is crucial as EPA does the quantitative uncertainty analysis.

- **Juleen Lam:** Can you speak about the broader plans of the agency about integrating this informally as a part of risk assessment and dose-response assessment? Will this be the primary approach? Do you know if there are conversations about integrating this into risk-assessment framework?
  - **Todd Blessinger:** There are conversations happening and the agency wants to generate feedback and stimulate discussion. We are going to apply this method to chloroform. It is a broad topic and there are several issues that must be worked out before it is applied.
  - **Samantha Jones:** Addressing all questions that come up is a priority. We are working on uncertainties as well as how this could be of use to other agencies.
- **Chris Gennings:** You were talking about HDMI where the numerator and denominator are random variables. Is assessment factored into the denominator? I am thinking about risk-assessment for single chemicals.
  - **Todd Blessinger:** The difference is treating them as random variables rather than field values. The sub chronic to chronic data was collected previously where the datasets were analyzed, and BMD were taken from each. Ratios were taken and estimated a distribution of ratios, and it was approximately normal to the parameters used. It is using empirical data, but you can insert other assessment factors. It can be done, but a different software must be used. There is no limitation on the uncertainty factors you can include.
- **Chris Gennings:** It seems reasonable to think about things as having a lot of uncertainty, but there could be complexities with this. How does the idea of uncertainty relate to the regulatory aspect?

  - **Todd Blessinger:** Regarding BMDS, there is uncertainty expressed in terms of benchmark dose level (BMDL). You are correct that uncertainty is a broad topic that will require collaboration within our office and other program office, regions, and various stakeholders.
- **Rick Becker:** From a philosophical standpoint, when you extend this approach to the entire population, is it an underlying assumption that no matter how small the exposure is, there is some percent of the population that will be impacted? That is tipping the biology a bit. If you take it all the way out, there will be potential for increased incidence.
  - **Todd Blessinger:** This is something I have never thought of but should be considered.
  - **Rick Becker:** If you are at or below the reference dose, the decision is that there is not a risk of anybody in that population. This approach has programmatic and technical challenges in terms of implementation. We also must be careful of going from rat to human studies.
  - **Todd Blessinger:** You are correct, I agree.
- **Donna Vorhees:** In terms of biological plausibility, some people truncate distributions. I was looking at your underlying paper and it mentions the ability to modify the model in the future to eliminate assumptions. What work have you done internally to evaluate those assumptions?
  - **Todd Blessinger:** We are at the beginning stage of this.
  - **Samantha Jones:** That is where we would encourage the public discourse around these methodologies. You will see this in the coming months.
- **Chris Gennings:** It sounds like there is opportunity for scientists to dive into what EPA wants and needs. How do these projects get started? Sometimes quantitative methods do not start that way.
  - **Samantha Jones:** ORD and the program partners drive research. We understand what the problems and gaps are, and we bring that back to ORD with ways to fix them. We are driven by clients within the agency, but we also know that you must think about the future.
  - **Beth Owens:** Also, as a program it allows us to better serve the program offices and stakeholders by being scientifically sound and defensible.
- **Daland Juberg:** For Charge Question 3, when you do dose response modeling, are you doing this for well-studied chemistry?
  - **John Vandenberg:** There is a lot of planning that takes place as some chemicals have large amounts of evidence to work with. There is a wide range of data availability. We want to provide what the program offices need.
  - **Samantha Jones:** We are limited to some extent about what is available. It does vary, but the idea is applying dose response methodologies is a priority.

- **Daland Juberg:** In output 3.5, integration or incorporation of epidemiology studies was discussed. How much work is being done with epidemiology side of the equation?
    - **John Vandenberg:** In our Integrated Science Assessment, we represent the response of population and exposure response information. Risk exposure is done by EPA's Office of Air.
- **Jeff Gift:** Some studies have been done to show that you might see a dose response if you evaluate the intake versus the water concentrations. We are looking closely at that.
- **Dale Johnson:** In terms of human exposure, what kind of data is available?
    - **John Vandenberg:** There is a lot of work going into how to estimate the hotspot. Biomonitoring data is used if available. For others, it may not be clear as it varies depending on the pollutants.
- **Dale Johnson:** Annie Jarabek mentioned collaboration between FDA using vaping approach. Is that a way to use the information?
    - **Annie Jarabek:** FDA is using MPPD and extending it to vaping looking at dynamics of the heat and vapor and particle in a vaping liquid. It was mostly devoted to the dynamics of the particles I was referring to.
- **James Stevens:** Does the information flow? Are there areas we can help to make sure that the exciting science is delivered and has impact?
    - **John Vandenberg:** The matrix works surprisingly well. This reflects the community within ORD and across EPA centers. There is a balancing act of tremendous skill sets and prioritizing the areas we think will be the most impactful. Lack of resources can be an issue sometimes.
    - **Samantha Jones:** How the agency works internally is important. EPA has long standing relationships with program partners. Hearing opinions and ideas from program partners has been key for our success. EPA tries to prioritize and focus on the most prevalent. Within HERA, there is a need to move the science forward.
- **James Stevens:** Jeff Frithsen mentioned having high priority deliverables for TSCA. Are you able to manage the resources internally?
    - **Jeff Frithsen:** We do not have the resources to answer everything. We are faced with new requests daily. The other direction is that we must balance the needs of all our partners in how and when we respond.
    - **James Stevens:** If you cannot do everything, how do you allocate your resources to multiple customers? Do you feel that is achievable within the matrix?
    - **Jeff Frithsen:** Yes, it is achievable from the point of delivering the highest priority science and highest priority topics. There are some topics that slip through the bottom.
    - **Jennifer Orme-Zavaleta:** We are part of a government organization with resources coming from congress. Congress identifies those targets and priorities for us to address and administration further refines them. As we receive and

allocate resources, it is meeting those overarching priorities. It depends on the level of effort and decisions to be made in the selected timeframe. There are several factors that play into this. There is flexibility with the process, but it is largely directed by administration.

- **James Stevens:** I am not talking about being provided more resources. How do we make the tradeoff decisions to provide certain customers one thing, others another? Your feedback helps us with strategy implementation. I find many things you define as products as activities, which is normal for some areas. NAMs have be developed and validated. Making the Strategic Plan a more intentional document is important and requires effective management instruction.
    - **Jennifer Orme-Zavaleta:** The focus of the question is on the science. As we look at priorities and resources, we check our capabilities and capacity, and have conversations with the individual program. I think this is to be considered in the next round of EPA Strategic Plans, and perhaps a companion implementation document.
- **Katrina Waters:** Going back to Day 1, in terms of NAMs, there are several outcomes that are publications. I think as you go forward with next EPA Strategic Plan and implementation plan, it should be clear if outcomes/deliverables are the activities? How do you articulate those deliverables?
- **Juan Colberg:** I want to comment on CSS Charge Question 1. The analytical method or any method we develop must have deliverables that we plan on. The metric and prioritization are important aspects. Discussions about specific functions of NAMS are relevant for us to make good recommendations.
- **Dale Johnson:** It is important to look at the right kind of case studies and then provide an analytical verification of NAMS. How are the selection of case studies done? Who makes that decision?
    - **Samantha Jones:** From a HERA perspective, most of it will be driven from the individuals doing the work. If it is an ongoing assessment, there will be a real-world application. Also, for us, it has been helpful to engage at the strategic level and the research teams to get feedback on best ways to move forward.

BOSC Subcommittee Discussion and Questions and Answers

Katrina Waters, Chair

Closing Statements and Responses

Samantha Jones, National Program Director, Health and Environmental Risk Assessment Research Program

Jeff Frithsen, National Program Director, Chemical Safety for Sustainability Research Program

Dr. Jones and Dr. Frithsen discussed how the three main topics discussed were the CSS/HERA program interactions, strong evolving connections between both programs, and how the CSS

program and the HERA program share a common objective. Many of ORD's scientists conduct research for both programs to ensure properly communicated information.

BOSC Subcommittee Deliberation

Katrina Waters, Chair

Dr. Katrina Waters suggested that the group go into the documents for each charge question. If committee members were not in that group, she asked them to leave comments with their initials over the next week. She explained that, for example, in her group, she does not feel that she is an expert on SeqAPass; therefore, if someone else is, they could provide that information. She said this was especially important for CSS Charge Question 1 because there were no members able to attend. Dr. Waters clarified that her goal is to have a final draft for each question's responses prepared before the next meeting so that trends and concerns can be narrowed in on for recommendation to the EPA.

Dr. Waters then asked the committee members if there were last questions for each other or for EPA staff.

- **Chris Gennings:** Are any of our recommendations going to be included in the next StRAP? Can we sort of add those things even though it is not really a part of our charge questions, for example sort of working in mixtures?
    - **Katrina Waters:** We were asked to provide feedback on the implementation of the StRAP and if we have any improvements or questions about the outcomes and tools presented. If there are suggestions of future areas to consider, that can be included in the suggestions, but should not be in the recommendations as that was not the charge.
- **James Stevens:** Should we take special considerations as all the members of this BOSC are turning over in a year or two?
    - **Bruce Rodan:** There is technically an expiration of the BOSC in March 2022, but we do have the option to renew. We need to hear from the incoming administration and see what they want to do with the committees. We do want to keep continuity and we extend out to March 2022 to ensure that there was coverage from beginning to end of the StRAP.
- **Jeff Frithsen:** How can we increase the confidence in NAMs which goes back to validation? What did you see in the sessions that you liked and what did you notice was missing? Those sorts of things are helpful to us. We also know that conventional validation is not going to be the same here in fit-for-purpose NAMs.
    - **James Stevens:** I like what you said about using confidence, that is a better way to think about it. We will use that as we move forward.
    - **Bruce Rodan**: Giving honest and clear answers to help us to respond to is helpful. We do have a metric of our partners rating how satisfied they are in the products that were created.
    - **Jennifer McPartland:** Can you give us that metric?

    - **Bruce Rodan:** That is the ORD metric, it is a metric of the satisfaction of our partners with the products given to them. I will make sure it gets to you. We make sure that we get the feedback and solve any problems to make sure the product is meeting their needs.
- **Daland Juberg:** Chris and I were talking about HERA Charge Question 3, and mixtures came up again, I wanted to let you know that it may look like we keep bringing it up and wanted to note that this is not just an agency issue but a larger scientific community issue that we will all need to work on, not just on you.
    - **Katrina Waters:** I did notice that over the years the BOSC has kept bringing it up, but we do recognize that if your partners are focused on single chemicals at a time that ties your hands. We have seen work in these presentations that keep these ideas alive, and if we can provide suggestions to be included in StRAPs that could keep these ideas moving that would be great.
- **Katrina Waters:** I noticed in CSS Charge Question 3 that has implications to HERA, when you think about developing a new tool, which I am sure comes from a particular partner or need, but how much of a landscape analysis is done to see where a commercial tool or something else are almost ready that could be adapted. Is there a process of how EPA addresses this?
    - **Jeff Frithsen:** We must be careful to not look like we are endorsing a tool, and things must be available to everyone. Sometimes we pay for it to be available to the world, and more. We try our best to not recreate things that already exist but sometimes we must make it freely available and totally transparent.
    - **Kris Thayer:** I agree with that philosophy. Anytime we look at a tool or needing a tool we do look. We have found many times that there are not really tools already out there, or that they are too specific that they cannot really be used. Any time that we use a tool we make sure it is at least interoperable if not totally open source.
    - **Katrina Waters**: There may be a point that in the development of something that even if something has been worked on but noticing that there is something out there that maybe we do not continue the development of something.

Adjourn

The meeting adjourned at 4:40pm, Eastern Time.

<u>Thursday, February 25, 2021</u>

Welcome – Day 5

Opening Comments

Tom Tracy, Designated Federal Officer, Office of Science Advisor, Policy, and Engagement

Katrina Waters, Chair

The subcommittee convened at approximately 11:00 a.m. Eastern Time. Dr. Waters reviewed the meeting goal and provided an overview of the meeting purpose. Mr. Tracy asked the subcommittee if any technical issues needed to be addressed.

Charge Question Workgroups and Report Out Discussion

The subcommittee divided into six breakout groups to continue work for their respective charge question. Upon returning, each workgroup presented the draft strengths, suggestions, and recommendations for each of the six charge questions, CSS Charge Questions 1-3, and HERA Charge Questions 1-3, to the BOSC subcommittee.

CSS Charge Question 1:

Dr. Bahinski reported the subgroup's draft strengths, suggestions, and recommendations.

Dr. Bahinski highlighted the draft strengths, which included implementation of partner feedback to ensure implantation of NAMs needs are met and development of the SeqAPASS method to predict species sensitivity.

He presented the workgroup's draft suggestions of providing guidance on exceptions to current methods, further collaboration with other agencies regarding NAMS, and additional information on ORD plans to expand the SeqAPASS database and prioritize species.

Dr. Bahinski offered the workgroup recommendations of defining analysis modalities, presentation of clear deliverables (i.e., battery of testing, risk assessment and test for chemicals, mixtures and metabolisms), and mapping of an overall data leverage plan.

- **James Stevens:** CSS Charge Question 1 also covered the toxicogenomic initiatives, correct?
- **Katrina Waters:** Yes, CSS Session 1 and the concurrent presentations and research activities to highlight NAMs development for hazard evaluation, exposure, ecotoxicology, and human-system models.
- **Jim Stevens:** We will add some additional comments to address the other NAMs areas.
- **Jane Rose:** I suggest additional clarification to further clarify on the current methods bullet by adding wording to specify "read across" methods.
- **Juan Colberg**: To provided further clarification this recommendation is to create guidance within the methods so the users can understand the limitations based on the grouping, specifically with isomers.
- **Jim Stevens:** This suggestion involves the managing the ability to cut down data space to limit the false discovery rate. I will move the bullet to the suggestions and edit.
- **Katrina Waters:** I suggest you pull in the comments about developing a full list of NAMs and move the comment on registration and acceptance as a sub-bullet under the deeper dive to present clear deliverables, including battery of testing, risk -assessment.
    - **Tony Bahinski:** The bullet on data mapping is for ORD to provide a better overview of the current NAMs development.

  - **Jim Stevens:** The "building capacity and building confidence", might overlap with CSS Charge Question 2, and suggest can we pull into the Executive Summary a section of overlapping issues. Do you have a sense of if there is anything missing? Is this the intent of CSS Charge Question 1?
    - **Tony Bahinski:** Yes, CSS Charge Question 1 is asking if ORD is identifying the right NAMs and are they being characterized correctly? The answer is yes. The presentation on the complex *in vitro* models and looked appropriate, *in silico* models, Tom Knudsen's presentation, we did not see anything missing.
    - **Richard Di Giulio:** Agree. I was impressed by the ecotox component, especially SeqAPASS, and bringing in more of the -omics.
- **Katrina Waters**: I noticed the continued recognition and discussion of mixtures and how we are viewing it as a priority as an important application of the NAMS and impact to certain system. CSS can lead the field on how these might be used.

CSS Charge Question 2:

Dr. Stevens explained the workgroup addressed key areas including improved use of RACTs, building confidence in NAMS, capturing response in complex systems and transition from NAMs to complex systems, building on the AOP initiative and increasing confidence.

Dr. McPartland discussed the draft strengths and recommendations. The BOSC subcommittee discussed the various approaches for categorizing TSCA chemicals into varying risk bins and levels of confidence.

- **Jane Rose:** Perhaps we need to use the concept of tiering to the suggestion of assessing confidence.
- **Jennifer McPartland:** We need to look at other data to characterize chemicals and the level of confidence in the existing battery of NAMs. Data needs to be brought in earlier in the process.
  - **Gina Solomon:** Does it make more sense to direct this suggestion to CSS or is more in the domain for the TSCA program and how TSCA chemicals are prioritized?
  - **Jim Stevens:** CSS oversees the TSCA program and the methodologies that exist within TSCA. Our remit is on the methodologies and how they are being implemented. We will take this input and present back a revised suggestion.
  - **Richard Becker:** CSS presented a case study, so this is "in bounds" to address. The complexity could be different for different models.
  - **Katrina Waters:** And ORD has a role in supporting the decision making for pre-prioritization.
  - **Juleen Lam:** Maybe frame the suggestion with the case example, so it is not specific to TSCA program.

- **Jennifer McPartland:** There is the issue of the binning of chemicals and the computational methods first and then review of additional data to address the adverse outcomes.
- **Gina Solomon:** I think we have two issues. The first issue is to make sure bullet is directed to CSS; the second issue is if there are other data sources, should they or not be brought in at this binning stage. The suggestion needs to be more direct and clearer as to what the suggestion is.
- **Richard Becker:** This is discussion of suggesting ORD should do a deep dive and address domain of applicability.

Dr. McPartland reviewed the three draft recommendations: RACTs be jointly utilized by CSS and HERA, determine a more structured process for evaluating a method, restructure table to identify activities, deliverables, and milestones.

- **Katrina Waters**: I suggest we make the second bullet clearer and rewrite to include what action the BOSC is asking CSS to perform. The third bullet, the format of the table should be a suggestion and the need for CSS to provide a clear list of what a NAMS is and the types of NAMs as a recommendation.

CSS Charge Question 3:

Dr. Jane Rose reviewed the strengths, suggestions, and recommendations. She described how the CSS/HERA subcommittee observed three databases and models from EPA presentations. The first being CompTox chemicals dashboard and I give credit to the development of this database and repository of tools and data that can be linked out to other models. We recognize and applaud the EPA for recognizing the need to upgrade the underlying data structure and technology for future applications This will be important piece of work moving forward.

The second presentation was around SeqAPass and this was where we asked for help form others in the group – so Richard or others in the group we would ask for more feedback. It was clear from the presentation that this is a powerful tool for future research, but we missed in the presentation how this was applied into risk assessment or regulatory context and how the community may use it.

The third presentation focused on factodum a program that combines ingredient combinations. A strength that came out of this but this tool may only be available within EPA so we wanted to better understand how that decision was made or whether that would be true moving forward. For the dashboard because it is so critical, that training needs are often a resource burden, so there may be ways to creatively train users.

For factodum, one thing that came up was whether the data streams that are fed into factodum are the most up to date and curated data sets. Are there other places that for example ingredients or product information could be accessed. We provided examples of other databases that provide product information that were not already considered. A common theme that comes out in our recommendations is there a way that we can use technology like Google analytics to better

understand how users are using tools and it may better inform EPA where to focus on changes or improvements and focus resources within EPA.

I think with SeqAPass we had already talked about that.

I think one of the other questions that had come up was again around the theme of has EPA looked to other approaches, tools, databases, that are already out there that could help to inform the development of their database, or maybe even to not create a new tool or database if one already exits. One example was GenRA and were there other tools that already existed that could have been used instead.

I am going to move to the recommendations and then we can discuss everything together. We are likely going to combine these recommendations together and our recommendation is really recognizing that developing these resources are labor and resource intensive, so helping the agency to try to define priorities by using things like google analytics to identify what tools and what parts of tools that are being utilized the most. Back to the theme of making sure that we are focusing and building on the right things, is an external landscape analysis done to see if EPA has identified private tools that could be used instead of building their own?

Questions from the Group:

- **James Stevens:** SeqAPass, I think it is a good tool, but it is targeted toward species differences and I would like to see EPA apply it. It seems a bit targeted toward the MIE end of the AOP spectrum and I would like to see some application in other areas. Is there an application where a lack of homology does in fact lead to a difference in risk between species? And how do we incorporate protein database information. When does the linear sequence homology matter and show a difference?
    - **Jane Rose:** where does SeqAPass fail and you would need more than that, like 3D protein structure?
- **Jennifer McPartland:** I noticed that you used a word "commercially available," and I wonder if there is a technicality to whether EPA can use that if it is not publicly available.
    - **Katrina Waters:** I believe the HERA program must have that transparency, as Factodum is not publicly available so CSS is able to use it as they do not have the same restrictions. The point is that there is not only a cost to develop, but to maintain and I think having the discussion to have about when to build a tool for each problem v. trying to find an existing tool or part of a tool.
    - **Jennifer McPartland:** Maybe keep the suggestion in there and the EPA can decide when they can or cannot use the tool but the idea of looking and considering it is important.

HERA Charge Question 2

Dr. Juleen Lam presented a HERA program overview, and he described how the HERA program has made substantial advancements in systematic review activities. He also highlighted a strength that HERA is actively engaged in meaningful collaboration with other groups external and internal to EPA. Dr. Lam shared relevant activities and products, which demonstrated

success implementing these tools in systematic review and incorporating mechanistic information. Current activities coordinate ecological and dose response information in the systematic review. Dr. Lam shared the suggestion that HERA focus on developing the ontology language, and he noted how there are areas to focus on and consider while developing this. Dr. Lam discussed a challenge the CSS/HERA Subcommittee identified, which entails increasing seamless transition between tools.

Dr. Thayer mentioned how the IRIS handbook and that may be a place where this information is already addressed but was not reviewed here. We do think that it is important that this is done and addressed, however. HERA did consider expanding dose response to systematic review but there was not a list of information on how this is going to be accomplished. These plans should be expanded on and mapping these efforts to products and such is important.

Expanding systematic review into mechanistic and *in vitro* studies. In one presentation they did look into how to appraise *in vitro* tools critically and our recommendation is that HERA should look at this and consider existing *in vitro* tools (such as OHAT tool) and make see if there are other tools that could be used, for example dentistry has a tool for *in vitro* appraisal and could that be incorporated or modified. One thing to make sure to cover is that you should always consider just the *in vitro* perspective and not always thinking how it relates back to animal as there are certain characteristics that may need to be addressed that only relate to *in vitro* tools and not to animal and may be missed.

Questions:

- **Katrina Waters:** The second recommendation could be better suited under HERA Charge Question 3 or to make it connect somehow there as that question talks more about their section, the third bullet may fit better under Charge Question 1 and so should similarly be linked or moved there as appropriate.
    - Juleen Lam noted that they will make sure that these bullets harmonize with the other two questions.

HERA Charge Question 3

Presented by Daland Juberg

Dr. Juberg explained how in general, EPA saw that there was good progress being made in modeling and such.

- **Katrina Waters:** it may be good to work on the formatting of the recommendations to make sure it is clear what is an actionable item. The last bullet should be a suggestion as it appears that they are trying to do it but may not be enough.
    - **Daland Juberg:** I will incorporate these thoughts and any others into our draft.

- **Katrina Waters:** Maybe the second and third bullets under recommendations could be sub bullet sunder the first? It may be better to have a single recommendation of the overarching point and then using the others as specific points or considerations.

Adjourn

The meeting adjourned at 5:00 p.m. Eastern Time

Thursday, March 11, 2021

Welcome – Day 6

Tom Tracy, Designated Federal Officer, Office of Science Advisor, Policy, and Engagement

James Stevens, Vice Chair

The CSS/HERA Subcommittee convened at approximately 2:00 p.m. Eastern Time. The CSS/HERA Subcommittee decided to improve the recommendations by dividing into charge questions workgroups. Dr. Stevens suggested editing the recommendations and moving additional justification text to the narrative or suggestions sections. The workgroups met for approximately 20 minutes. The CSS/HERA Subcommittee resumed and addressed the recommendations for each of the six charge questions CSS Charge Questions 1-3, and HERA Charge Questions 1-3.

Charge Question Consensus and Draft Recommendation Discussion

HERA Charge Question 3

Dr. Juberg discussed the updates made to the recommendations. The BOSC workgroup proposed a recommendation to establish routine strategies for comparing traditional animal-based PODs/RFDs to analysis of human epidemiological data when available. Dr. Stevens suggested rewriting the sub-bullets and incorporate them as rationale for a stronger overall recommendation with supporting suggestions.

- **Jennifer McPartland:** What does the term "categories" refer to? Is it the health end points? It is a bold statement to say that NAMs-derived PODs are "health protective" and hesitant to go that far.
    - **Rick Becker:** "Categories" is meant to indicate the type of chemicals, without naming the specific category of chemical. I was thinking about the recently SOT-awarded paper by Katie Paul Friedman referencing the NAM PODs, with IVIVE conversations, which provided lower health-based exposure values that traditional PODs. We could add this reference.
    - **Jennifer McPartland:** I suggest you qualify and add specificity to better define what is meant by categories. We also should modify the statement and provide some reference on how the NAMs derived PODs are protective across the board.
- **Daland Juberg:** We will take the sub-text to expand the narrative to refer to one overarching recommendation. This reorganization and addition of a third data stream NAMs helps to highlight an important facet.

- **Jim Stevens:** We have had so much discussion in previous years on exposure and establishing dose-response relationships this is worth highlighting in the report.

HERA Charge Question 2

Dr. Lam reported on the changes made to the three recommendations. The workgroup will keep the first recommendation, which focused on the challenges in utilization of tools at certain stages to guide tool improvement for future harmonization. She reviewed the second recommendation to see if it overlapped in HERA Charge Question 3 and the workgroup agreed to keep under this question.

She mentioned the recommendation for HERA to identify and review *in vitro* critical appraisal tools. She referred to the list to identify various fields where these tools are aligned with other efforts.

- **Jim Stevens:** Do you think there is redundancy between CSS and HERA with tool development? Is there a duplication of effort?
    - **Juleen Lam:** Not entirely, but part of the recommendation is to encourage HERA to ensure the development of tools is not done in silo, and not start from scratch in the development of a new tool. I will specify the NTP tool is in draft.
    - **Rick Becker:** I understand your point about the consistency in topic areas, like you listed for dentistry. Regarding my suggested edit, perhaps you can still list the topic by changing to "e.g., environmental health."

HERA Charge Question 1

Dr. Vorhees explained how the workgroup implemented the suggestions from the BOSC subcommittee. She explained the recommendation refers to HERA's need to regularly access the reliability for HERA's specific purposes and own uses in risk assessment.

- **Rick Becker:** My comment was trying to state that multiple plans are not needed since EPA has a workplan to develop scientific guidance.
    - **Dale Johnson:** The key thing is the validation and the usage of NAMs is something that HERA must do, while working with CSS to develop. It needs to be information coming from both groups, but specific to HERA's goals.
    - **Jim Stevens:** I suggest changing the wording to make it "update the HERA strategy" versus just "the strategy." This nuance specifies how HERA is evaluating their own program needs.
    - **Rick Becker:** Should we also add "HERA" to the risk assessment portion of the sentence?
        - **Jim Stevens:** We should make the change "For use by HERA in risk assessment."

CSS Charge Question 3

Dr. Jane Rose presented CSS Charge Question 3 and reviewed the CSS/HERA subcommittee's recommendations made. She noted that the first recommendation related to HERA Charge

Question 2 and that the second recommendation focuses on the use of analytics methods to determine where users are most using tools and inform ways and places to upgrade the tools. The group reviewed the recommendation that Rick Becker added. Dr. Rose noted that there was a similar point made in the suggestions section. Dr. Becker noted that it was important enough to put here that his own experience in using non-curated data is that it can cause extensive issues. He noted that curation can be very difficult and costly, but without it, a dataset may have little confidence. Without putting this as a recommendation, this may not be a priority for EPA and would not be accomplished. This includes biological and chemical information but did not want to be that specific to become limiting.

- **Dale Johnson:** I think we could combine the second and third recommendations.
    - **Jim Stevens:** I think that doing that would diminish and cloud them.
- **Jane Rose:** I think just cleaning up and rephrasing the first sentence would make the third recommendation clearer and that it is an important addition.

Dr. Becker reviewed the CSS Charge Question 2. The first is that the RACTs should be utilized and that joint representation from CSS and HERA on the RACTs of joint interest would be helpful. The second is that methods to improve confidence in NAMs should be developed (e.g., statistical and precision model performance analyses). The third is to explain how work products are different from publications.

Dr. Colberg reviewed CSS Charge Question 1. The question asked them to confirm the direction that CSS was taking. A common theme that we saw was around how in depth the presentations were and that we recommend a deeper dive in the Fall 2021 presentation. To assess a methodology, we really need to be able to see what it can do. Allowing for specific deeper dives into topics of interest would be very helpful.

- **Jim Stevens:** I did not mean for the third point to be a separate point as I think it is more repetitive of the first point. I can work with you to determine how to best phrase the inclusion of this information as I meant to highlight a topic that could be of interest but of high level of effort. The short version is that where we have these deliverables we would just like a little more detail and maybe we include a specific reference to transcriptomics here.
- **Jim Stevens:** When is the deadline of a consolidated draft?
    - **Tom Tracy:** The absolute final is due in the first week of May. There is a little bit of wiggle room in when we have the drafts together and could be iterative.
    - **Jim Stevens:** By next Wednesday everyone needs to review and make their edits. Then Savannah Bertrand and Tom Tracy can put the document together into one document, accept any remaining changes, copy over the comments, and then send to ICF staff to review and reformat. Blank sections for the narrative introduction and conclusion will be included. Then Dr. Waters and Jim Stevens can return a more final draft back to the subcommittee for review.

Adjourn

The meeting adjourned at 5:00 p.m. Eastern Time.

Appendix A: Agenda

United States Environmental Protection Agency
Board of Scientific Counselors (BOSC)
Chemical Safety and Sustainability/Health and Environment Risk Assessment Subcommittee (CSS/HERA)
Meeting Agenda
February 2-5, February 25, March 11, 2021
Virtual

FEBRUARY 2, 2021

TIME (EST)	AGENDA ACTIVITY	PRESENTER
12:00 - 12:10	Meeting kick off/FACA rules/expectations/logistics	Tom Tracy, DFO, OSAPE
12:10 - 12:15	ORD Welcome	Jennifer Orme-Zavaleta, ORD Principal DAA for Science
12:15 - 12:25	Subcommittee Chair Opening Remarks and Introductions	Katrina Waters, Chair
12:25 - 12:45	CSS NAMs Research and Development Portfolio: Connecting the Dots to Relevance and Acceptance	Jeff Frithsen, NPD, CSS
12:45 - 1:05	HERA Advancing the Science and Practice of Assessments	Samantha Jones, NPD, HERA
1:05 - 1:20	Translating Strategy into Action: Research Implementation Plans in ORD	Jill Franzosa, ACD, CCTE
1:20 - 1:50	Evolution of NAMs in EPA: From Research to Application	Rusty Thomas, CD, CCTE
1:50 - 2:15	BOSC Subcommittee discussion and Qs/As	Katrina Waters, Chair
2:15 - 2:30	NAMs Research Introduction with Charge Question	Jeff Frithsen, NPD, CSS
2:30 - 2:45	**BREAK & Transition to Virtual Break-out Rooms**	
CSS SESSION 1: CONCURRENT PRESENTATIONS ON NAMS RESEARCH Note: Each research topic will be presented in 25 minutes including time for specific questions.		
2:45 - 4:00	**SESSION A: Emerging Approaches to Hazard Testing**	
	1. High Throughput Transcriptomics	Logan Everett, CCTE
	2. High Throughput Phenotypic Profiling	Joshua Harrill, CCTE
	3. Metabolic Augmentation in *in vitro* Systems	Chad Deisenroth, CCTE
	SESSION B: NAMs for Exposure	
	1. High Throughput Exposure Models (SEEM)	John Wambaugh, CCTE
	2. High Throughput Toxicokinetic Models and IVIVE	Barbara Wetmore, CCTE
	3. Non-Targeted Analysis	Jon Sobus, CCTE
	SESSION C: NAMs for Ecotoxicological Applications	
	1. Approaches and Models for Species Extrapolation	Carlie LaLone, CCTE
	2. Novel *in vitro* Methods for Ecological Species	Brett Blackwell, CCTE
	3. High Throughput Transcriptomics: A Multi-Species Approach	Kevin Flynn, CCTE
	SESSION D: System-specific Models and Approaches	
	1. Respiratory tract models	Shaun McCullough, CPHEA

	2. Inhalation models	Mark Higuchi, CPHEA
	3. Neurovascular Unit Modeling and Blood Brain Barrier Function	Tom Knudsen, CCTE
4:00 - 5:00	BOSC Subcommittee discussion and Qs/As	Katrina Waters, Chair
5:00	**ADJOURN**	

FEBRUARY 2, 2021

TIME (EST)	AGENDA ACTIVITY	PRESENTER
12:00 - 12:10	Public comments	Tom Tracy, DFO, OSAPE
12:10 - 12:15	BOSC Subcommittee Chair Opening Remarks	Katrina Waters, Chair
CSS SESSION 2: APPLICATIONS OF NAMS TO AGENCY AND STATE PROGRAMS		
12:15 - 12:30	NAMs Applications Introduction with Charge Question	Jeff Frithsen, NPD, CSS
12:30 - 1:00	OCSPP-TSCA Inventory: Prioritization Proof of Concept	Richard Judson, CCTE
1:00 - 1:30	Developmental Neurotoxicity (DNT) *in vitro* Battery as an Alternative to DNT *in vivo* Guideline Studies Used by OPP	Tim Shafer, CCTE
1:30 - 2:00	Chemicals of Emerging Concern: A Prioritization Case Study with Minnesota Department of Health	Kristin Isaacs, CCTE
2:00 - 2:30	Application of NAMs and AOPs to Surface Water Surveillance and Monitoring in the Great Lakes (EPA Region 5) and a Western River (EPA Region 8)	Dan Villeneuve, CCTE
2:30 - 2:45	**BREAK**	
2:45 - 3:15	BOSC Subcommittee discussion and Qs/As	Katrina Waters, Chair
CSS SESSION 3: DEMONSTRATIONS OF TOOLS		
3:15 - 3:30	NAMs Tools Demo Intro with Charge Question	Jeff Frithsen, NPD, CSS
3:30 - 4:00	CompTox Chemicals Dashboard	Tony Williams, CCTE
4:00 - 4:30	SeqAPASS	Carlie LaLone, CCTE
4:30 - 5:00	Factotum: Curation of Exposure-Relevant Public Data	Kristin Isaacs, CCTE
5:00 - 5:30	BOSC Subcommittee discussion and Qs/As	Katrina Waters, Chair
5:30	**ADJOURN**	

FEBRUARY 4, 2021

TIME (EST)	AGENDA ACTIVITY	PRESENTER
12:00 - 12:05	Meeting kick off/FACA rules/expectations/logistics	Tom Tracy, DFO, OSAPE
12:05 - 12:15	BOSC Subcommittee Chair Opening Remarks	Katrina Waters, Chair
12:15 - 12:25	Connecting Assessment Needs to HERA Research	Samantha Jones, NPD, HERA
12:25 - 12:35	CPHEA Implementation and Workforce planning	Wayne Cascio, CD, CPHEA
12:35 - 12:50	BOSC Subcommittee discussion and Qs/As	Katrina Waters, Chair
HERA SESSION 1: Applying NAMS to Inform HERA Assessments		

12:50 - 1:00	Applying NAMs to Inform HERA Assessments with Charge Question	Luci Lizarraga, CPHEA
1:00 - 1:20	Advancing Read-across in HERA	Luci Lizarraga, CPHEA
1:20 - 1:40	Filling Metabolism Data Gaps in Read-across	Matthew Boyce, CCTE
1:40 - 2:00	Adverse Outcome Pathway (AOP) Footprinting for Mixtures	Jason Lambert, CCTE
2:00 - 2:40	BOSC Subcommittee discussion and Qs/As	Katrina Waters, Chair
2:40 - 2:50	**BREAK**	
HERA SESSION 2: Advancing Systematic Review Methods		
2:50 - 3:05	Advancing SR Methods and Tools Intro with Charge Question	Kris Thayer, CPHEA
3:05 - 3:25	Organizing and Evaluating Mechanistic Evidence	Catherine Gibbons, CPHEA
3:25 - 3:45	Automated/Machine Learning approaches	Michele Taylor, CPHEA
3:45 - 4:05	Semantic Ontology Mapping	Michelle Angrish, CPHEA
4:05 - 4:25	PFAS 150 systematic evidence maps	Laura Carlson, CPHEA
4:25 - 5:00	BOSC Subcommittee discussion and Qs/As	Katrina Waters, Chair
5:00	**ADJOURN**	

FEBRUARY 5, 2021

TIME (EST)	AGENDA ACTIVITY	PRESENTER
12:00 - 12:05	Meeting kick off/FACA rules/expectations/logistics	Tom Tracy, DFO, OSAPE
12:05 - 12:20	BOSC Subcommittee Chair Opening Remarks	Katrina Waters, Chair
HERA SESSION 3: Advancing Dose-Response Analyses and Tools		
12:20 - 12:35	Advancing Dose-Response Intro with Charge Question	John Vandenberg, CPHEA
12:35 - 12:55	Multi-path Particle Dosimetry Model	Annie Jarabek, CPHEA
12:55 - 1:15	Bayesian Model Averaging and BMDS 3.2	Allen Davis, CPHEA
1:15 - 1:35	Approximate Probabilistic Analysis (APROBA)	Todd Blessinger, CPHEA
1:35 - 2:10	BOSC Subcommittee discussion and Qs/As	Katrina Waters, Chair
CSS-HERA Closing		
2:10 - 2:30	Closing Statements and Responses	Samantha Jones, NPD, HERA Jeff Frithsen, NPD, CSS
2:30 - 5:00	BOSC Subcommittee Deliberations	Katrina Waters, Chair
5:00	**ADJOURN**	

Appendix B: Participants

BOSC Chemical Safety for Sustainability and Health and Environmental Risk Assessment Subcommittee Members:

Katrina Waters, *Chair*
James Stevens, *Vice Chair*
Anthony Bahinski
Richard Becker
Juan Colberg
Richard Di Giulio
Chris Gennings
Paul Gilman*
Dale Johnson
Daland Juberg
Juleen Lam
Timothy Malloy
Jennifer McPartland
Jane Rose
Gina Solomon
Ponisseril Somasundaran
Donna Vorhees
Clifford Weisel
Mark Wiesner

**BOSC Executive Committee Chair*
*** did not attend February 25*
****did not attend March 11*

EPA Designated Federal Officer (DFO): *Tom Tracy, Office of Science Advisor, Policy, and Engagement*

Presenters:

Michelle Angrish, *Center for Public Health and Environmental Assessment*
Brett Blackwell, *Center for Computational Toxicology and Exposure*
Todd Blessinger, *Center for Public Health and Environmental Assessment*
Matthew Boyce, *Center for Computational Toxicology and Exposure*
Laura Carlson, *Center for Public Health and Environmental Assessment*
Wayne Cascio, Center Director, *Center for Public Health and Environmental Assessment*
Allen Davis, *Center for Public Health and Environmental Assessment*
Chad Deisenroth, *Center for Computational Toxicology and Exposure*
Logan Everett, *Center for Computational Toxicology and Exposure*
Kevin Flynn, *Center for Computational Toxicology and Exposure*
Jill Franzosa, Assistant Center Director, *Center for Computational Toxicology and Exposure*
Jeff Frithsen, National Program Director, *Chemical Safety for Sustainability*
Catherine Gibbons, *Center for Public Health and Environmental Assessment*

Joshua Harrill, *Center for Computational Toxicology and Exposure*
Mark Higuchi, *Center for Public Health and Environmental Assessment*
Kristin Isaacs, *Center for Computational Toxicology and Exposure*
Annie Jarabek, *Center for Public Health and Environmental Assessment*
Samantha Jones, National Program Director, *Health and Environmental Risk Assessment*
Richard Judson, *Center for Computational Toxicology and Exposure*
Tom Knudsen, *Center for Computational Toxicology and Exposure*
Carlie LaLone, *Center for Computational Toxicology and Exposure*
Jason Lambert, *Center for Computational Toxicology and Exposure*
Luci Lizarraga, *Center for Public Health and Environmental Assessment*
Shaun McCullough, *Center for Public Health and Environmental Assessment*
Jennifer Orme-Zavaleta, Principal Deputy Assistant Administrator for Science, *Office of Research and Development*
Tim Shafer, *Center for Computational Toxicology and Exposure*
Jon Sobus, *Center for Computational Toxicology and Exposure*
Michele Taylor, *Center for Public Health and Environmental Assessment*
Kris Thayer, *Center for Public Health and Environmental Assessment*
Rusty Thomas, Center Director, *Center for Computational Toxicology and Exposure*
John Vandenberg, *Center for Public Health and Environmental Assessment*
Dan Villeneuve, *Center for Computational Toxicology and Exposure*
John Wambaugh, *Center for Computational Toxicology and Exposure*
Katrina Waters, Chair
Barbara Wetmore, *Center for Computational Toxicology and Exposure*
Tony Williams, Center for Computational Toxicology and Exposure

Other EPA Attendees:

Linda Adams
James Avery
Savannah Bertrand
Heidi Bethel
James Brown
Timothy Buckley
Amy Carpenter
Kelly Carstens
Bryant Chambers
Dan Chang
Brian Chorley
Krista Christensen
Bryan Clark
Jessica Conley
John Cowden
Taukecha Cunningham
Daniel Dawson
Michael DeVito
Michael Hornung
Andrew Hotchkiss
Michael Hughes
Victoria Hull
Sid Hunter
Scott Jenkins
Hyunsu Ju
John Kenneke
Elaina Kenyon
Channa Keshava
Nagu Keshava
Barbara Klieforth
Andrew Kraft
David Lattier
Candice Lavelle
Janice Lee
Monica Linnenbrink
Susan Makris
Mary Ross
Zachary Rowson
Elizabeth Sams
Kathryn Saterson
Paul Schlosser
Laurel Schultz
Rachel Shaffer
Dahnish Shams
Andy Shapiro
Avanti Shirke
Alysha Simmons
Jane Ellen Simmons
Steven Simmons
Nisha Sipes
Kimberly Slentz-Kesler
Marci Smeltz
Darcie Smith
Vicki Soto

Kathie Dionisio
Steven Dutton
Peter Egeghy
Aimen Farraj
Briana Foley
Stiven Foster
Chris Frey
David Gallegos
John Gamble
Barbara Glenn
Michael Goldsmith
Colin Guider
Annette Guiseppi-Elie
Maureen Gwinn
Paul Harten
Susan Hester
Dale Hoff
Kristen Hopperstad
Melissa Martin
Ardra Morgan
Jonathan Mosley
Anuradha Mudipalli
Jessica Murray
Johanna Nyffeler
Tom O'Farrell
Russell Owen
Beth Owens
Stephanie Padilla
Grace Patlewicz
Amanda Persad
Allison Phillips
Katherine Phillips
Jocylin Pierro
Kathleen Raffaele
Glenn Rice
Caroline Ring
Jessica Soto Hernandez
Adam Speen
Zachary Stanfield
Caroline Stevens
James Stevens
Tammy Stoker
Joseph Tietge
Rogelio Tornero-Velez
Miguel Torres
Emily Trentacoste
Michael Troyer
Elin Ulrich
Suryanarayana Vulimiri
Leah Wehmas
Chelsea Weitekamp
Amina Wilkins
Douglas Young

Other Attendees:

David Bottimore
Allen Davis
Helen Goeden
Maria Hegstad
Daland Juberg
Joseph Manuppello
Taylor Meredith
Bruce Rodan
James Smith
Scarlett VanDyke
Quinn Weinberger
Linda Wilson
Tiffany Yelverton

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Leah West