Advancing Chemical Risk Evaluations Through Use of New Approach Methods (NAMs): Challenges and Opportunities

20-21 June 2022 | Yokohama, Japan

International Council of Chemical Associations

Long-Range Research Initiative

National Institute of Technology and Evaluation

Introduction

The ICCA LRI & NITE workshop "Advancing Chemical Risk Evaluations Through Use of New Approach Methods (NAMs): Challenges and Opportunities" was held June 20-21, 2022, in Yokohama, Japan. The workshop was co-organized with the Japanese National Institute of Technology and Evaluation (NITE) and hosted by the Japan Chemical Industry Association (JCIA).

The conference was organized as a hybrid workshop to permit in person participation from those who were able to travel and to permit internet participation for those who could not attend in person. This structure maximized participation and fostered meaningful interactions face-to-face and through internet exchanges. Figure 1 shows the diversity of attendees organized by industry and job title.

As shown in Figure 1, 379 people registered for the workshop, with roughly 2/3 of the registrants from Japan, and 1/3 of the registrants from other countries around the world.

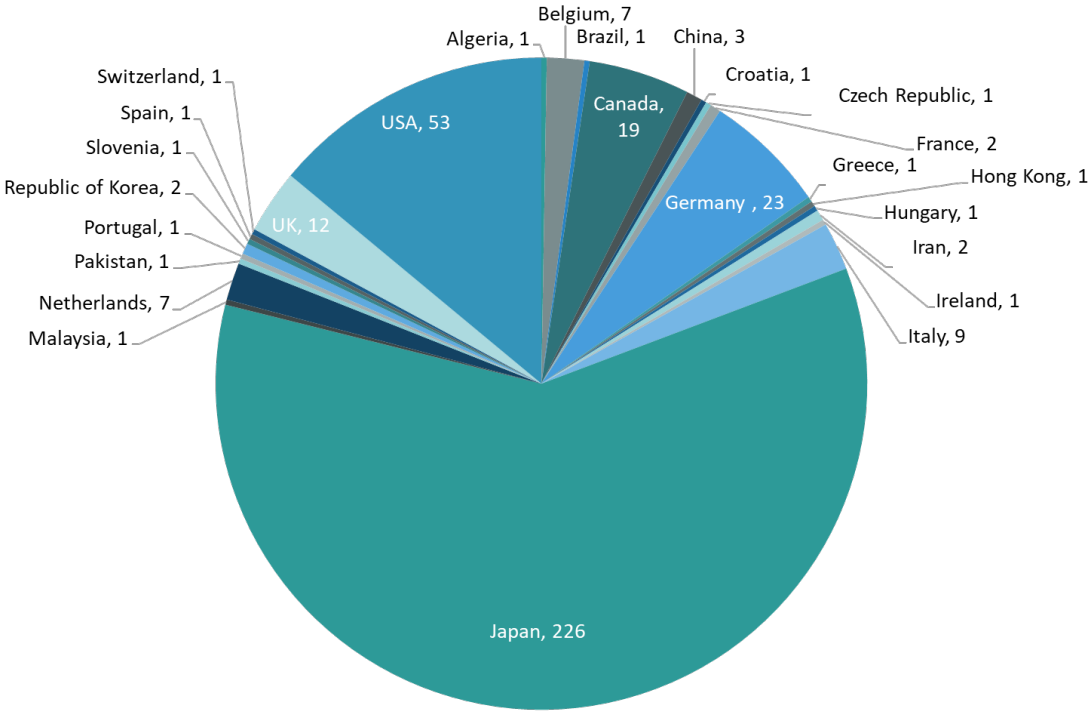

Fig 1. The geographic distribution of workshop registrants is displayed in the chart above.

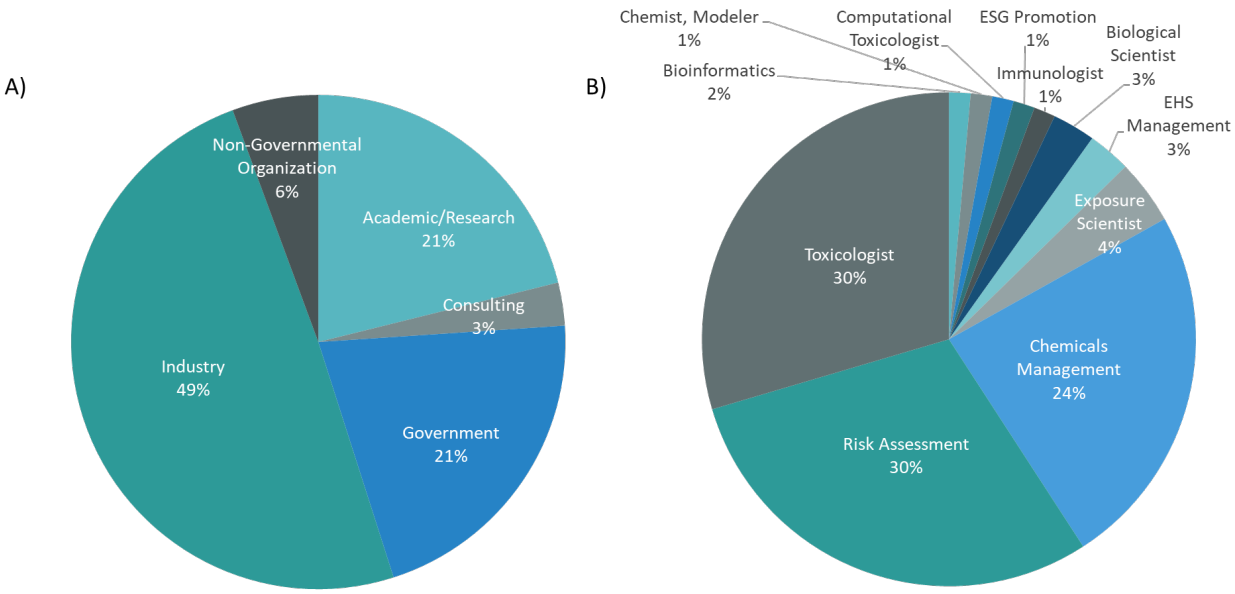

Fig. 2. The breakdown of attendees responding to the survey by A) industry, and B) job title, is displayed in the charts above (n=71).

Background

Significant investments in research over many years by governments and the private sector to improve our understanding of how chemicals can cause toxicity have generated new methods to predict and identify hazards of chemicals. While considerable progress has been made, this effort is still halfway to the goal. Energized by the 2008 publication of the U.S. National Research Council (NRC) report *Toxicity Testing in the 21st Century: A Vision and a Strategy*, alternative approaches that use computational profiling and measurements of biological activity in cellular pathways have emerged. These approaches have rapidly advanced our understanding of biological pathways in chemical toxicity.

- The European Chemicals Agency (ECHA) has encouraged the use of alternatives to animal tests in REACH (Registration, Evaluation, Authorization and Restriction of Chemicals). ECHA also prepares a bi-yearly report on progress made since 2007.
- In the U.S., amendments to the Toxic Substances Control Act (TSCA) enacted in 2016 required the Environmental Protection Agency (U.S. EPA) to develop, evaluate, and use alternatives to animal tests.
    - In 2019, the U.S. EPA administrator issued a directive pledging to reduce EPA's requests for, and funding of, mammalian studies by 30% by 2025 and to eliminate all mammal study requests and funding by 2035.
    - U.S. EPA then issued a NAMs workplan with specific objectives, deliverables, and timelines to achieve these goals over the ensuing years.
- Currently, the European Food Safety Authority (EFSA) is also developing its "EFSA Strategy 2027", which includes development and refinement of scientific methodologies and new approaches and tools for risk assessment that are in line with the 3Rs principle (Replacement, Reduction, and Refinement of animal testing).
- Japanese ministries (e.g., MAFF, METI, and MHLW) have also identified this challenge and continue to support and encourage public and private sectors to develop new methods.

Integration of information and knowledge from diverse methods is needed to ensure that a solid scientific foundation underlies the use of results from these new approaches for chemical safety assessment. Examples of these methods include computational methods, molecular and cellular pathways, pathogenesis of adverse effects, exposure science, and epidemiology.

Several recent initiatives provide important ideas and lessons that can be applied to accelerate the use of new approach methods in chemical risk assessments. This workshop was developed to share information on NAMs for chemical risk assessment and their pilot applications to help all stakeholders take stock of where the regulatory science community is at today. Additionally, the workshop will help define the paths forward to accelerate the development and application of NAMs for specific decision contexts, explore knowledge gaps, and identify the challenges and opportunities for future research in developing and applying NAMs in chemical safety assessment.

Conference Sessions

The conference commenced with opening remarks from Mr. Hideo Shindo (Director General, JCIA) and Dr. Fumihiko Hasegawa (President of NITE).

See Appendix 1 for the complete agenda including abstracts for each presentation.

NOTE: presentation materials will be available through September 30, 2022 on the workshop website here[1].

Session I: Setting the Stage

Session I consisted of four speakers who provided an overview on their perspectives of challenges faced in transitioning to NAMs from hazard evaluation and risk assessments based on traditional *in vivo* toxicity testing. These talks summarized numerous initiatives launched by their organizations, progress made to date, and strengths and limitations of NAMs. These speakers also highlighted additional research that is needed to help develop the scientific confidence to enable NAMs to be used in lieu of animal toxicity tests in chemical evaluations.

- Dr. Kathleen Plotzke (The Dow Chemical Company, USA) *Developing and Implementing a Scientific Confidence Framework for New Approach Methodologies (NAMs)* – Proposed a flexible Scientific Confidence Framework (SCF) consisting of seven components to evaluate NAMs and gave suggestions to make the framework semi-quantitative.
- Mr. Mitsuho Miyahara (Ministry of Economy Trade and Industry, Japan) *Outlook on Chemical Risk Assessment with New Approach Methodologies under the Regulation in Japan* – Gave Japan's perspective on the current situation and future outlook on chemical risk assessment with NAMs using Chemical Substances Control Law (CSCL).
- Dr. Russell Thomas (U.S. Environmental Protection Agency) *The Development, Evaluation, and Application of New Approach Methods at USEPA* – Provided a seven-step plan for application of NAMs to regulatory decision making and finding practical solution for applying NAMs in quantitative risk evaluations.
- Dr. Elisabet Berggren (Joint Research Centre (JRC))- European Commission) *Advancing Chemical Risk Evaluations through Use of New Approach Methods* – Gave the EU's perspective on advancing chemical risk evaluations to reach sustainable development goals by confidently implementing NAMs.

Session II: Poster Session

Thirty poster presentations were included in the conference. These presentations covered a wide range of topics and focus areas including NAMs for HH1 (6packs), HH2 (HH assess), HH3 (HH predictions), ecotox, and exposures. Posters were presented both in-person and virtually. Using a web platform, virtual participants were able to pose questions and discuss with poster authors.

Poster abstracts are included in Appendix 2.

[1] https://web.cvent.com/event/d4366e52-35c6-42a9-8dcb-2ec58b08b85d/websitePage:499a9959-269f-420d-9a05-6deeabd693e6

Session III: Using New Approach Methods to Predict Repeat-Dose and Complex Apical Endpoints

In this session, three speakers presented their research on developing NAMs to predict complex *in vivo* endpoints. These speakers covered such topics as chronic toxicity, liver toxicity, kidney toxicity, and developmental neurotoxicity for human health safety assessments. In addition, a fourth speaker in this session described the development and use of the KAshinhou Tool for Ecotoxicity.

- Dr. Kimito Funatsu (Nara Institute of Science and Technology, Japan) *Development of safety prediction system (AI-SHIPS) for industrial chemical compound using AI by METI* – Outlined the development of mechanism-based toxicity-prediction system (AI-SHIPS), including Three-Layers Model for constructing predictive model for toxicity.
- Dr. Ellen Fritsche (IUF Leibniz Research Institute for Environmental Medicine, Germany) *An in vitro battery for developmental neurotoxicity evaluation: from basic science to a regulatory paradigm shift in 21st century Toxicology* – Discussed developmental neurotoxicity *in vitro* battery (DNT IVB) as a valuable tool for hazard characterization and prioritization from its setup, applicability domains, and gaps perspective.
- Dr. Corie Ellison (The Procter & Gamble Company, USA) *Internal Threshold of Toxicological Concern (iTTC): Where We Are Today and What Is Possible in the Near Future* – Shared the current Internal Threshold of Toxicological Concern (iTTC) project in terms of comparing human exposure, selecting chemicals, reviewing preliminary model output, and usage of iTTC for human PK data.
- Dr. Hiroshi Yamamoto (National Institute for Environmental Studies (NIES), Japan) *Potential use of in silico methods for the ecological risk assessment in the chemical management in Japan* – Described the performance of KAshinhou Tool for Ecotoxicity (KATE) and the limitations of ecotoxicity QSAR systems in the chemical management in Japan.

Key Points from Session III Presentations and Discussions

- For prediction of repeated-dose toxicities, it is critical for AOPs to clearly establish the causal relationships that link chemical information to specific key events and the adverse effect.
- *In silico* methods have great promise, but it is important for stakeholders to understand the advantages and the limitations of *in silico* methods to ensure appropriate regulatory and product stewardship use.
- One to one replacement of a repeat dose animal study with a NAM is not realistic. Therefore, development and use of a battery of *in silico* and *in vitro* NAMs that evaluate Molecular Initiating Events and Key Events is a pragmatic way to address this challenge. Consideration needs to be given to use of a battery that encompasses sufficient biological pathways.
- While use of *in silico* and *in vitro* NAMs for hazard identification appears possible today for certain mechanisms of action, there are many challenges encountered when addressing complex endpoints, such as developmental neurotoxicity (DNT).
    - There is ongoing work at OECD to develop and validate a NAMs-based DNT battery, with the goal to eventually deploy this battery for regulatory and product stewardship uses,

but many questions remain.
    - Research to address complex endpoints needs to include, for example:
        - Consideration of the number and types of AOPs that need to be developed and used, not just for specific pathway modes of action, but also for generalized systemic toxicity and how these AOP's should be deployed for regulatory use.
        - Consideration of the range of chemical concentrations to use to develop dose response data; to include a rationale for the selection of the highest *in vitro* concentration that will be tested.
        - Consideration of methods that can be used to evaluate and distinguish pathway specific responses from non-specific responses.
        - Development and use of methods such as in vitro to in vivo extrapolation PBPK modeling to enable *in vitro* concentrations to be related to realistic exposure conditions *in-vivo*, and to assist in equating *in vitro* concentrations to *in vivo* limit doses.
- The iTTC (internal Threshold of Toxicology Concern) holds considerable promise to ground truth in *in vitro* NAM results and to interpret human biomonitoring studies.
    - The iTTC approach can be deployed to evaluate aggregate human exposures.
    - Further research to expand and refine the use of iTTC for risk evaluations for dermal and inhalation exposures is anticipated.
    - For regulatory use of the iTTC, could one conclude that a chemical which only produces bioactivity *in vitro* at a concentration above the iTTC poses no appreciable risks to human health?
    - What battery of NAMs would be needed to confirm that a chemical has no (or very low) bioactivity, such that an iTTC can be used with confidence?
- Use of the KATE *in silico* model in ecological risk assessment is useful, particularly for prioritization of chemicals, to identify those that require further evaluation, and for contributing to the weight of evidence approach for the determination of PNECs for regulatory use. However, the extent of available data is limited for constructing prediction modeling of chronic exposure effects.

Session IV: Integrating Exposure Assessment NAMs (Internal & External) into Risk Evaluations

Three speakers presented their research on developing PBPK models to improve quantitative derivation of exposures for use in risk evaluations. In addition, a presentation was given describing EAS-E suite, an online platform containing a compendium of exposure models.

- Dr. Shino Kuwa (National Institute of Technology and Evaluation, Japan) *Practical Use of Toxicokinetics Information for a Read-Across of Repeated Dose Toxicity – A Case Study Using Halogenated Aliphatic Compounds Hepatoxicity Category* – Presented a case study aimed at utilizing physiologically based kinetic (PBK) model and showing practical use of toxicokinetics information for a read-across of repeated dose toxicity.
- Dr. Jun Abe (Sumitomo Chemical Co., Ltd., Japan) *A case study of human risk assessment of a substance using a PBPK modeling technique* – Demonstrated the feasibility of approach of using

chimeric mice to obtain human hepatic parameters to accurately predict the pharmacokinetics using a PBPK modeling technique.

- Dr. Katharina Schwarz (Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Germany) *In vitro Data to Parameterise PBPK Models For Inhalation Exposure (Cefic LRI project B21)* – Described the results of inhalation-focused physiologically based pharmacokinetic (PBPK) model (lung PBPK model) for humans and discussed future steps of this model.
- Dr. Jon Arnot (ARC Arnot Research & Consulting, Canada) *Development and Evaluation of a Holistic and Mechanistic Modeling Framework for Chemical Emissions, Fate, Exposure, and Risk* – Explained a new modeling framework called PROTEX-HT (PROduction-To-Exposure High-Throughput model), which simulates aggregate human exposure and ecological exposure, and a new online platform to aid evaluations for ecological and human health assessment called EAS-E Suite (Exposure And Safety Estimation Suite).

Key Points from Session IV Presentations and Discussions

- Exposure is one-half of the equation for determining risk and must be addressed in transforming risk assessment to use of NAMs.
- The Age-dependent Biologically Effective dose Evaluation Tool (ABEET) incorporates a generic human PBPK model.
    - ABEET holds great promise to provide quantitative ADME information for read-across of repeated dose toxicity for substances that lack sufficient hazard data.
- Chemicals with complex kinetics can pose significant challenges for the development of laboratory animal PBPK models and human PBPK models.
    - For chemicals with simple kinetics, PBPK parameters (e.g., hepatic clearance) can be obtained from *in vitro* studies using rodent and human hepatocytes.
    - However, for chemicals with complex kinetics, a chimeric mouse model with humanized liver is an approach that can overcome many of the challenges.
- Many exposures in the workplace, in homes, and in the ambient environment occur via inhalation. An inhalation-focused PBPK has been developed and shown to successfully predict respiratory uptake and bioavailability from gases, vapors, aerosols and solid particles for a variety of exposure durations.
    - A web-based platform is under development, and once released will enable widespread use of this model.
- The PROduction-To-Exposure High Throughout (PROTEX-HT) model enables efficient calculation of screening-level exposure estimates based on input information of chemical structure and production volume. These exposure estimates can be used for premanufacture predictions as well as existing chemicals.
    - Exposure estimates can be integrated with toxicity values to enable risk-based prioritization and decision making.
    - PROTEX-HT and other models are freely available to all user through the EAS-E suite platform https://arnotresearch.com/eas-e-suite/.

Session V: The challenges towards improved risk evaluations - Quantitative Human Risk Assessment

Four speakers described their research in applying NAMs in developing quantitative risk evaluations.

- Dr. Leslie Recio (ScitoVation LLC, Durham, NC USA) *Integrating New Approach Methodologies into Quantitative Risk Assessments* – Explained toxicogenomics and presented a case study on *in vivo* acrylamide toxicogenomics study looking at mode of action and benchmark dose.
- Dr. Satoki Fukunaga (Sumitomo Chemical Co. Ltd., Japan) *Evaluating the human relevance of chemically induced liver tumors in rodents – Quantitative risk assessment based on the mode of action* – Presented quantitative assessment and human relevance analysis of chemically induced liver tumors in rodents based on MOA using Compound X.
- Dr. James Sherman (Celanese Corporation, USA) *Chemical Risk Assessment: Formaldehyde as a Case Example Use of SILMS to Inform Toxicology Testing Needs and Quantitative Risk Evaluations* – Gave an overview of SILMS (Stable Isotope Labeled Mass Spectroscopy) using example of formaldehyde to inform toxicology testing needs with molecular dosimetry data generated by SILMS and incorporating it into quantitative risk evaluations.
- Dr. Carl Westmoreland (Unilever, UK) *Opportunities for NAMs in an EU regulatory context* – Talked about Unilever's tiered and exposure-driven approaches to assess safety of their products to consumers and workers using newer techniques such as Next Generation Risk Assessment (NGRA), which integrates NAM data from various sources into safety decision-making process.

Key Points from Session V Presentations and Discussions

- *In vitro* to *in vivo* extrapolation (IVIVE) modeling is a critical tool for deployment with *in vitro* NAMs.
  - By using IVIVE to calculate equivalent *in vivo* doses, one can compare toxicity points of departure derived from traditional animal toxicity tests to PODs derived from *in vitro* NAMs.
  - Good correlations have been shown for a number of test compounds using liver cell assays and lung cell assays (that use exposure via the air liquid interface), showing that NAMs can be predictive and protective
- Mechanistic NAMs (*in silico* and *in vitro*) are critical for use in quantitative assessment for interspecies relevancy in carcinogenesis.
  - NAMs can be used quantitatively for evaluating a range of postulated Modes of Action and establishing human relevancy of animal study results.
  - When coupled with *in vivo* results, these NAMs can be used to establish quantitative species differences.
- The Stable Isotope Labeled Mass Spectroscopy (SILMS) is an extremely sensitive and specific analytical NAM for measuring biomarkers of exposure.
  - For the formaldehyde case study, SILMS shows that there is a clear threshold dose for exposure to exogenous formaldehyde.
  - Integrating these NAM results with knowledge of the mode of action shows that the

way formaldehyde causes cancer is through cytotoxicity and regenerative proliferation and that this mode of action only operates when exogenous exposures exceed a threshold level.

- The Next Generation Risk Assessment (NGRA) approach is an exposure-led, hypothesis-driven risk assessment approach that integrates NAMs for evaluation of chemical product safety for ingredients in cosmetic and consumer products without relying on animal toxicity tests.
    - Case examples have been developed using the NAMS to generate bioactivity points of departure. These are then integrated with exposure assessment calculations or measurements to calculate bioactivity to exposure ratios or margins of safety.
    - Research is showing that the NGRA approach can applied today for many types of chemical products, and research is underway to extend the approach to cover other routes of exposures and effect endpoints.

Organizing Committee

The organizing committee was responsible for deciding the theme and planning the program for the conference. The committee was comprised of scientific experts and organizational members from the Japan Chemical Industry Association (JCIA), the National Institute of Technology and Evaluation (NITE), the American Chemistry Council (ACC), the European Chemical Industry Council (Cefic), and the National Institute of Health Sciences (NIHS), and Together, their efforts ensured a well-balanced, high-quality technical program.

Appendix 3 contains a complete list of the 2022 ICCA-LRI & NITE Workshop committee members.

Post-Event Survey Results

Survey respondents were asked to comment on satisfaction with the sessions and presentations, and the overall workshop. Survey respondents were also asked to provide feedback on additional topics to cover in future conferences. **The survey results reported an overall workshop satisfaction of 98%.**

Appendix 4 highlights the feedback from the post-event survey, including short answer responses on future suggestions for improvements to the workshop.

APPENDIX 1. Complete Agenda Including Abstracts

Advancing Chemical Risk Evaluations Through Use of New Approach Methods (NAMs): Challenges and Opportunities

Day 1: Monday, 20 June 2022

Registration

11:30-12:40 (JST)
Foyer

Check-in

Welcome

12:40-13:00
Ballroom &Zoom

Welcome and Opening Remarks

Hideo Shindo – Director General, JCIA

Dr. Fumihiko Hasegawa – President, NITE

Session I: Setting the Stage

Session Chairs: Hideo Shindo (JCIA) & Dr. Fumihiko Hasegawa (NITE)

13:00-13:30
Ballroom &Zoom

Setting the Stage: Developing and Implementing a Scientific Confidence Framework for New Approach Methodologies (NAMs)

Dr. Kathleen Plotzke – The Dow Chemical Company, USA

The dynamics and complexities of functional biology and homeostasis in whole organisms create significant challenges in transitioning from lab animal-centric toxicity testing methods to assessment strategies based on new approach methodologies (NAMs). Nevertheless, considerable progress is being made. The LRI programs, and others, have developed approaches for using computational, in vitro, and exposure NAMs to fill data gaps, conduct risk-based prioritization, and to improve integrated approaches to testing and assessment. However, to put NAMs into use requires regulatory acceptance - demonstrating that a NAM has achieved a requisite degree of scientific confidence for one or more specific applications before it can be used to support product stewardship or regulatory decisions. 'Fit- for-Purpose Validation' (FFPV) has emerged, and while various components of such a process have been discussed, a uniform approach for FFPV has yet to be adopted. Accordingly, we have proposed a scientifically rigorous, yet flexible framework, that can be widely applied to evaluate most, if not all, types of NAMs. This NAM Scientific Confidence Framework (SCF), centered on explicitly applying the scientific method, consists of 7 components: 1) problem formulation and proposition statement (description of the intended use of the NAM for a specific decision context) 2) the biological relevance & plausibility of the NAM; 3) assay performance (documentation of sensitivity, specificity, reliability, & domain of applicability of the NAM); 4) documentation of the performance of the inference (prediction) model imbedded in the NAM (based on the NAM response – predicted outcome response relationship); 5) dissemination of the data, inference models, etc. to support independent replication; 6) a narrative rationale making the case that there is / is not sufficient scientific confidence in the NAM to support the specific application for the chemistry domain of interest; and 7) verification through independent scientific peer review. Time permitting, we will illustrate application of this NAM Scientific Confidence Framework to the evaluation of 2) the purported key characteristics of carcinogens for classifying chemicals and b) to use of the Threshold of Toxicological Concern (TTC) and points of departure from ToxCast data for risk-based prioritization / deprioritization.

13:30-14:00
Ballroom &Zoom

Outlook on Chemical Risk Assessment with New Approach Methodologies under the Regulation in Japan

Mitsuho Miyahara – Ministry of Economy Trade and Industry, Japan

The New Approach Methodologies(NAMs)are the technologies that have a great potential to increase quality and efficiency in chemical assessment and management. However, there remain difficulties for further developing NAMs and promoting practical use globally, especially introducing NAMs into regulations. In this session, he will describe current situation and outlook on chemical risk assessment with NAMs in Japan from the standpoint of regulatory authority. First, he will explain overview of Chemical Substances Control

Law(CSCL) which is one of chemical management laws in Japan. Evaluation for new chemicals and risk assessment for existing chemicals are conducted by the authority under CSCL. NAMs such as quantitative structure-activity relationships(QSARs)model and read-across are utilized partially for evaluation of chemical properties such as biodegradability and bioconcentration. Next, he will introduce activities on research and development of NAMs in Japan, such as weight of evidence approaches and development of a state-of-the-art hazard prediction system analyzing relationship between toxicity and chemical structure as well as other various factors by using AI technologies. These developments have certain achievements so far and are expected to contribute for further development to evaluate biodegradability, bioaccumulation and toxicity of chemicals under environment close to actuality. In addition, he will present Japan's contribution and cooperation to the OECD which is the center of the international discussions on the NAMs. Finally, he will raise major challenges and necessary future efforts to expand the application of NAMs to regulations, such as improving reliability, accumulating practical use cases, and creating guidelines regarding NAMs.

14:00-14:30
Ballroom &Zoom

The Development, Evaluation, and Application of New Approach Methods at US EPA

Dr. Russell Thomas – U.S. Environmental Protection Agency, USA

*Thousands of chemicals are used in commerce and are present in the environment, while more chemicals are introduced each year. Many of these chemicals have limited data that can inform potential human health and ecological risks. The continued development, evaluation, and application of emerging scientific and technological approaches are critically important to address these challenges. New Approach Methods (NAMs) is term that has been coined to capture many of these emerging approaches and they include a broad range of in vitro and in silico approaches for evaluating potential chemical hazards, identifying modes or mechanisms of action, extrapolating between internal and external doses, and estimating exposure. In many cases, the endpoints and uncertainties associated with these new methods are qualitatively and quantitatively different than the traditional approaches. The inherent differences have required new efforts to characterize the uncertainties in both the NAMs and traditional methods while developing new frameworks to build confidence in the approaches for application to a range of decision contexts. The US EPA continues to invest in NAM research and has released an updated Agency Work Plan outlining broad objectives for developing and applying NAMs as well as long-term and short-term strategies to achieve those goals. This presentation will provide an overview of the ongoing efforts at US EPA to develop and improve NAMs related to toxicology, toxicokinetics, and exposure; evaluate NAMs for fit-for-purpose regulatory application through case studies and scientific confidence frameworks; and outline a path forward for increasing integration into regulatory decisions. *The contents of the abstract and associated presentation do not necessarily reflect U.S. EPA policy.*

14:30-15:00
Ballroom &Zoom

Advancing Chemical Risk Evaluations through Use of New Approach Methods (NAMs): Challenges and Opportunities

Dr. Elisabet Berggren – Joint Research Centre (JRC) - European Commission

In the context of the EU Chemicals Strategy for Sustainability towards a toxic-free environment, aiming to better protect citizens and the environment and boost innovation for safe and sustainable chemicals, the discussion on how and when NAMs can be used in chemicals safety assessment has been further emphasised within the EU. Looking at the assessment of systemic health effects and long-term effects in the environment, there are many challenges how to confidently implement New Approach Methods. What level of validation or standardisation is needed to accept a NAM? We need to ensure that we maintain or higher the current level of safety while guaranteeing sustainability in use and exposure to chemicals, but still accepting that a one to one replacement of traditional animal methods is not possible. The NAMs can neither be used only to trigger further animal testing to confirm suspected adverse outcome, which could lead to more intense use of animal testing. In addition, to achieve safe and sustainable use of chemicals, the assessment need to be more efficient to cover the large number of chemicals on the market and not only a fraction. There is a need for common understanding and trust among stakeholders to progress the development of new approaches to testing and assessment for safer use of chemicals.

Session II: Poster Session – Development of NAMs and Case Studies with NAMs Session Chair: Dr. Tokuo Sukata (JCIA)	
15:00-15:10 ***Ballroom &Zoom***	**Overview of Poster Session** **Dr. Tokuo Sukata -** JCIA
15:10–15:30	**Afternoon Break**
15:10-18:15 ***Foyer &Box***	**Poster Session**
18:15	**Adjourn Day 1**

Day 2: Tuesday, 21 June 2022

Registration	
8:00-8:30 (JST) ***Foyer***	**Check-in & Coffee**
Welcome	
8:30-8:40 ***Ballroom &Zoom***	***Welcome and Agenda Review*** **Taketoshi Fujimori -** JCIA
Session III: Using New Approach Methods to Predict Repeat-Dose and Complex Apical Endpoints Session Chairs: Dr. Yuki Sakuratani (NITE) & Dr. Bruno Hubesch (Hubesch Consult)	
8:40-9:05 ***Ballroom &Zoom***	***Development of safety prediction system (AI-SHIPS) for industrial chemical compound using AI by METI*** **Dr. Kimito Funatsu** – Nara Institute of Science and Technology, Japan *The official name of this project is Development of next generation integrated chemical safety prediction system by using A.I. and chemical toxicity related big data. Backgrounds for establishment of this project are 1. Strengthen competitiveness of Japanese material/chemical industry, 2. Changes in the global competitive situation of business (the customer's request level is high (safety, SPEC), and the speed is particularly required), and 3. Trend of reducing, replacement and refinement animal testing (3R).* *In this project, we developed in silico prediction system for 28-day repeated dose toxicity test. As a way of proceeding, it was decided to develop a prediction system for hepatotoxicity in the first stage and a prediction system for hematological toxicity and nephrotoxicity in the second stage. Those three toxicities EP were chosen because most NOELs of those environmental chemicals are observed on these toxicities in the 28-day repeated dose study.* *The basic development policy and strategy for the construction of this toxicity prediction system AI-SHIPS are the following four points. First, in the previous SAR system, the relationship between chemicals information and prediction endpoint was a black box, but in this system, a prediction method was developed based on the toxicity expression mechanism, that is, AOP and mode of action. 2nd point is that the system incorporates environmental chemical PBPK predicting system. 3rd point is that we have prepared a data matrix including the latest toxicity research information and developed predictive models using the latest computational science methods such as machine learning.* *Finally, we also aimed to improve the accuracy of the prediction system by reflecting appropriate in vitro assay results such as the signal transduction mechanism etc.*

As a feature of this system development, we use high-quality, uniform about 2000 GLP-level 28 days repeated-dose toxicity test HESS, CSL and REACH EP information as training data sets.
Furthermore, "Funatu's three-layers model; chemical substanceà in vitro à in vivo" is adopted as basic pillar for development of the in vivo prediction model.
Finally, an in vivo prediction model is constructed using the in vitro predicted values obtained by in vitro prediction models. In other words, in order to construct a systemic toxicity prediction system based on the toxicity onset mechanism, we emphasize the relationship information between chemical substances and MIK and KE, and build a system to predict endpoints at the in vivo level by making full use of this information. Therefore, the basic concept of Funatsu's three-layers model was established. This has made it possible to fully utilize the reactivity information of chemical substances and living protein such as enzymes, receptors for the construction of more accurate prediction methods.

9:05-9:30
Ballroom &Zoom

An in vitro battery for developmental neurotoxicity evaluation: from basic science to a regulatory paradigm shift in 21st century Toxicology

Dr. Ellen Fritsche – IUF Leibniz Research Institute for Environmental Medicine, Germany

Testing for developmental neurotoxicity (DNT) is currently performed in rats according to OECD/US-EPA guidelines. These methods are very costly, take a long time and have uncertainties in their interpretation. To overcome these issues and allow a large number of chemicals to be tested for DNT, a DNT in vitro testing battery (DNT IVB) has been assembled under the guidance of the European Food Safety Authority (EFSA) in collaboration with the Danish- and US-Environmental Protection Agency and under the umbrella of the Organisation for Economic Cooperation (OECD). Despite the large coverage of neurodevelopmental key events in the DNT IVB, some gaps were already identified. These concern radial glia, astrocyte and microglia-related endpoints.
The DNT IVB consists of test methods based on primary human neural progenitor cells (hNPC), human induced pluripotent stem cell (hiPSC)-derived neural crest cells and neurons, as well as LUHMES cells and model the key neurodevelopmental processes hNPC proliferation, migration and differentiation into neurons and oligodendrocytes, neurite morphology, neural crest cell migration and neurite outgrowth. This IVB was challenged with 120 chemicals. In addition, a hiPSC-based test method for human neuronal network formation was set up and challenged with 28 pesticides. To close the identified IVB gaps, we started setting up methods concerning radial glia, astrocytes and microglia within the LRI AIMT11 project.
Concentration-response curves reveal benchmark concentrations (BMCs) for the 120 compounds in the individual test methods. Classification models for data interpretation were applied. For interpretation of compound results across the whole battery, respective most sensitive endpoints (MSEs) were determined. Battery results were used in two case studies, i.e. hazard characterization of deltamethrin and flufenacet in an Adverse Outcome Pathway-informed Integrated Approach for Testing and Assessment (by EFSA) and flame retardant prioritization (by the US-National Toxicology Program). For the newly developed methods on radial glia, astrocytes and microglia, we established respective marker expression over development and responses to first compounds.
These data demonstrate the successful set-up of a DNT-IVB that can be used for different regulatory purposes. An OECD guidance document has been prepared (Crofton & Mundy 2021) that informs on use and interpretation of the DNT-IVB for regulatory application and highlights DNT ICB gaps. Increasing trust in battery performance by closing these gaps, testing more chemicals and lab-to-lab transfer will aid its implementation into regulation.

9:30-9:55
Ballroom &Zoom

Internal Threshold of Toxicological Concern (iTTC): Where We Are Today and What Is Possible in the Near Future

Dr. Corie Ellison – The Procter & Gamble Company, USA

The Threshold of Toxicological Concern (TTC) is an important risk assessment tool that establishes acceptable low-level exposure to chemicals with limited toxicological data. Traditionally, the TTC has been used in food safety and consumer product safety evaluations for chemicals with low exposure potentials. However, in cases where the scientific confidence of the TTC approach is appropriate for a given decision context, the TTC can be applied more widely. One of the next steps in the continued evolution of TTC is to develop the concept further so that it is representative of internal exposures. This refinement of TTC based on plasma concentrations referred to as internal TTC (iTTC) requires a significant amount of data and computational tools that can be used to convert the chemical-specific external dose NOAELs in the TTC database to an estimated internal exposure for each chemical. To achieve an iTTC that is suitable for human safety risk assessment, a multi-stakeholder collaboration between Cosmetics Europe Long-Range Science Strategy program, American Chemistry Council Long-Range Research Initiative, and the Research Institute for Fragrance Materials (RIFM) was established. The chemicals selected to generate the iTTC database come from multiple existing TTC/chemical databases, including Munro et al. (1996), COSMOS (Yang et al., 2017) and the RIFM database (Patel et al., 2020). For the 1,251 chemicals in the combined database, a literature search was conducted to identify existing in vivo and in vitro pharmacokinetic (PK) data.

Additionally, chemical space analysis was conducted for the 1,251 chemicals using molecular and PK descriptors so that the combined database could be reduced to approximately 300 chemicals for final inclusion in the iTTC database. In vitro oral absorption data for each chemical in the iTTC database and in vitro hepatocyte clearance for chemicals lacking literature values are being generated. Iterative batch processing physiologically based pharmacokinetic (PBPK) modeling is being used for the iTTC database chemicals to establish the best conditions for PBPK modeling and to make quantitative comparisons between the PBPK model simulations and the existing PK data. In addition to this work bringing us closer towards establishing the final iTTC values, it has provided an opportunity to generate new structured data repositories for PK data and has helped inform approaches for in vitro to in vivo PBPK modeling for a wide range of chemicals. The current presentation will share the status of the iTTC project and will provide a representative example use scenario for iTTC.

9:55-10:20
Ballroom &Zoom

Potential use of in silico methods for the ecological risk assessment in the chemical management in Japan

Dr. Hiroshi Yamamoto – National Institute for Environmental Studies (NIES), Japan

In Chemical Substances Control Law (CSCL), there has been long needs from industry side for the use of in silico methods not only for bioaccumulation and biodegradation tests but also for ecological risk assessment. KAshinhou Tool for Ecotoxicity, QSAR-based ecotoxicity prediction system researched and developed by National Institute for Environmental Studies (NIES) under the contract with Ministry of the Environment (MOE) Japan, first released in 2008, and later updated for several times before the English version (KATE2017) was first released in 2018. The latest version of KATE2020 ver.3.0 (https://kate.nies.go.jp/onnet2020-e.html) was released in March 2022.

The main features of KATE2017 and 2020 are the categorization of chemicals based on substructure and the reactivity, and the toxicity values for input chemical can be predicted for fish, daphnid and alga for both acute (EC50 or LC50) and chronic (NOEC) using linear regression with the explanatory variable, octanol-water partitioning coefficient (log P). Training dataset used was over 1000 chemicals mainly those obtained by MOE in GLP labs with relatively high reliability in conformity with OECD guideline for testing of chemicals Nos. 201, 202, 203, 210, and 211. Only for fish acute toxicity tests, additional training dataset was provided from US Environmental Protection Agency (USEPA) from Fathead Minnow Database. The external validation with test dataset from OECD SIDS database, we found that the relatively better prediction accuracy with nearly 80% are within the range of one order of magnitude difference from the measured value while ECOSAR of USEPA showed relatively less accuracy with large applicability domain.

As for the use of in silico methods such as QSAR (including KATE and ECOSAR) and category approach in CSCL, KATE and ECOSAR have long been used only as a reference for the registration of new chemicals while these predictions can be better used in the designation of priority assessment chemicals after the proper guidance is established. For the detailed risk assessment of the priority assessment chemicals, the use of in silico predictions for the weight of evidence approach for the determination of PNEC.

In the other chemical risk assessment framework, such as Initial Environmental Risk Assessment of Chemicals, which has been conducted since 2002, even vigorous use of in silico methods is expected to fill in the gap of test data. Since this is the screening level risk assessment for the prioritization of air and water quality standards (or guidance values) and emerging chemicals of concern such as pharmaceuticals and personal care products (PPCPs), further detailed risk assessment and management should be conducted in the higher tier risk assessment, anyway.

10:20-10:40
Ballroom &Zoom

Q&A and Panel Discussion

Dr. Kimito Funatsu – Nara Institute of Science and Technology, Japan
Dr. Ellen Fritsche – Leibniz Research Institute for Environmental Medicine, Germany
Dr. Corie Ellison – The Procter & Gamble Company, USA
Dr. Hiroshi Yamamoto – National Institute for Environmental Studies (NIES), Japan

10:40-11:00 **Morning Break**

Session IV: Integrating Exposure Assessment NAMs (Internal & External) into Risk Evaluations
Session Chairs: Dr. Tomoya Yamada (Sumitomo Chemical) & Dr. Richard Becker (ACC)

11:00-11:25
Ballroom &Zoom

Practical Use of Toxicokinetics Information for a Read-Across of Repeated Dose Toxicity – A Case Study Using Halogenated Aliphatic Compounds Hepatoxicity Category

Dr. Shino Kuwa– National Institute of Technology and Evaluation, Japan

The study aimed to utilize the physiologically based kinetic (PBK) model to explain the variability and uncertainty of toxicokinetics between the target substance and its analogs in read-across explicitly for subcategorization of analogs and uncertainty characterization through a case study. In this case study, read-across of compound category for hepatotoxicity by halogenated aliphatic compounds in HESS Category was attempted and 1,3-dichloropropane (CAS RN 142-28-9) was chosen as the hypothetical target substance Traditionally, PBK models have been developed for individual substances with enough kinetic data but the recently published OECD guidance document for the PBK model introduces the assessment framework for "data-poor" situations. In this study, a tool incorporating a generic human PBK model, Age-dependent Biologically Effective dose Evaluation Tool (ABEET), was modified to calculate blood and liver concentrations in rats during repeated dose toxicity tests for the target substance and analogs without kinetic data. The predictive capability was validated using read-across approach described in the OECD guidance document for the PBK model. The AUC24h (Area-Under-the-Curve over 24 hours) of the liver concentration at 1 unit dose was chosen as a toxicokinetic indicator for read-across. Substances with toxicokinetic dissimilar characteristics from the target substance that may affect their toxicity were detected and excluded from the analogs by characterizing the correlation between the AUC24h of liver concentration and hepatotoxicity. In this case-study, PBK model contributed to provide quantitative ADME information for read-across and to reduce and describe the uncertainty.

11:25-11:50
Ballroom &Zoom

A case study of human risk assessment of a substance using a PBPK modeling technique

Dr. Jun Abe – Sumitomo Chemical Co., Ltd., Japan

It is generally known that interspecies differences in efficacy or toxicity occur when significant pharmaco/toxicokinetic and pharmaco/toxicodynamic variations arise among species. The use of kinetic data to describe the internal dosimetry of non-pharmaceutical compounds is becoming more important; however in most cases, it is impossible to obtain kinetic data experimentally in humans. To overcome this issue, physiologically based pharmacokinetic (PBPK) model has been being utilized. In the kinetic research in humans using PBPK models, it is common to initially develop an animal PBPK model using the available in vitro and in vivo kinetic data from the same animal species, and then to convert the animal model to a human model by applying human in vitro data as the input parameters. For the compounds which only have simpler elimination route, e.g. hepatic metabolism, many examples are reported to successfully predict the human kinetics thus PBPK has been regarded in the practical level. However, when the target compound exhibits complicated kinetics, it is difficult to reproduce its kinetics only by the combination of in vitro experiments.
Here, we present a case study that the reliable human exposure assessment was achieved for a compound which has very complicated kinetics. It has already been revealed that the compound immediately metabolized in mammals and its major metabolite (Metabolite A) exhibits quite complicated kinetic profiles; e.g. active hepatic uptake via transporters followed by hepatic metabolism and biliary excretion. For the accurate prediction of the kinetics of Metabolite A in humans, we adopted the use of chimeric mice with humanized liver alongside the in vitro hepatocyte uptake assay, and obtained some key human parameters, i.e., hepatic uptake intrinsic clearance and hepatic elimination intrinsic clearance via biliary excretion and metabolism. Finally, all human parameters which were determined to have higher influence by sensitivity analysis were obtained without applying simple extrapolation without biological basis (i.e. assuming no species difference between humans and mice), and the exposure of Metabolite A in human could be evaluated.
We believe this approach which utilizes the chimeric mice to obtain human hepatic parameters can accurately predict the pharmacokinetics, but further proof of this concept was still desired. In order to demonstrate the feasibility of the concept, we applied the similar approach to predict the rat kinetics. The chimeric mice with rat liver were prepared and the rat hepatic parameters were obtained. Finally, the kinetics of Metabolite A in rats were predicted without any rat kinetic parameters obtained from in vivo rat experiment. The data showed that the kinetics of Metabolite A in rats in vivo were well reproduced with the PBPK model referring the parameters obtained from the chimeric mice, suggesting the concept of this approach is well validated.

11:50-12:15 ***Ballroom &Zoom***

In Vitro Data To Parameterise PBPK Models For Inhalation Exposure (Cefic LRI project B21)

Dr. Katharina Schwarz – Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Germany

Use of chemicals in the consumer and occupational environment requires a risk assessment considering both the hazard of and exposure to a substance. In both cases, the inhalational route is of major importance. Usually the internal dose is not known due to absence/lack of in-vivo data describing systemic uptake. Up to know, the inhaled dose, or the dose deposited in the respiratory tract is typically considered as the relevant dose for the risk assessment process. This is a pragmatic approach which, however, often results in uncertainties and conservative assumptions.

To bridge the gap between external exposure concentrations to internal concentrations inside the body, an inhalation-focused physiologically based pharmacokinetic (PBPK) model for humans has been developed to provide a more refined determination of the systemic available dose. This PBPK model was aimed to be publicly available and to deliver information relevant for regulatory needs.-

The new model divides the lung into several sub-compartments due to its heterogenic structure and different local clearance / uptake mechanisms. It considers deposition and dissolution, mucociliary clearance and macrophage uptake as well as absorption into blood. The attached systemic part consists of a generic state-of-the-art design that includes various tissues. Substance-specific respiratory input parameters such as lung permeability, particle dissolution and gas uptake, can be generated in vitro. Transfer rates/permeability coefficients across lung epithelial cells are determined in an vitro inhalation testing with suitable human cell and tissue barrier models for the pulmonary and tracheobronchial regions utilizing the P.R.I.T.® ExpoCube®. Dissolution rates of poorly soluble particles are measured in simulated biological fluids. For integration of substance-unspecific clearance processes like mucociliary and macrophage-mediated clearance existing literature data are used. Systemic parameters can be mostly obtained using established in-silico tools. Several substances were used to investigate the applicability of this model: highly soluble nicotine, poorly soluble toluene (vapor), and bio-inert iron oxides and polystyrene (aerosol). Predicted lung retention or blood kinetics for these substances were compared to available clinical data and show very good agreement with both the quantitative concentration levels as well as the qualitative concentration or lung burden profile.

A particular focus has been on the analysis of the sensitivity of the input parameters and their individual relevance regarding the systemic uptake kinetics/lung retention for the different substance classes. The identification of the key determinants is aimed at minimizing the number of input parameters required to optimize usability of the PBPK model.

The results indicate that the lung PBPK model has a wide applicability domain and can successfully predict respiratory uptake and bioavailability from gases and vapors to liquid and solid particles for exposure durations of a single or a couple of breaths to several hours and clearance for up to several months. Future steps of the lung PBK model comprise the inclusion of different human subpopulations, lung metabolism, the diseased lung, and application for rats, all of which were already explored in the project. A user-friendly application for the PBPK model has been developed and will be made publicly available shortly.

12:15-12:40 ***Ballroom &Zoom***

Development and Evaluation of a Holistic and Mechanistic Modeling Framework for Chemical Emissions, Fate, Exposure, and Risk

Dr. Jon Arnot – ARC Arnot Research & Consulting, Canada

Thousands of new and existing chemicals are subject to ecological and human health risk assessment; however, extensive data gaps for exposure estimation in particular present obstacles to addressing regulatory objectives. The Exposure And Safety Estimation (EAS-E) Suite freely accessible on-line platform (www.eas-e-suite.com) facilitates regulatory objectives and the safe and sustainable production and use of chemicals in society. EAS-E Suite contains databases of chemical information and various models and tools to support chemical evaluations (i) retrospectively for existing chemicals and use, and (ii) prospectively (forecasting) for new chemicals and for proposed changes of use of existing chemicals. The PROduction-To-EXposure High Throughput (PROTEX-HT) in EAS-E Suite quantifies relationships between production volumes, chemical emissions throughout the lifecycle, fate, and transport in natural and manufactured environments (e.g., homes), persistence, bioaccumulation, toxicokinetics in ecological receptors and humans and aggregate human and ecological exposures to estimate the potential for effects. PROTEX-HT requires only two chemical-specific pieces of information as input parameters: production volume and structure. The PROTEX-HT model predictions are evaluated using 95 chemicals for which human biomonitoring data are available. Seventy-nine percent and 97% of the PROTEX-HT human exposure predictions were within one and two orders of magnitude, respectively, of independent human exposure estimates inferred from biomonitoring data from the US population. The results show how PROTEX-HT in EAS-E Suite can support: (i) the screening and ranking of chemicals based on various exposure and risk metrics, (ii) setting chemical-specific maximum allowable tonnage based on user-defined toxicological

thresholds, and (iii) identifying the most relevant emission sources, environmental media, and exposure routes of concern. The results also show that high chemical tonnage does not necessarily result in high exposure or health risks. PROTEX-HT enables efficient screening-level evaluations of existing and premanufacture chemicals in various exposure- and risk-based contexts for holistic and effective chemicals management.

12:40-13:00
Ballroom &Zoom

Q&A and Panel Discussion

Dr. Shino Kuwa – National Institute of Technology and Evaluation, Japan
Dr. Jun Abe – Sumitomo Chemical Co., Ltd., Japan
Dr. Katharina Schwarz – Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Germany
Dr. Jon Arnot – ARC Arnot Research & Consulting, Canada

13:00-14:15 **Lunch Break**

Session V: The challenges towards improved risk evaluations - Quantitative Human Risk Assessment

Session Chairs: Dr. Hajime Kojima (NIHS) & Dr. Bruno Hubesch (Hubesch Consult)

14:15-14:40
Ballroom &Zoom

Integrating New Approach Methodologies into Quantitative Risk Assessments

Dr. Les Recio– ScitoVation LLC, Durham, NC

Over the last decade, a significant shift has emerged in the safety assessment chemicals with an aim to need to reduce reliance on animal testing and to develop highly predictive human-based biological models. New in vitro human-relevant biological test systems integrated with in silico methods, collectively referred to as new approach methodologies (NAMs) are revolutionizing the practice of toxicology with a significant movement away from the traditional high cost and ethical concerns of animal testing. The goals for implementation of NAMs are to efficiently identify potential chemical hazards and to gather dose-response data to inform human-relevant safety assessment. NAMs are gaining importance as an alternative to traditional in vivo toxicity testing. However, replacement of traditional animal toxicity tests for toxicology safety assessments with NAMs can encounter significant regulatory hurdles including how accurately NAMs predict responses known to occur in traditional regulatory testing and more importantly what can occur in humans are the biggest challenges for acceptance of NAMs in place of animal data by regulatory agencies. To overcome this challenge, Scitovation is focused on human relevant airway and hepatocyte models integrated with apical measures of cellular health status and genomics-based presumptive modes of action (MOA) to develop quantitative estimates of relevant dose responses to derive benchmark dose and point of departure (POD) estimates. Significant concordance of in vivo derived POD estimates for gene expression changes and apical measures of toxicity in animals or cancer outcomes have been observed for several chemicals. Scitovation scientists are using NAMs-based approach with in vitro to in vivo extrapolation (IVIVE) modeling of dose-response and POD estimates "reverse dosimetry" to assess how well IVIVE aligns with in vivo exposures expected to give similar tissue dose. A recent case study has shown that IVIVE modeling of air:lung interface exposures to 1,3-dichloropropene and calculated rat equivalent inhaled concentrations across the air ways (nasal to alveolar epithelium) were qualitatively in accordance with empirically derived based air way PoD estimates. The IVIVE predicted PoD concentrations for 1,3-dichloropropene were slightly higher than the PoDs observed from in vivo subchronic studies. This case study implementing a NAM-based chemical risk assessment approach for inhalation toxicology has provided insight into the predictive power of this approach and opportunities to increase confidence in the predictivity of NAMs. These data and others support the use of NAMs in chemical safety testing as a more efficient and human relevant alternative to rodent in vivo inhalation studies.
We thank the American Chemistry Council Long-Range Research Initiative funding and PETA Science Consortium International for donation of the Vitrocell® exposure system.

14:40-15:05
Ballroom &Zoom

Evaluating the human relevance of chemically induced liver tumors in rodents – Quantitative risk assessment based on the mode of action –

Dr. Satoki Fukunaga – Sumitomo Chemical Co. Ltd., Japan

Some chemicals are known to interfere with hepatic heme biosynthesis in rodents, mainly by inhibiting particular enzymes of the heme biosynthesis pathway, which leads to accumulation of porphyrins in many tissues including the liver (chemically induced porphyria) and ultimately the development of liver tumors. Compound X is not genotoxic and induces liver tumors in male mice via a porphyria-mediated cytotoxicity mode of action (MOA) with the following key events (KEs): (#1) inhibition of mouse protoporphyrinogen oxidase (PPO); (#2) accumulation of porphyrins; (#3) hepatocellular injury; with (#4) subsequent regenerative cell proliferation; and ultimately (#5) development of liver tumors. These key events for the porphyria-mediated cytotoxicity MOA in rodents are considered to have some similarities with human

porphyria leading to liver tumorigenesis, and therefore this carcinogenicity MOA is considered qualitatively plausible in humans.
In the case of a carcinogenicity MOA in rodents which is qualitatively relevant to humans, quantitative assessment between rodents and humans for each KE needs to be considered to address human relevance, in accordance with the International Programme on Chemical Safety (IPCS) Human Relevance Framework. Thus, we conducted a quantitative assessment and human relevance analysis based on the MOA, taking into account quantitative toxicokinetic (TK) and toxicodynamic (TD) differences.
Compound X is immediately metabolized and exists as Metabolite A in mammals, and is actively taken up into hepatocytes via transporters, which corresponds to TK. In vitro TK assay using hepatocytes revealed a 6- to 13-fold lower hepatic uptake of Metabolite A in humans than in mice, which was caused by a significant active uptake of Metabolite A via transporters in mice but with less of a contribution of transporters in humans. Also, in vitro TD assay using hepatic PPO revealed that 14-fold weaker inhibition of PPO (KE #1) by Metabolite A in humans than in mice.
Quantitative differences in the subsequent KEs were also assessed by in vivo studies using the chimeric mouse with humanized liver (PXB mouse), where the mouse liver is repopulated by human hepatocytes at a ratio of around 90%. By administration of Compound X to PXB mice in which human hepatocytes from three donors were transplanted, slight porphyrin accumulation (KE #2) occurred, but neither hepatocyte injury (KE #3) nor subsequent regenerative proliferation (KE #4) was observed. Although there are no data on liver tumor formation in humans (KE #5), it is highly unlikely that Compound X will induce liver tumor in humans via this MOA since key events #3 and #4 are unlikely to occur in humans.
Overall, quantitative assessment of the MOA demonstrates that Compound X has the potential to induce mouse liver tumors through a nongenotoxic, porphyria-mediated cytotoxicity MOA with a clear threshold, and this MOA is considered not relevant to humans based on the marked TD and TK differences between mouse and human, utilizing the IPCS human relevance framework. Consequently, a linear low-dose extrapolation approach is not appropriate for cancer risk assessment, but instead a nonlinear, threshold margin of exposure risk assessment is appropriate.

15:05-15:30
Ballroom &Zoom

Chemical Risk Assessment: Formaldehyde as a Case Example Use of SILMS to Inform Toxicology Testing Needs and Quantitative Risk Evaluations

Dr. James Sherman – Celanese Corporation, USA

Recent mass spectrometry advancements have significantly enhanced the reliable characterization and quantification of biomarkers of exposure. As such, it is now possible to characterize molecular dosimetry to the molecular target, in potential target organs, throughout the dose-response curve. For chemicals with both endogenous and exogenous exposures, this has greatly increased the understanding of added and relative risk, as well as helped to define thresholds for potential genotoxic and carcinogenic effects. This presentation will provide an overview of Stable Isotope Labeled Mass Spectroscopy (SILMS) and its use in understanding the dose-response for formaldehyde-related nasal tumors and potential genotoxic effects of inhaled formaldehyde at relevant levels of human exposure. Additionally, a case study of using SLIMS to reduce uncertainty related to toxicokinetics and toxicodynamics will be discussed in relation to directing/reducing the need for further animal testing and decreasing uncertainty when deriving acceptable exposure limits.

15:30-15:55
Ballroom &Zoom

Opportunities for NAMs in an EU regulatory context

Dr. Carl Westmoreland – Unilever, UK

At Unilever, our Safety and Environmental Assurance Centre (SEAC) is a team of over 160 safety and environmental sustainability scientists based in Bedfordshire in the UK. We use the latest techniques, deep scientific expertise and an evidence-based approach to ensure that Unilever products are safe for consumers and workers and better for the environment.
To assure that our products are safe for our consumers and the workers that make them, we use a tiered and exposure-driven approach to all our human health safety assessments. We use a wide range of non-animal approaches to assess the safety of our products.
This talk will demonstrate the non-animal approaches we use in our safety assessments concentrating on some of the newer techniques we have developed and are applying in Next Generation Risk Assessment (NGRA). NGRA provides a way to integrate New Approach Methodology (NAM) data from various sources into the safety decision-making process, allowing for safety assessments to be conducted without the use of animal data. Recently, the Internal Cooperation for Cosmetics Regulation (ICCR) outlined nine principles for the use of NGRA to make decisions on consumer safety of ingredients in cosmetics products[1]. These principles have been incorporated into the most recent update of the European Commission's SCSS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation[2] and used by Unilever in several published case studies in the areas of systemic toxicity[3] and skin allergy[4]
Whilst the adoption of NAMs for decisions on consumer safety in the EU continues to progress, there has been slower progress with the acceptance of NAMs for chemical registration in the EU[5], though significant opportunities exist to increase their use and acceptance[6].

15:55-16:15 ***Ballroom &Zoom***	**Q&A and Panel Discussion** **Dr. Les Recio** – ScitoVation LLC, Durham, NC **Dr. Satoki Fukunaga** – Sumitomo Chemical Co. Ltd., Japan **Dr. James Sherman** – Celanese Corporation, USA **Dr. Carl Westmoreland** – Unilever, UK
Closing: Closing Remarks and Next Steps	
16:20-16:35 ***Ballroom &Zoom***	***Wrap-up Session*** **Dr. Tatsuhiro Niino -** Mitsubishi Chemical Corporation
16:35-16:45 ***Ballroom &Zoom***	***Closing Remarks*** **Dr. Fumihiko Hasegawa** – President, NITE **Hideo Shindo** – Director General, JCIA
16:45	**Adjourn**

APPENDIX 2. Complete List of Posters Including Abstracts

Poster Session Abstracts

Poster ID	Presenting Author and Affiliation	Poster Title
1	**Yusuke Yamamoto** *Fuji Film*	Application of ADRA to various sensitization assessments
2	**Hideyuki Mizumachi** *Kao*	Reconstructed human epidermis-based skin sensitization method called Epidermal Sensitization Assay (EpiSensA): validation study and development of defined approach
3	**Takayuki Abo** *Kao*	The Short Time Exposure test method that the applicability domain was expanded and the defined approach in assessing eye irritation potential with combination of in vitro test methods as future new guideline
4	**Izuru Mizoguchi** *Tokyo Medical University*	A novel alternative method for evaluation of sensitizing potential by T cell
5	**Etsushi Kuroda** *Hyogo college of medicine*	Development of an evaluation for inflammatory particles based on the activation of alveolar macrophages
6	**Hajime Kojima** *NIHS*	Update on Japanese projects regarding the New Approach Method
7	**Yuhji Taquahashi** *NIHS*	A new approach to predicting lethality in acute toxicity studies; Make vital signs simple to measure
8	**Yusuke Okubo** *NIHS*	Establishment of developmental toxicity test based on the integration of FGF signal disruption effects for safety evaluation of drugs and chemicals using human iPS cells
9	**Scott Slattery** *ScitoVation*	NAM-based prediction of point-of-contact toxicity in the lung: a case example with 1,3-dichloropropene
10	**Jamie Scaglione** *ScitoVation*	Development and use of human 2D and 3D hepatic co-culture models as alternatives to traditional hepatotoxicity testing
11	**Sylvia Escher** *Fraunhofer Institute for Toxicology and Experimental Medicine, Germany*	Zet-O-Map- Identify principal molecular drivers of (dys)morphogenesis in zebrafish
12	**Yuhei Nishimura** *Mie University*	Generation of a triple-transgenic zebrafish line for assessment of neuroinflammation in developmental neurotoxicity
13	**Yuko Sekino** *The University of Tokyo*	Proposal of a new AOP for the neurotoxicity and developmental neurotoxicity assessment of glutamate receptor binding agonists that cause learning and memory impairment
14	**Yaichiro Kotake** *Hiroshima University*	Searching for developmental neurological evaluation indices for chemical toxicity assessment

15	**Keishi Ishida** *Gifu Pharmaceutical University*	Validation of brain neuronal differentiation reporter mice for improved developmental neurotoxicity evaluation
16	**Michael Black** *ScitoVation*	Application of toxicogenomics in next-generation risk assessment and predictive toxicology
17	**Mark Viant** *University of Birmingham, UK*	MetAbolomics ring-Trial for CHemical groupING (MATCHING)-Assessing the Reproducibility of Metabolomics Within a Regulatory Context Through a Multi-laboratory Ring-trial (LRI C8)
18	**Maiko Okamoto** *Kao*	Prediction of Repeated Dose Toxicity with Read-across Based on IATA
19	**Bruno Hubesch** *Hubesch Consult*	Linking LRI AMBIT3 chemoinformatic system with the IUCLID6 substance database to support read across of substance endpoint data and category formation
20	**George Daston** *Procter & Gamble, US*	Toxicogenomics and Read Across
21	**Masashi Kamo** *AIST*	Addressing some issues to expand the use of species sensitivity distributions in ecological risk assessment
22	**Kota Hirasawa** *Sumitomo Chemical*	Parameter acquisition with humanized chimeric mice, for human exposure prediction using a PBPK model
23	**Aldert Piersma** *RIVM, Netherlands*	Developing a quantitative AOP for liver-mediated thyroid modulation after prenatal exposure to a xenobiotic compound in the rat
24	**Jeremy Fitzpatrick** *ScitoVation*	Release of Population Life-course Exposure to Health Effects Model (PLETHEM): An Online Tool for PBPK Modeling version 3.2
25	**Hiroaki Todo** *Josai University*	In silico estimation of skin permeation of chemicals with their diffusion and partition parameters
26	**Masanori Niwano** *Sumitomo Chemical*	An application of a 3D hydrodynamic ocean model to predict chemical behavior at waters off Niihama in Seto Inland Sea
27	**Yuriko Ishikawa** *AIST*	Development and application of a spatio-temporal chemical concentration estimation model for watershed risk assessment
28	**Kazuhide Kimbara** *Graduate School of Integrated Science and Technology, Shizuoka University*	A multi-model system for predicting biodegradation rates and the presence of residual substances of chemicals using chemical descriptors and 3D structures
29	**Kaku Oguro** *NITE*	Evaluation of slow-stirring method for determining log Kow (n-octanol/water partition coefficient) of surfactants
30	**Jon Arnot** *ARC Arnot Research and Consulting*	EAS-E Suite: A data integration and modelling framework for chemical safety and sustainability

1 – Application of ADRA to various sensitization assessments

Yusuke Yamamoto[1], Masaharu Fujita[1], Toshihiko Kasahara[1], Yasuhiro Katsuoka[1]

[1]*FUJIFILM Corporation, Safety Evaluation Center, Kanagawa, Japan*

The Amino acid Derivative Reactivity Assay (ADRA) is an *in chemico* test method that evaluates the binding of chemicals to proteins, which is a molecular initiating event (Key event 1) in the adverse outcome pathway (AOP) of skin sensitization. In Key event 1, since sensitizers mainly bind to cysteine and lysine in proteins, ADRA uses cysteine and lysine derivatives *N*-(2-(1-naphthyl)acetyl)-L-cysteine (NAC) and *N*-(2-(1-naphthyl)acetyl)-L-lysine (NAL) as nucleophiles and predicts skin sensitization from the reactivity of these nucleophiles with the test chemical. ADRA was adopted in the OECD test guidelines (TG 442C) in 2019. The following three studies have been conducted so that ADRA can be used for various evaluations: 1) application to mixture evaluation, 2) establishment of photoallergy evaluation method, 3) establishment of respiratory sensitization evaluation method. In this presentation, we report on these three studies.

Evaluation of the mixture by ADRA requires test conditions that do not use molecular weight for preparing test chemical solution and are not interfered by the peaks derived from the components of the mixture in HPLC measurements. Therefore, we attempted to evaluate the mixture by combining the evaluation conditions using the test chemical solution prepared at the weight concentration and the measurement of NAC and NAL by the fluorescence detection. As a result, it was confirmed that the sensitizer contained in the mixture solution of known non-sensitizer could be detected as in the case of the sensitizer alone, and both NAC and NAL could be quantified selectively even in the plant extract having many peaks. We are currently applying for revision to the test guidelines that add this evaluation method.

In the early stage of photoallergy, photo-excited chemicals bind to proteins. Therefore, the test chemical and NAC and NAL were reacted under photo and non-photo irradiation conditions, and it was verified whether the photoallergic potential could be evaluated from the difference in depletion between the photo and the non-photo irradiation conditions. As a result, the predictive capacity vs human in 59 chemicals was as high as 81.4% (48/59).

It is known that many respiratory sensitizers bind to lysine residues in proteins. Focusing on this feature, it was verified whether respiratory sensitizers could be detected by selectively evaluating the reactivity to NAL. As a result, all 29 respiratory sensitizers could be correctly classified, except for substances outside applicability domain.

From the above, it was shown that by applying the ADRA technology, it is possible to evaluate a wider variety of substances such as mixtures, and it may be used for toxicity evaluation other than skin sensitization.

2 – Reconstructed human epidermis-based skin sensitization method called Epidermal Sensitization Assay (EpiSensA): validation study and development of defined approach

Hideyuki Mizumachi[1], Sho Suzuki[1], Masaaki Miyazawa[1]

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The development of strategy for non-animal safety assessment has recently been stimulated, and seven effective alternative methods for skin sensitization have already been adopted to OECD test guideline. In addition, testing strategies combining multiple assay results, Defined Approaches (DAs), have also been adopted to OECD test guideline. However, there are some common limitations on existing alternative methods. One of them is the applicability for lipophilic chemicals (e.g., LogKow >3.5) because all existing methods are based on aqueous system such as culture medium or buffer. Furthermore, it is easy to understand that DAs also have the same limitation. Moreover, pre/pro-haptens can be common limitations because existing methods are based on cell line or in chemico method. On the other hand, lipophilic chemicals can be directly applied on reconstructed human epidermis (RhE) like animal test because RhE have stratum corneum. In addition, RhE may be effective to detect pre/pro-haptens because they have metabolic capability like real human skin (Oesch et al., 2018). EpiSensA, developed by Kao Corporation, is RhE-based assay and the promising method to overcome these limitations. Regarding the method of EpiSensA, a test chemical is exposed on the RhEs and incubated for 6 hours, and the fold inductions of four marker genes of RhEs (*ATF3*, *GCLM*, *DNAJB4*, and *IL-8*) are measured by real-time PCR. When at least one out of the four marker genes exceed the respective cut-off values, the test chemical is judged as positive. In the past, 136 chemical datasets including 69 lipophilic ones and 37 pre/pro haptens have been tested by EpiSensA, and an accuracy of 83% and broad applicability domain for various chemicals were confirmed.

The international validation study in which three laboratories participated was performed under the JaCVAM using 27 chemicals. As a result, within- and between-laboratory reproducibility met the target criteria (85% and 80%, respectively), and the validation management team concluded that sufficient reproducibility was confirmed.

Moreover, EpiSensA-based DA called RhE-based Testing Strategy (RTS) for hazard and potency identification was developed. RTS is simple score-based DA and consist of EpiSensA and the Times Metabolism Simulator platform used for predicting Skin Sensitization (TIMES-SS, OASIS-LMC). Results of EpiSensA and TIME-SS were converted to scores (from 0 to 3) based on the minimum estimated concentration at which any of four marker genes exceeds the respective cut-off (min.EC value) and potency prediction of TIMES-SS, respectively. The hazard and potency identification of RTS was performed from the sum of scores. The predictive performance of RTS was compared to existing DAs adopted as OECD Guideline No.497. It was confirmed that RTS had broader applicability domain than existing DAs at the 136-chemical dataset. Furthermore, the hazard and potency accuracies of RTS were 85% and 74% for GHS categorization based on LLNA results, respectively. In addition, it was confirmed that the predictive performances were comparable to or greater than those of existing DAs such as 3 out of 3 DA or ITS v1/v2 at 136 chemicals.

3 – The Short Time Exposure test method that the applicability domain was expanded and the defined approach in assessing eye irritation potential with combination of in vitro test methods as future new guideline.

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The Short Time Exposure (STE) test method has been adopted as test guideline 491 by Organisation for Economic Co-operation and Development (OECD) since 2015. The STE test method can identify chemicals that induce serious eye damage, defined as Category 1 by the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS), and chemicals that do not cause eye irritation or serious eye damage, defined as No Category by GHS. However, any in vitro test methods including the STE test method can't identify chemicals that induce eye irritation, defined as Category 2 by GHS. In addition, the STE test method had possessed two out of applicability domains.

For two applicability domains, the one of two applicability domains was highly volatile substances (6kPa, 25 degrees C). In 2020, TG 491 was updated for expansion of the out of applicability domain for highly volatile substances. This update led that the STE test method can apply the almost all liquids. The point of update is that all highly volatile substances employ mineral oil (CAS RN 8042-47-5) as a solvent. As a results for highly volatile substances, the accuracy, false positive rate, false negative rate was updated from 75% (15/20), 7.7% (1/13), 57.1% (4/7) to 95.0% (19/20), 7.7% (1/13), 0% (0/7) (Abo et al., 2018).

For the classification of Category 2, two defined approaches for liquids other than surfactants were proposed and discussed in OECD expert group. These two defined approaches would be accepted in 2022 at the earliest. The one of two defined approaches is the combination of the STE test method (TG 491) and the Bovine Cornea Opacity and Permeability Laser-Light Based Opacitometer test method (BCOP LLBO, TG 437). This defined approach classifies GHS No Category or Category 1 by the STE test method first, continuously to classify GHS Category 1 or Category 2 by the BCOP LLBO test method. The accuracies are 81.2% for Category 1, 56.3% for Category 2, and 85.3% for No Category (Alépée et al., 2019). Furthermore, using the above defined approach, the principle was demonstrated by case studies with chemicals.

In conclusion, it is indicated that the defined approach can identify three GHS categories including Category 2 for eye irritation potential with updated the STE test method. Thus, to identify three GHS category can be to lead completely alternative to in vivo tests. After this defined approach has been adopted as guideline by OECD, it should be further extended for the availability to be adopted to authority bodies in various countries.

4 – A novel alternative method for evaluation of sensitizing potential by T cell

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There are mainly two types of allergic responses: one is skin sensitization, that is an allergic response in the skin following skin contact such as allergic contact dermatitis, and another is respiratory sensitization, that is an allergic response in the airways caused by inhalation, mostly asthma. Because a worldwide movement emerged to limit the use of animal models in safety testing of chemicals, several *in vitro* assays to predict the skin and respiratory sensitizing potential of chemicals have been developed. However, none of these alternative methods can distinguish between chemical respiratory sensitizers and skin sensitizers. The adverse outcome pathway (AOP) for skin and respiratory sensitization was described to accurately develop alternative methods for evaluation of them. The AOP contains four key events (KE). All currently developed and validated in vitro assays are based on KE1~3, and there is no validated in vitro assay based on KE4 which is the activation of T cells so far.

To mimic the human airway upper epithelium, we recently developed a novel three-dimensional DC coculture system consisting of upper airway epithelial cells, immature DCs, and lung fibroblast cells cultured in individual scaffolds. In the recent study, to improve the versatility, peripheral monocyte-derived immature DCs were similarly replaced with immature DCs derived from monocyte-derived proliferating cells called CD14-ML established by introducing genes related to the cell cycle and survival and lung fibroblast cell lines were removed from this system. Moreover, to recapitulate in vivo activation of naive $CD4^+$ T cells by DCs that are stimulated with chemical sensitizers in the upper airway epithelium and then migrated into draining lymph nodes, we sought to establish a new 2-step DC/T coculture system by further adding peripheral allogeneic naive $CD4^+$ T cells onto the DCs stimulated in the DC coculture system. In this DC/T coculture system, mRNA up-regulation of Th2 maker IL-4 in the T cells representing the KE4 was successfully used to discriminate respiratory sensitizers from skin sensitizers 5 days after the stimulation. Finally, when allogenic Th2 cell lines established by repetitive stimulation of allogenic $CD4^+$ T cells with DCs were used instead of primary naive $CD4^+$ T cells, selective mRNA up-regulation of IL-4 was observed by the stimulation with respiratory sensitizers 12 hours after the stimulation. Furthermore, Up-regulation of IL-4 at protein level was detected by ELISA 48 hours after the stimulation. Currently, we are continuing to evaluate and improve this 2-step DC/T coculture system.

5 – Development of an evaluation for inflammatory particles based on the activation of alveolar macrophages.

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It has been reported that inhalation of fine particles, such as PM2.5 and sand dusts, are associated with allergic airway inflammation. So far, many studies have demonstrated that some particles stimulate immune systems to induce allergic immune response in the airway. These inflammatory particles are thought to be adjuvants, which induce or enhance antigen-specific immune responses. In general, pathogen-associated molecular patterns (PAMPs) such as LPS and bacterial DNA stimulate innate immune cells such as dendritic cells and macrophages and then activated innate cells induce acquired immune responses. The same way as PAMPs, several inorganic particles also function as adjuvant, however, the mechanisms for the adjuvant activity of particles remains unclear.

As inhaled particles are thought to settle deep in the lungs and to engulfed by alveolar macrophages to clear them from the lungs, we investigate the mode of action of inhaled particles on the activation of alveolar macrophages. we used aluminum salts (alum) as inflammatory particles and previously demonstrated that alum stimulated alveolar macrophages to induce cell death and then released interleukin-1alpha (IL-1α) as dead cell-derived factor, called damage-associated molecular patterns (DAMPs). We also observed that released IL-1α was involved in induction of serum immunoglobulin E (IgE). However, non-inflammatory aluminum oxide (Al_2O_3) particle did not induce cell death and IL-1α. These results suggest that alveolar macrophage death by inflammatory particles and subsequently released DAMPs are closely linked to allergic immune responses. Based on these findings, we thought these phenomena could be applied to an evaluation method to identify “danger” particles.

In this presentation, we will introduce our recent studies regarding the mechanisms of DAMPs release from inflammatory particle-stimulated alveolar macrophages and the application to evaluation method to identify inflammatory particles.

6 – Update on Japanese projects regarding the New Approach Method

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We have ongoing projects that are developing New Approach Methods (NAMs) for systematic toxicology. One NAM is to develop in vitro immunotoxicity test methods for the Organisation for Economic Co-operation and Development (OECD)Test Guideline (TG) and the other is to develop non-animal development and reproductive toxicity test methods for the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) S5(R3) guideline and OECD TG. The development of these NAMs includes the following steps: 1) adverse outcome pathway (AOP), 2) detailed review paper, 3) test methods based on AOP, 4)validation study of test methods for developing TGs, and 5) integrated approaches to testing and assessment (IATA). I believe that the NAMs developed on these steps may enable risk assessment of a chemical with non-animal test methods in near future.

7 – A new approach to predicting lethality in acute toxicity studies; Make vital signs simple to measure

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The endpoint of acute toxicity is "death", which is the ultimate toxicity, and the LD50 value, introduced by Trevan (1927), have been used because of the convenience of showing the toxicity intensity of compounds as a common index. On the other hand, the acute toxicity test was criticized for scientific, economic, and animal ethical reasons (Zbinden 1981), the OECD TG401 using many animals was deleted in 2002, and an improved method with a reduced number of animals was adopted (OECD TG420, TG423 and TG425). However, the criticism from the viewpoint of animal welfare has not converged; the animal mortality is still used as the endpoint (TG423, TG425), or evident toxicity (TG420), which is a less objective endpoint and depending on experimenter. Moreover, the significant shortcoming of acute toxicity study is that it is not useful for the medical treatment of human poisoning because the cause of death, target organs, etc. are not considered at all. In order to solve these issues concerning acute toxicity study, it is great way to obtain valid result with precisely measure and quantify the signs of toxicity over multiple items and make rational criteria for determining the acute toxicity intensity of substances and toxic targets. This will be, however, a complicated and expensive test compared to acute toxicity in which death is used as endpoint. In other words, it is the development of a test method that obtains vital signs from animals corresponding to tests that human acutely poisoned patients undergo in emergencies. For modernization of acute toxicity, we are focusing on vital signs and wearable devices with cutting-edge sensors; the method makes vital signs simple to measure. We established the measurement of ECG and EEG using a novel material, carbon-nanotube yarn (CNT-Y) made from high-purity, highly crystalized, double-walled carbon nanotubes. CNT-Y is an advanced material with excellent electrical conductivity and flexibility; hence, it could potentially be used as a novel electrode for biopotential measurements. CNT-Y is flexible like silk thread, it functions as an electrode when sewn directly to the skin. We obtained a clear ECG waveform from rats and a guinea pig; the QRS amplitude was ~1.4 mV. In rats, we obtained an EEG waveform with an amplitude of ~150 μV; the peak frequency was 0.8 Hz and the range was ~3 Hz according to power spectral density analysis. In the guinea pig, we obtained an EEG waveform with an amplitude of ~500 μV; the first peak was 0.1 Hz, the second peak was 1 Hz, and the range was ~3 Hz. These results show that CNT-Y could be used in toxicology studies to easily and inexpensively obtain high-resolution biological signals. The less invasiveness and easy to measure biopotential are expected to have a ripple effect on toxicity tests. (Health and Labour Sciences Research Grant, Japan)

8 – Establishment of developmental toxicity test based on the integration of FGF signal disruption effects for safety evaluation of drugs and chemicals using human iPS cells

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The number of man-made chemicals including medicines has increased exponentially recently, and exposure to some of them is possible to induce fetal malformations. Although the toxicities of chemicals have been tested in animals, chemicals that are not teratogenic in rodents can cause severe malformations in humans, owing to the differences in the susceptibility to the teratogenicity of chemicals among species. One possible cause of such species differences, other than pharmacokinetics, could be the difference in sensitivity to such chemicals at the cellular level. To clarify this possibility, in vitro assays using human cells would be a powerful tool. In particular, the analysis of intracellular and intercellular signaling pathways in vitro may be useful as one of the important factors that may generate differences in cellular susceptibility to chemicals among species. Because these signaling pathways play important roles in both developmental processes and their robustness from intrinsic and extrinsic noises. We hypothesized that the developmental toxicity itself means a result that the signal pathways were disrupted regardless of the causes. Then, we established a signal reporter assay system based on real-time monitoring and the time-accumulation of the signal disruption over time, rather than the classical endpoint detection of the signal disruption. This approach was useful for detecting signal disruptions caused by the malformation chemicals, including thalidomide[1, 2]. The human iPSC-based signal disruption assay could be a promising tool for the initial screening of developmental toxicants.

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Keywords: Developmental toxicity testing, Alternative assays, Signal disruption, Human pluripotent stem cells, FGF signaling

9 – NAM-based prediction of point-of-contact toxicity in the lung: a case example with 1,3-dichloropropene

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Time, cost, ethical, and regulatory considerations surrounding in vivo testing methods render them insufficient to meet existing and future chemical safety testing demands. There is a need for the development of in vitro and in silico alternatives to replace traditional in vivo methods for inhalation toxicity assessment. Exposures of organotypic airway epithelial cultures to gases or aerosols can assess tissue responses and in vitro-in vivo extrapolation can align in vitro exposure levels with in-life exposures expected to give similar tissue exposures. The airway epithelium varies along its length, with different regions composed of different cell types. Therefore, in this study a volatile compound of known toxicity, 1,3-dichloropropene (1,3-DCP), was administered as a vapor at the air liquid interface to five organotypic in vitro airway epithelial cell culture models—MucilAir of nasal, tracheal, or bronchial origin, SmallAir, and EpiAlveolar—that represent five regions of the airway epithelium—nasal, tracheal, bronchial, bronchiolar, and alveolar. Toxicity was monitored in these cultures 24 hours after acute exposure using an assay for transepithelial electrical conductance (for epithelial barrier integrity) and the LDH release assay (for cytotoxicity). Finally, an airway dosimetry model for 1,3-DCP vapor was developed to predict in vivo external exposure scenarios that would produce toxic local tissue concentrations as determined by in vitro experiments. Measured in vitro points of departure (PoDs) for all tested cell culture models were similar (109—194 ppm). Calculated rat equivalent inhaled concentrations varied by model according to position within the airway, with nasal respiratory tissues being the most proximal and most sensitive tissue (191 ppm), and alveolar epithelium being the most distal and least sensitive tissue (427 ppm). These predictions are qualitatively in accordance with empirically determined in vivo PoDs. The predicted PoD concentrations were close to, but slightly higher than, PoDs reported for in vivo subchronic studies.

10 – Development and use of human 2D and 3D hepatic co-culture models as alternatives to traditional hepatotoxicity testing

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New approach methodologies (NAMs) are gaining importance as an alternative to traditional in vivo toxicity testing, as the latter involves high cost and time requirements as well as ethical considerations. Regulatory agencies and industry are developing biologically relevant in vitro cell models for efficiently and accurately predicting the toxicity of chemicals. The liver has been a major focus of these efforts, yet there are currently no in vitro alternatives for hepatotoxicity testing accepted by regulators. While hepatocytes are the primary component of the liver, the liver non-parenchymal cells (NPCs) also play an important role in hepatotoxicity. Our goal was to develop 2D and 3D in vitro models using co-cultures of cryopreserved human hepatocytes and NPCs as an alternative to traditional toxicity testing of pharmaceutical compounds and commodity chemicals. These models are characterized by the presence of hepatocyte-specific markers (Albumin, CYP3A4) and NPC-specific markers (Desmin, ACTA2, LYVE1) using qRT-PCR and by immunostaining for albumin, LYVE1, and Vimentin. The utility of these 2D and 3D hepatic co-culture models to measure the cytotoxicity response of compounds was evaluated by studying dose-response profiles of 12 compounds. As a control model for comparison, human hepatocytes plated as mono-cultures were also assayed. The compounds tested were acetaminophen, aflatoxin B1, tamoxifen, acetochlor, flusilazole, carbaryl, bisphenol A, triflumizole, ethofumesate, ametryn, dimethenamid, and sucrose with 9 concentrations per compound including an appropriate media control. Cytotoxicity and viability were measured using CellTox Green Assay and CellTiter Glo Assay, respectively. Also, the number of dead cells were measured using high content imaging techniques. We will categorize the test compounds as having low, medium, or high cytotoxicity using the dose-response curves and IC50 values. We also observed that the co-culture model produces more consistent and reproducible dose-response curves, and for some compounds (aflatoxin B1 and ametryn) presence of NPCs helps reduce the cytotoxic effect of the chemical. Species comparison by implementing the ‘parallelogram approach’ for IVIVE comparisons is in progress using data generated in ScitoVation’s rat 2D and 3D models. Further investigation and ongoing detailed analysis of 2D and 3D co-culture systems seek to determine the optimal system for capturing response to chemical exposure that is most representative of in vivo response.

11 – Zet-O-Map - Identify principal molecular drivers of (dys)morphogenesis in zebrafish

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The zebrafish embryo-toxicity test (ZET) is a promising alternative test for developmental toxicity, since abnormal developments can be directly observed microscopically in the transparent developing embryo; its biology has shown to be relevant to humans and zebrafish cells are compatible with high throughput sequencing (Hoffmann et al., 2021). The combination of transcriptome and morphological changes will allow a better understanding of the mechanisms of teratogenicity, an essential prerequisite for the integration of this test as an alternative method in human risk assessment.

The zet-o-map project aims to identify predictive time-resolved transcriptome biomarkers for teratogenic effects. For this purpose, published data such as peer-reviewed publications and data from the Gene Expression Omnibus (GEO) repository were integrated into two databases:

1) The Zebrafish Teratogens database (ZeTera), which includes morphological changes per target, dose level and timepoint. Data curation included the development of ontologies.

2) Transcriptome ZET data from array-based (e.g. Affymetrix/Agilent) as well as from high-throughput RNA sequencing approaches (e.g. Illumina).

Overall data from various experimental settings were considered up to a treatment period of five days. Experiments include chemical perturbations carried out at specific stages of embryonic development but also experiments on unperturbed zebrafish embryos. All transcriptome data were reanalysed starting with raw data files. For high throughput transcriptome, an adapted version of the recently proposed Omics Data Analysis Framework (ODAF) workflow was used (Verheijen et al., 2022).

In total, metadata for 1523 GEO series were identified comprising data from 41 platforms (array based or high-throughput RNA-sequencing). A subset of 392 GEO series from the 11 most frequently used platforms were reanalysed, resulting in about 300 compounds with transcriptome data.

The comparison of 1400 chemicals with morphological effect data, collected in ZeTera, with the 300 compounds with omics data resulted in an overlap of 92 “data rich” compounds. The majority of these chemical stressors are teratogenic compounds, which induced morphological alterations in zebrafish embryos. For the identification of developmental-stage specific transcriptome biomarkers, we grouped data rich compounds into read-across categories with similar modes of action. A data gap analysis revealed that transcriptome data for several relevant time points are lacking for many individual compounds as well as for categories with similar modes of action.

The above outlined approach, as well as the spatio-temporal maps are exemplified for selected read-across groups and further analyses approaches e.g. the integration of data into knowledge maps are discussed.

Key words: zebrafish embryo-toxicity test, developmental toxicity, transcriptomics, morphological alteration

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12 – Generation of a triple-transgenic zebrafish line for assessment of neuroinflammation in developmental neurotoxicity

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The incidence of neurodevelopmental disorders such as autism, attention deficit hyperactivity disorder, and learning disabilities is increasing year by year and has become a major social problem. Although chemical exposure during development has been suggested to increase the risk of developing these neurodevelopmental disorders, the detailed mechanisms underlying developmental neurotoxicity remain largely unclear. Various molecular initiating events are involved in the developmental neurotoxicity mechanisms of chemical substances, but different molecular initiating events often exert toxicity through a common key event. Developing a test method that can evaluate such a common key event is an effective strategy that will lead to the elucidation of the adverse outcome pathway of chemical substances. Impaired differentiation of neural stem cells into neurons and astrocytes, and neuroinflammation mediated by microglia, which are resident immune cells of the brain parenchyma, are attracting attention as the key event common to the developmental neurotoxicity of chemical substances. We have developed a transgenic zebrafish line selectively expressing fluorescent proteins mVenus, mCherry, and Cerulean in microglia, astrocytes, and neurons. Using in vivo fluorescence microscopy, we have assessed the change of microglia, astrocytes, and neurons in the transgenic zebrafish developmentally exposed to neurotoxicants, especially focusing on ethanol. In this presentation, we demonstrate how the triple-transgenic zebrafish line can be used to assess neuroinflammation in developmental neurotoxicity.

13 – Proposal of a new AOP for the neurotoxicity and developmental neurotoxicity assessment of glutamate receptor binding agonists that cause learning and memory impairment.

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The purpose of our LRI project is to propose an adverse outcome pathway (AOP) in which the molecular initiating event (MIE) is the binding of a compound to the glutamate receptors, resulting in the adverse outcome (AO) defined as learning and memory impairment. First, we have established an algorithm for high content imaging to detect dendritic spines with drebrin immunoreactivity, and an image analysis method by machine learning. Then, morphological changes of dendritic spines were quantitatively evaluated using frozen hippocampal nerve cells from rat embryos and neurons derived from human iPS cells. We will validate the possibility as an alternative method for developmental neurotoxicity/neurotoxicity testing, using known compounds with learning and memory impairment.
In Japan, a study group of the Ministry of Health, Labor and Welfare has reported the hazard risks of chronic use of cannabis during juvenile. However, the mechanisms of the risks are still unclear. In this study, we have investigated if the chronic exposure to cannabis during juvenile has hazardous effects, using cultured rat hippocampal neurons. The cultured rat hippocampal neurons in 96-well plates were prepared from frozen embryonic rats' hippocampal neurons (SKY neuron, AlzMed, Inc., Tokyo). At 7, 14, or 21days in vitro, CP55940 (from 100 nM to10 μM) was applied depending on the schedule. After the immunocytochemical staining to visualize drebrin, MAP2, and cell nucleus, the quantitative analyses of neuron number, dendrite length, and drebrin clusters were performed using our algorithms. CP55940 significantly changed distribution of drebrin as well as increased the numbers of drebrin clusters. Further, treatment of 10 μM CP55940 at 7, 14 days in vitro significantly reduced neuron numbers. It has been discussed how cannabinoids affect the juvenile brain, and lead to cognitive dysfunction and emotional abnormality in adulthood. Because drebrin plays a key role in dendritic spine formation of neurons and the morphological plasticity of dendritic spines, changes in drebrin distribution might change glutamate signaling. Identification of drebrin clusters and analysis of their numbers and distribution are promising to detect hazard risks of cannabis.
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14 – Searching for developmental neurological evaluation indices for chemical toxicity assessment

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Although there are standardized methods for evaluating the effects of chemical substances that are considered to be highly sensitive during development on the cranial nerves, they require a great deal of labor and involve the sacrifice of a large number of experimental animals, and a simpler and more accurate evaluation method is desired. In recent years, basic research in the field of neuroscience has revealed a number of molecules involved in neurite outgrowth and neuronal circuit reorganization, but few attempts have been made to evaluate chemical effects using these molecules as indicators. The purpose of this study was to identify key molecules in neurodevelopment that can be used as better indicators to assess neurotoxicity of chemicals during development and to clarify their usefulness in assessing neurotoxicity of chemicals during development.
We established culture conditions for primary cultured rat cerebral cortex neurons and examined changes in mRNA expression encoding 12 molecules that play key roles in neurodevelopment. The results showed that there were three major patterns: genes whose expression was low in early culture and increased with culture, genes whose expression was maximal in mid-culture, and genes whose expression was maximal in early culture and then decreased with culture. Next, methylmercury, tributyltin, and acrylamide were used as known developmental neurotoxicants, and the effects of these molecules on gene expression were examined. The results showed that the expression of three genes, *Dlg4*, *Syp*, and *Bdnf*, fluctuated at specific time points. By examining whether these genes are also altered by many other suspected developmental neurotoxicants, useful developmental neurotoxicity markers may be identified.

15 – Validation of brain neuronal differentiation reporter mice for improved developmental neurotoxicity evaluation.

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Developmental neurotoxicity (DNT) caused by environmental chemicals is a great concern because the number of patients with neurodevelopmental disorders has been increasing in recent years. Although the in vivo DNT guideline test has been updated in 2007, it still has some disadvantages of high-cost and poor time efficiency. To overcome the disadvantages of in vivo DNT tests, in vitro or in silico DNT tests are proposed as alternatives; however, it is still difficult that the results of these tests would reflect the central nervous system of mammals, and may overlook the secondary effects caused by hypothyroidism, etc. Thus, a new technology is required that can more easily detect chemical-induced DNT in in vivo. Here, we created a transgenic (Tg) mouse expressing the reporter gene (luciferase; Luc) under the control of a promoter of neuronal differentiation marker and evaluated its usefulness in detecting DNT. First, the chronologic changes of brain Luc activity were evaluated using in vivo bioluminescence imaging and in vitro Luc assay of brain tissues. Luc activity peaked immediately after birth, decreased with age and reached plateaus after the weaning period. Because this temporal pattern of Luc activity is analogous to the generally recognized temporal changes of the dendritic spine numbers in the developing brain, the changes in Luc expression are thought to reflect the state of brain neural differentiation. Rodents, prenatally exposed to sodium valproate (VPA), are established as an animal model that exhibit some autistic spectrum disorder-like symptoms. Therefore, we administered VPA (500 mg/kg i.p.) to pregnant mice at gestation day 12.5 and evaluated the temporal changes of in vivo luminescence in the developing brain of Tg pups. In vivo luminescence in the brain of VPA-treated group significantly decreased from postnatal day 4 to weaning compared to vehicle-treated group. Furthermore, we performed Nissl staining in the prefrontal cortex at 8 weeks postpartum and confirmed that the number of neurons in VPA-treated group was significantly decreased compared to vehicle-treated group. Consistent with this, Luc activity in the same brain area of VPA-treated group was also decreased. These results suggest that Luc expression in Tg mice may be a useful marker reflecting the state of brain neural differentiation. In vivo luminescence using our Tg mice could be a potential tool for detecting chemical-induced DNT on developing brain easily.

16 – Application of toxicogenomics in next-generation risk assessment and predictive toxicology

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Transcriptomics in toxicology uses quantitative measures of cellular mRNA transcripts to measure changes in gene expression as a response to exposure to a chemical. Changes in gene expression have proven highly informative data for determining cellular modes of action (MOA) and determining points of departure (POD) for hazard assessment and comparative potency of congeners. Benchmark dose modeling (BMD) combined with conventional ontology enrichment to derive points of departure (POD) based on functional cellular pathway changes allows for the biologically relevant POD values from short term bioassays (in vivo and in vitro) to increase throughput at reduced cost. In vivo toxicogenomic studies also offer a path forward to reduce or eliminate animal testing. Transcriptomic values were used in a study of the food contaminant acetamide to derive short term POD values for risk assessment. The overall consistency of these transcriptomic POD values with those for increased cell proliferation based on Ki67 labeling indicate transcriptomic POD values offer valid and useful alternatives to conventional toxicology POD estimates. Similarly, in vitro exposures to ketoconazole and phenobarbital in 3 cancer cell lines (HepG2, MCF7 and Ishikawa cells) indicated that transcriptomic POD values provide a consistent and reliable means of deriving POD values for toxicogenomics from very short-term in vitro assays. Comparative analyses of differentially expressed genes provides a mechanism for transcriptomic based read-across comparing alterations in genes between compounds. ScitoVation developed a quantitative mechanism, the modified Jaccard index (MJI)to compare transcriptomic derived MOA analyses between compounds. Use of the MJI offers another tool for transcriptomic read across by quantitatively comparing the similarity of functional ontology enrichment using differential gene expression and conventional ontology enrichment analysis. This takes the comparison of changes in individual genes to a comparison of cellular functional affects as well. Transcriptomic raw data is often publicly available (e.g., NCBI's Gene Expression Omnibus, GEO). However interactive or comparative forms of processed data or analyses results are not. We are also building an analyses pipeline around a MySQL database that captures both raw transcriptomic data, processed data and analysis outputs to build out a comparative database and comparative analyses toolkit. Combined with visualization tools such as our MoAviz interactive ontology enrichment viewer application, capturing the entirety of toxicogenomic experiments in a relational database will provide a system for comparative transcriptomic read-across and MOA investigation, along with POD derivation and comparison with similar compounds or compounds with similar cellular biological response.

17 – MetAbolomics ring-Trial for CHemical groupING (MATCHING) - Assessing the Reproducibility of Metabolomics Within a Regulatory Context Through a Multi-laboratory Ring-trial (LRI C8)

Mark R. Viant (University of Birmingham, UK), on behalf of the MATCHING team

Metabolomics has reached a critical point in determining its value to regulatory toxicology. Building on 20 years of metabolomics research, in the last few years the first metabolomics data to support grouping and read-across has been submitted to ECHA; metabolomics best-practice and reporting guidelines for various applications including chemical grouping have been published in *Nature Communications*[1] by the MEtabolomics standaRds Initiative in Toxicology (MERIT) consortium; and an OECD project to develop an *Omics Reporting Framework* is nearing completion.[2] Given these and other examples of its increasing relevance to chemical safety regulations, an assessment of the reproducibility of metabolomics in the context of *chemical grouping* - across multiple labs - is required. The overarching aim of the Cefic-funded MATCHING international ring-trial is to determine whether this technology can demonstrate high reproducibility in grouping, and hence high reliability, or whether refinements in analytical or data analysis practices are needed.

A consortium of multiple industrial, government and academic partners is undertaking this metabolomics ring-trial, including:

- BASF Metabolome Solutions, Germany
- Imperial College London, UK
- University of Birmingham, UK
- Syngenta, UK
- Environmental Protection Agency, US
- National Toxicology Program, US
- Vrije Universiteit Amsterdam, Netherlands

With the aim to demonstrate that these multiple metabolomics laboratories, each analysing and reporting metabolomics data from a single rodent toxicity study, can arrive at the same regulatory conclusion (i.e., the same grouping of chemicals), a series of specific objectives that were organised into three work packages were developed:

1. Prepare quality-checked plasma samples for the ring-trial, derived from 28-day rodent tests using eight chemicals selected from the MetaMap®Tox database by BASF SE and ECHA.

1. Conduct a *blinded* metabolomics ring-trial and report the findings of the grouping study, using OECD guidelines, to ECHA.

1. Collate and evaluate all ring-trial findings, review all statistical analyses and the reporting template used, and disseminate the project outcomes to regulators and industry including recommending any refinements in analytical or data analysis practices.

To date, objective one has been completed, and objective two largely completed, with five of the ring-trial partners presenting results to ECHA in June 2022. Ultimately, a demonstration of high reproducibility of the findings from the grouping case study should help to facilitate the uptake of metabolomics by the chemical industry.

References: [1] Viant, M. R. *et al.* Use cases, best practice and reporting standards for metabolomics in regulatory toxicology. *Nat. Commun.* **10**, 3041 (2019). [2] Harrill, J. A. *et al.* Progress towards an OECD reporting framework for transcriptomics and metabolomics in regulatory toxicology. Regul. Toxicol. Pharmacol. **125**, 105020 (2021).

18 – Prediction of Repeated Dose Toxicity with Read-across Based on IATA

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To develop an alternative strategy for repeated dose toxicity, it is needed to take various factors such as toxicokinetics and toxicodynamics into account in the strategy. Under these circumstances, Integrated Approaches to Testing and Assessment (IATA) which makes an integrated hazard and exposure prediction using New Approach Methods (NAMs) for predicting toxicological effects attracts attention. In the NAMs, read-across which predicts the toxicological effect from structural analogs is predominantly used for assessing systemic toxicity in regulatory use. However, read-across has risk of error in prediction of systemic toxicity due to its uncertainties. It is considered that the slightly structural changes might lead to change of biological responses as the uncertainties. To reduce the uncertainty, comparing the biological similarities based on adverse outcome pathway (AOP) with in vitro and in silico in addition to structural similarities have been discussed. Although it is considered that this concept is useful for predicting systemic toxicity, there is no established concrete strategy due to a lack of case studies. Therefore, we have confirmed the utility of this concept and extracted key considerations for developing a novel strategy through establishing the read-across case studies. In the case studies, we compared the biological similarity with in vitro and in silico among structural similar chemicals which have in vivo data. Then we confirmed the prediction accuracy with real data and extracted key considerations. As a results of case studies, it was confirmed that the combination of in vitro and in silico enhance the prediction accuracy from viewpoint of quality and quantity. The data showed that gene expression array plays the vital role for predicting putative AOP and selecting suitable in vitro test for comparing similarity of toxicological hazard among structural similar chemicals. Furthermore, pharmacokinetic modeling and simulation contributes to comparing the toxicological intensity among the chemicals which have similar toxicological hazard. We considered that these key considerations contribute to the development of new alternative strategy for repeated dose toxicity. In this presentation, we would like to discuss the detail of case studies and key considerations of read-across based on IATA.

19 – Linking LRI AMBIT3 chemoinformatic system with the IUCLID6 substance database to support read-across of substance endpoint data and category formation

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Read-across and category formation are indispensable techniques in safety assessments of chemicals. The read-across approach is used on average in 20% of the Endpoint Study Records, while (Q)SAR is used in less than 1% of the dossiers, according to European Chemical Agency reports. Although many tools are available, only a limited number is capable to provide easily accessible data on substance identity, composition together with chemical structures and high-quality endpoint data.

The AMBIT software, funded initially within the CEFIC LRI programme, provides a web service and user friendly web interface to a chemical database, various chemical structure search facilities and toxicity prediction models. The AMBIT data model supports substances with complex compositions and substances experimental data which allows importing data from the International Uniform Chemical Information Database (IUCLID6) as well as other sources. The chemical structures already contained in AMBIT are automatically linked to constituents/impurities/additives of the imported substances. The flexible data storage and visualization allows for user friendly presentation of study data (physicochemical properties, environmental fate, ecotoxicological and toxicological information) and composition. Comprehensive assessment workflows are developed for read-across and category formation based on all the data available in AMBIT. The assessment workflow facilitates the search for target and source structures through multiple similarity methods, generating data matrices, gap filling and generating assessment reports with predefined formats automatically. The enhanced AMBIT facilitates drafting and improves quality for read-across and category formation and will be a useful tool for experts responsible for substance assessments.

The current landscape of chemical databases is in order of many thousands, distributed under different licenses, and are being continuously updated and may be of interest in different use cases. In addition to REACH study results https://iuclid6.echa.europa.eu/reach-study-results and OpenFoodToxv2, AMBIT3 will follow stakeholder's recommendation and integrate additional sources as e.g. ECHA's REACH 2018 dataset, US EPA CompTox Dashboard, EFSA's OpenFoodTox 2 dataset and the RepDose database.

20 – Toxicogenomics and Read Across

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Read across continues to be the most commonly used non-animal alternative for systemic toxicity assessment of new chemicals. Read across guidelines indicate that in addition to chemical structural similarity, supporting data should be provided to show that the biological activity of analogs is similar. It is well established that exposure to virtually any toxicant produces characteristic changes in gene expression. It is possible to evaluate gene expression across the genome (transcriptomics), potentially making it a useful technique for demonstrating toxicological similarity among analogs (toxicogenomics). Our lab has developed a large database of transcriptomic signatures for toxicants using a number of cell types that cover a broad range of biology relevant to toxicology. This database, along with a large database (LINCS) on pharmaceuticals and gene knockouts curated by the Broad Institute makes it possible to compare transcriptomic profiles of new chemicals with those already in the database using a statistical approach coined connectivity mapping (CMAP). We used this approach to characterize similarities among groups of similar chemicals that target different toxicity endpoints, including phthalates (liver toxicity), nitrobenzenes and nitrotoluenes (testicular toxicity) and branched carboxylic acids (developmental toxicity). We will focus on the results for the carboxylic acids for this poster. 2-Propylpentanoic acid (valproic acid, VPA) is a human developmental toxicant. The transcriptomic profiles of several other alpha-branched carboxylic acids along with one branched alcohol and a straight-chain acid were compared to VPA. Profiles were generated using the L1000 platform in four cell types – MCF7, A549, HepG2 and iCell cardiomyocytes – exposed to three concentration of each chemical for 6 hours. Toxicogenomic profiles were similar among three of the acids: VPA, 2-ethylhexanoic acid (a rodent developmental toxicant), and 2-propylnonanoic acid, but not for the other compounds. Connectivity mapping mapped the profiles for these three compounds to histone deacetylase inhibitors. Molecular docking studies on three isoforms of this enzyme show that VPA and the two acids with similar transcript profiles have chemical features that allow them to occupy the active site, while the other compounds tested have features that exclude them from the active site. We generated in vitro toxicokinetic data that indicate that all chemicals tested have similar absorption and hepatic clearance. PBPK modeling was used to extrapolate from measured in vivo Cmax data for VPA and ethylhexanoic acid to predict Cmax for the other compounds and to reverse calculate NOAELs for those compounds. This research demonstrates the utility of toxicogenomics to support read across, and of in vitro and in silico toxicokinetics to make the read across useful for risk assessment.

21 – Addressing some issues to expand the use of species sensitivity distributions in ecological risk assessment

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Species sensitivity distribution (SSD) has long been discussed for its use in ecological risk assessment. SSD is a distribution of toxicity data (e.g., no observed effect concentration and half lethal/effect concentrations) derived from ecotoxicity tests. A concentration leading to a protection of 95% species (HC5) is estimated and the HC5 itself or HC5 divided by some magnitude of assessment factor is often used as a predicted no effect concentration (PNEC) in ecological risk assessment.

For the statistical distribution of SSD, the log-normal or log-logistic distribution is often employed. These statistical models have only two parameters (mean and standard deviation), but their accurate estimation requires a large sample size. Such the sample size is rarely available, and there are concerns about the accuracy, and the concern is a critical barrier for the use of SSD in practice. In this study, we aimed to resolve the concern by two approaches. The first is to build models that estimate two parameters of the distribution even when the data is limited. The second is to quantify the degree of uncertainties for the estimated SSD.

In the first approach, we first collected quality-assured acute toxicity data for a total of 60 chemicals, and estimated the means and the standard deviations of SSDs and HC5 values (data-derived HC5). We then developed linear multiple regression models to estimate the means and standard deviations of SSDs using readily obtainable descriptors such as logKow as predictor variables. As has been reported previously, the model only with these descriptors had lower predictability. We then reconstructed the multiple regression models by adding the means and standard deviations calculated from the toxicity data of three species selected from algae, crustaceans, and fish to predictor variables. The HC5 values derived from the resulting best models were within the 95% confidence intervals of data-derived HC5, and their ratios generally fell within a factor of 10. For the chronic SSD derivation, we examined the relationships between the acute and chronic SSDs. The HC5 values derived from chronic SSDs were, on average, 10-fold lower than acute ones. We suggest that multiplication of the acute HC5 by a factor of 0.1 is a defensible way to obtain a first approximation of the chronic HC5.

A theoretical analysis was carried out for the second approach. We examined the extent to which the estimated HC5 differed from the population HC5. The deviation between the population and the estimated HC5 varied depending on the sample size and the estimated standard deviation. These quantitative results allow us to equivalently compare different types of uncertainty: uncertainty due to small sample size and uncertainty due to large variability in susceptibility (large standard deviation). The results also allow us to discuss quantitatively how large the assessment factor should be.

22 – Parameter acquisition with humanized chimeric mice, for human exposure prediction using a PBPK model.

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Human internal dosimetry of chemicals is essential in the risk assessment. While human toxicokinetics data of chemicals are hardly obtained intendedly, the use of physiologically based pharmacokinetic (PBPK) modeling has become important for predicting human internal dosimetry. For the reliability of the predicted human TK data, it is important to acquire sensitive parameters by the well-validated methods (e.g. *in vitro* to *in vivo* extrapolation), not to apply simple extrapolation based on an organ weight scaling factor without biological basis (e.g. baseless assumption that there is no species difference in the parameters). However, when the compound exhibits complicated pharmacokinetics via active uptake, metabolism, and biliary excretion in liver, it is difficult to obtain these hepatic parameters only by the *in vitro* experiments. Compound X is rapidly metabolized to Metabolite A in mammals. Metabolite A is eliminated from the systemic circulation by biliary excretion and metabolism in liver. Although uptake of Metabolite A by transporters is observed in mouse primary hepatocytes, significantly less of it is observed in human primary hepatocytes. We evaluated the mouse-human interspecies TK difference by the use of PBPK modeling into which is incorporated the mechanism-based descriptions of relevant ADME processes (i.e., absorption, distribution, metabolism, and excretion).
First, we developed a precise mouse PBPK model using the available *in vitro* and *in vivo* TK data from mice. The predicted Metabolite A exposure in mice was within about 0.9-fold and 1.1-fold of the actual values. Then we converted a mouse PBPK model to a human PBPK model by applying the input parameters of humans. To obtain human hepatic parameters, i.e., hepatic elimination intrinsic clearance via biliary excretion and metabolism, we used chimeric mice with humanized liver as a model to reproduce the complicated pharmacokinetics of Metabolite A in humans. The validity of this approach using chimeric mice cannot be fully verified because human TK data for verification cannot be obtained experimentally. However, we validated this approach by quantitatively verifying the prediction accuracy when performing rat PBPK modeling with the same strategy using chimeric mice with rat hepatocytes. As the results of this verification, Metabolite A exposure in rats were precisely reproduced by using such techniques, although it tended to be slightly overestimated. This can lead a conclusion that the human TK data would also be predicted accurately and conservatively by the same methods using chimeric mice, which would preferable in the view of human risk assessment. In this way, after verifying the prediction accuracy of the PBPK model in multiple animal species and the parameter acquisition method, we predicted Metabolite A pharmacokinetics in humans. Comparing the predicted Metabolite A exposure in human liver with the measured values in mice, we demonstrated a clear interspecies difference of approximately 4 times lower C_{max} and AUC in humans. This is the first report to present the approach to evaluating target tissue dosimetry of chemicals in humans by the PBPK model in combination with the novel method using chimeric mice and the verification of this technique.

23 – Developing a quantitative AOP for liver-mediated thyroid modulation after prenatal exposure to a xenobiotic compound in the rat

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Xenobiotic-induced hepatic metabolism induces phase I and phase II enzymes, which in turn may cause enhanced clearance of thyroid hormones. In humans, thyroid hormone is needed for orderly brain development during the first trimester of pregnancy, when the fetus is entirely dependent on the maternal transfer of triiodothyronine (T3) and thyroxine (T4). This project aims to monitor relevant maternal and offspring liver and thyroid parameters after prenatal exposure to a model liver enzyme inducer in rats using BMD modelling in order to define quantitative relationships early in the AOP. A broad dose range up to 300 mg/kg bw/day of pregnenolone-16α-carbonitrile (PCN), a model pregnane-X-receptor agonist and UGT1A isozyme inducer, was administered orally to female rats (Wistar Han) from gestational day (GD) 6 to GD 20. Upon the completion of dosing on GD 20 pregnant rats in each treatment group were evenly divided into two cohorts. One cohort was terminated at GD21 and maternal and fetal parameters assessed. The other cohort was terminated at weaning, and dam and pup parameters were assessed. Dose-response analyses were performed using PROAST software (Slob, 2002). Pregnancy rate, body weight and food consumption were unaffected throughout the study. Dam liver and thyroid weight were dose-relatedly reduced at GD21, and this effect had disappeared at weaning. In general, responding parameters showed full sigmoid dose-response curves with effects appearing at the mid dose range and plateauing at the higher dose levels. Litter size and pup body weight gain until weaning were unaffected by treatment. At GD21, liver T3 decreased in the dams while liver T4 concentrations were increased, accompanied by upregulation of relevant Cyp enzymes, glucuronidases, sulfatases, and D3 activity. Serum T3 and T4 decreased at GD21, with no effect on TSH. These parameters had all returned to control values at weaning. Fetal brain at GD21 and pup blood and brain at weaning did not show changes in thyroid hormone concentrations. The data confirm that PCN acts as a strong phase II liver enzyme inducer in adult rats, followed by secondary modulation of the thyroid axis. The interpretation of possible brain developmental effects of thyroid hormone modulation (also if mediated by liver metabolic changes) observed in this study protocol in the light of hazard and risk assessment meets with limitations. Furthermore, effects on offspring brain development in this study cannot as yet be excluded and this requires further analyses.

24 – Release of Population Life-course Exposure to Health Effects Model (PLETHEM): An Online Tool for PBPK Modeling version 3.2

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An outstanding challenge in the acceptance of alternatives to animal testing is the systematic incorporation of computational models into risk-based decision-making pipelines. This can be achieved by linking exposure estimation methods, physiologically based pharmacokinetic (PBPK) modeling, and computational systems biology pathway modeling tools into a standardized framework. To that end, we have developed the Population Life-course Exposure to Health Effects Model (PLETHEM) suite, a modular open source modeling platform that provides users the ability to create and run PBPK models. The platform consists of a selection of different exposures routes, ability to define chemical parameters, QSAR models, life-stage specific physiological parameters, calculation of metabolism from different sources, uncertainty and variability calculation, an R-based engine to perform model simulations, and an interactive user interface to define and select parameter sets for the models. PLETHEM implements easy to use interfaces for a generic PBPK model and a high-throughput IVIVE model. The most recent iteration of PLETHEM updates the ecotoxicology module to increase stability and to include additional modeling output. The rapidPBPK module has been overhauled to increase stability and global sensitivity analysis capabilities. PLETHEM is available online at www.scitovation.com/plethem.

25 – *In silico* estimation of skin permeation of chemicals with their diffusion and partition parameters

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Skin permeation experiments are very important to evaluate the safety and effects of topically applied chemicals. Silicone membranes and three-dimensional cultured human skin models have been utilized for *in vitro* skin permeation experiments as alternative membranes, especially in the development of pharmaceutical and cosmetic products. *In silico* models, which estimate the skin permeability coefficient (*P*, in cm/s or cm/h) of a chemical based on its physicochemical properties, have also been used to evaluate efficacy and safety.

Skin concentration of chemicals (distributed in the lower layers of the stratum corneum) is important to calculate its dermal absorption as well as measurement of *P* value. Therefore, many methods to measure skin concentration have been reported, such as suction blister, punch and shave biopsies, autoradiography, tape-stripping, microdialysis, but are unfortunately expensive and time-consuming. Our research group have already reported that skin concentration as well as skin permeation of chemicals could be predicted with *in silico* model based on Fick's law of diffusion following dermal exposure to chemicals with wide range of lipophilicity. In addition, this method could be applied to estimate chemical permeation and concentration with different thickness of skin. Thus, this method should become a useful tool to assess the efficacy of topically applied drugs and cosmetic ingredients, as well as the risk of chemicals likely to cause systemic toxicity and skin disorder. However, permeation parameters, namely diffusion and partition coefficients, should be obtained by the curve fitting of observed skin permeation data to two-layered and one-layered diffusion models for whole and stripped skin to estimate skin concatenation and *P* values with *in silico* model based on Fick's law of diffusion. Therefore, a complete *in silico* model that does not rely on experimental measurements is needed as a further development.

In the present study, diffusion and partition coefficients of chemicals in stratum corneum and viable epidermis were estimated by physicochemical properties of chemicals. And estimation of skin concentration based on *in silico* model was compared with observed value following dermal exposure of chemicals. When permeation parameters were estimated by random forest method with highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO) and lipophilicity of chemicals as descriptors, each permeation parameters were well-fitted to the diffusion and partition coefficients that obtained from *in vitro* skin permeation experiment with excised human skin. Further experiment should be necessary with different lipophilicities of chemicals, but this present method to estimate skin permeation parameters would become a useful tool to assess the dermally exposed chemicals.

26 – An application of a 3D hydrodynamic ocean model to predict chemical behavior at waters off Niihama in Seto Inland Sea

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In order to develop a chemical fate model for assessing the effect to aquatic organisms as a final goal, a high-resolution hydrodynamic ocean model was applied to waters off Niihama in the middle of the Seto Inland Sea (SIS), Japan. For considering the effects of large spatial variability of salinity and water transparency on complex residual tidal flows in the middle SIS, the model incorporated estimated discharges of moderate size rivers and a formulation of heating depending on water transparency. Using this model, tidal current, temperature and salinity were firstly calculated in the area covering the middle of SIS, and then the behaviors at the waters off Niihama were simulated in detail by using results of the first calculation. The simulation was performed in July when concentration of released chemicals remains higher due to suppressed convective mixing and the effect of the chemicals to aquatic organisms was highly concerned. The model well reproduced spatial and temporal variations of tidal current, water temperature and salinity observed at the waters off Niihama as well as the mid SIS. Results from the simulation demonstrated that the waters off Niihama experienced fast water exchange by residual tidal currents enhanced at steep bathymetry and large vertical water displacement due to propagation of waves generated there in comparison with the middle of the SIS.

27 – Development and application of a spatio-temporal chemical concentration estimation model for watershed risk assessment

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Accurate chemical risk assessment in river basins requires exposure concentration data from different watersheds affected by varied anthropogenic and meteorological conditions, and the lack of these data makes risk assessment difficult. We have developed the AIST-SHANEL river model which can be applied to any river basin in Japan. The model estimates spatial and temporal river flow rates and chemical concentrations in the river water and sediment with a gridded spatial resolution of 250 m and a daily temporal resolution by using watershed characteristic data including meteorological, land-use, population, industry, and sewerage data. Chemical concentrations were calculated using the estimated river flow rates and discharge volumes and the dynamic mechanisms of advection, diffusion, adsorption and desorption of suspended solids (SS), settling and resuspension of SS, mass transfer between river water and sediment, and biodegradation were considered at different points in time for each grid cell. The physical parameters for analysis were vapor pressure, molecular weight, water solubility, organic carbon water partition coefficient, biodegradation rate estimated by the half-life of the chemical substance considering temperature dependence, and sewage treatment removal rate. Wet and dry deposition from the atmosphere was also included. This presentation shows examples of the application of the model to two chemicals, namely linear alkylbenzene sulfonates and bisphenol A in river basins in Japan and discusses the validity and improvement of the model for future use. The results provide useful information for understanding the potential risks predicted from temporal and spatial distribution of chemicals in river water and sediments. This also facilitates the evaluation of potential impacts of chemical substance substitutions, emission reductions, climate change, water saving measures, and improvements to wastewater treatment facilities, including sewage. Moreover, we are continually improving this model for watershed risk assessments for various contaminant types. For example, to predict the diffusion of chemicals due to a spill, the model has been modified to estimate concentration changes depending on precipitation, including localized heavy rainfall, using temporal resolutions of one minute and one hour. In another example, to evaluate the effectiveness of use of biodegradable plastics, the ability to calculate the concentrations using the dynamics of plastic particles is being improved. Although the present model targeted river basins in Japan, AIST-SHANEL can be applied to any river basin in the world if the necessary watershed data can be obtained. Due to limited available measurement data it is challenging to validate the model; however, we attempt to achieve this by using uncertainty and sensitivity analyses.

28 – A multi-model system for predicting biodegradation rates and the presence of residual substances of chemicals using chemical descriptors and 3D structures

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In Japan, according to the Act on the Evaluation of Chemical Substances and Regulation of Their Manufacture, etc., chemical substances are classified into ready biodegradable and non-biodegradable substances by the OECD test guideline 301C modified MITI I test, based on biodegradability and the presence of degradation residual substances. This test usually requires 28 days of continuous testing and is costly. Therefore, it is necessary to establish a model relating chemical properties to biodegradability and the presence of degradation residual substances in order to reduce costs. Many models have been proposed to estimate biodegradability and the presence of degradation residual substances, such as Lasso, XGboost and deep learning neural networks, but no model can estimate biodegradability and degradation residual substances with sufficient accuracy. Therefore, this study proposes an ensemble system including nine models for estimating biodegradability and eleven models for estimating the presence or absence of degradation residuals. Furthermore, a system is proposed to classify products into ready biodegradable and non-biodegradable products using the estimated biodegradability and the estimated degradation residual substances. The system used the chemical descriptors and steric structures of 4200 chemicals. Descriptors were generated by AlvaDesc software and three-dimensional structures were constructed by Gaussian software using the PM6 method. To obtain higher accuracy, chemicals were classified by estimating their computation residuals. The residuals were estimated by a deep learning neural network using chemical descriptors. The estimation accuracy of the system was 98% for the unlearned non-biodegradable products and 38% for the unlearned ready biodegradable products.

29 – Evaluation of slow-stirring method for determining log K_{ow} (*n*-octanol/water partition coefficient) of surfactants

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Surfactants are widely used both in industry and in everyday life. Thus, determination of their distribution in the environment is of particular importance. In environmental risk assessment of chemicals, the *n*-octanol/water partition coefficient (log K_{ow}) is a physicochemical property representing the hydrophobicity of chemicals and frequently used as a key parameter to estimate environmental fate, exposure, and toxicity. Existing experimental OECD methods for determining log K_{ow} such as shake flask method are not suitable for surfactants due to forming emulsion and micelle aggregation. “Slow-Stirring Method” can avoid turbulence with *n*-octanol and aqueous phases and thus is suggested by European Chemicals Agency as the best method in theory for surfactants. The slow-stirring method based on OECD TG 123 has been demonstrated to provide reliable data to highly hydrophobic chemicals but applicability for surfactants has not been validated. Recently, a developed method by other researchers has shown that the scope could be extended to non-ionic/ionic surfactants under the condition of less than critical micelle concentration (CMS) at which no micelles are present. However, the test conditions have not been established as there is currently no internationally standardized method.
The aim of this study was to evaluate the accuracy of the slow-stirring method for determining log K_{ow} of surfactants using six surfactants of three types: non-ion (tetraethylene glycol monooctyl ether, octaethylene glycol monolauryl ether), anion (sodium dodecyl sulfate, sodium dodecanoate) and, cation (dodecyltrimethylammonium chloride, hexadecyltrimethylammonium chloride). 300 mL test vessels with drain port near the bottom were prepared. The test substances were introduced into either *n*-octanol or aqueous phase and then stirred at 25℃. Samples of the two phases were taken and analyzed by LC-TOF/MS. Generally, log K_{ow} values showed a good agreement with literature values obtained by the slow-stirring method provided that a certain amount of test substances was added to the test vessels. In order to assess the influence of adding amount to the test vessels and the accuracy, log K_{ow} values were determined over a wide range of the concentration bellow the CMC. log K_{ow} values of three surfactants (tetraethylene glycol monooctyl ether, sodium dodecyl sulfate, and dodecyltrimethylammonium chloride) were almost identical within the studied range. These results indicate that the slow-stirring method generally allows for accurate determination of log K_{ow} of surfactants.

30 – EAS-E Suite: A data integration and modelling framework for chemical safety and sustainability

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There are significant data gaps in the information required to conduct reliable ecological and human health assessments for thousands of existing and new chemicals. The Exposure And Safety Estimation (EAS-E) Suite is a freely accessible on-line platform that addresses many data gaps and challenges for ecological and human health assessment and facilitates the safe and sustainable production and use of chemicals in society in a cost-effective manner. EAS-E Suite is comprised of (i) chemical information databases (e.g., physical-chemical properties, in vitro and in vivo toxicokinetics data, production volumes), (ii) quantitative structure-activity relationships (QSARs) for predicting chemical properties, biodegradation and biotransformation half-lives, and (iii) various mass balance models and tools for chemical hazard, exposure, and risk estimation. EAS-E Suite includes (i) the CiP-CAFE model for estimating life cycle chemical mode-of-entry and emission rates, (ii) the RAIDAR model for simulating chemical fate in the natural environment and exposures to a range of ecological receptors and far-field exposures to humans, (iii) the RAIDAR-ICE model for simulating indoor chemical fate and near-field human exposure simulations linking external and internal doses, and (iv) the PROTEX-HT model for aggregate human exposure and risk estimation. Other models in EAS-E Suite are (i) the in vitro mass balance model for simulating the fate of chemicals in in vitro bioassays, (ii) the EAS-E Suite and US EPA's high-throughput toxicokinetic models for forwards and reverse dosimetry in humans, rats, and fish, (iii) in vitro-in vivo extrapolation (IVIVE) models, (iv) bioaccumulation models for plants, plankton, invertebrates, fish, birds and mammals, (v) various dermal exposure models, and (vi) various environmental fate models. EAS-E Suite facilitates the application of these databases and models using either chemical name, CAS, or SMILES user supplied entry information. Furthermore, EAS-E Suite "autoparameterizes" the built-in tools and models for 70,000 discrete organic chemicals making it very easy for their application for experts and non-experts. If desirable the user has the option to enter alternative values for chemical information to the defaults provided by the system. The platform aids the critical scientific analysis and evaluation of data and models and provides knowledge transfer to multiple stakeholders fostering collaboration and consensus building. This poster summarizes key concepts of the EAS-E Suite platform showing how it can be applied for chemicals assessment and management objectives either retrospectively for existing chemicals and use, or prospectively for new chemicals and for proposed changes of use of existing chemicals, and for intelligent experimental test design.

EAS-E Suite quantifies and clearly demonstrates relationships between production volumes, chemical emissions throughout the lifecycle, fate and transport in natural and manufactured environments (e.g., homes), persistence, bioaccumulation, toxicokinetics and aggregate human and ecological exposures to estimate the potential for adverse effects at a screening-level. The recently developed PROTEX-HT system in EAS-E Suite consolidates the principal elements of the production-to-outcome continuum for tens of thousands of chemicals.

APPENDIX 3: Organizing Committee

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Logistical support for this conference was provided through contracts with MCI and ICF.

APPENDIX 4: Post-Event Survey Feedback

The appendix below includes data collected from the post-event survey. Survey respondents were asked to comment on satisfaction with the sessions and presentations and overall workshop. Survey respondents were also asked to provide specific feedback on additional topics to cover in sessions and future conferences. Short answer questions captured data on attendees' overall workshop suggestions and feedback and all responses are included below.

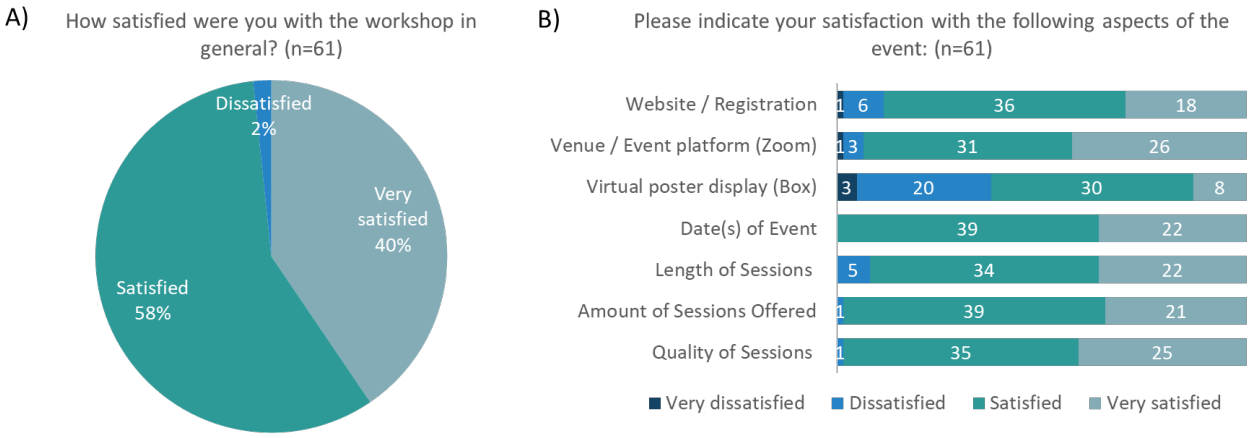

Fig 3. The overall satisfaction of polled participants is reported above.

Short Answer Feedback

What did you like most about the workshop?

- Broad overview on NAMs was provided
- Panel discussion of session 4
- The workshop was held in-person.
- Able to access it virtually
- AI-SHIPS
- Being able to communicate with various people.
- Conference presenters and dinner
- Discussion of NAMs from various viewpoints
- Diversity of topics
- F2F meeting at this season with these topics.
- General information about NAM
- Good spread of different topics and speakers, and easy to use virtual platforms
- Hear about new strategies for risk study.
- Held in hybrid was to promote the attendees who has some difficulty with traveling around. If it would be continued to be held in this way for the future event, it would be nice.
- Hybrid attendance, with Box and at venue.

- Under Covid-19, we had used only web meeting, however I could attend at the venue.
- Hybrid participation (online and in person)
- I could directly obtain the latest research trends related to the subject.
- I could get the updated specialized info. from global via online (on my desk).
- I liked that the sessions were held in a hybrid style, so I could join the sessions I wanted to attend between my desk work.
- I studied about development progress of NAMs new methods.
- Information concerning toxic effect of particles
- International/global character
- It brought me up to date very quickly and focused on what developments to expect in the immediate future
- iTTC, NAMs at EPA
- most NAMs topics
- NAM UPDATE
- PBPK work
- Presentations from experts and communication in person at the conference too,
- Selection of presenters
- Session 1; Session 3
- Session 3; iTTC
- Session V, 14:40-15:05
- Simultaneous interpretation
- Simultaneous translation
- Social networking at on-site.
- That the workshop was also conducted face-to-face.
- The application in chemical management
- The discussion with experts (both oral and poster)
- The fact that it has both virtual and FTF Sessions available, and a key summary of each session
- Varied topics and opinions, plus cutting-edge technology application
- Very interested in iTTC
- Very well organized
- Very well organized, interesting topics
- Web event!
- Workshop Topics
- Session 4; Session 5
- It's great to have a face-to-face discussion.

Do you have any other suggestions or feedback you would like to share?

- Box was inconvenient
- Ensure rapid participant sharing of all the presentation decks
- Hybrid meeting was a great idea and enabled a lot of people around the world to participate.
- I was very thankful to have been able to participate in the web event.
- It was unfortunate that government officials were not present on Day 2; it would be good to convey the conclusions of the workshop to the government officials who attended on Day 1 – this can be done by circulating the Workshop Summary.
- It would be great if university and high school students who are interested in the analysis of chemical substances could also watch (Online) and increase the number of participants by using SNS and sending

notifications of workshops to schools.
- Maybe better not starting on a Monday, so that week-end travel could be avoided
- Slides or recording should be shared for better understanding the content
- Thank you for arranging the hybrid workshop. We have active time at the workshop! However, on-site would be better than on-line because of social networking.

How could the workshop be improved?

- Better poster presentation, more personal interaction, maybe include short poster pitches
- Box does not work because of company's security
- By increasing posters and time to communicate with participants.
- Add translation to Day 1.
- Discussion time should be a bit longer
- For on-site attendee, the questions and answers from Zoom cannot be followed.
- I am happy if the poster presenter could provide the video presentation.
- I could not utilize BOX well.
- I do not have any comments.
- I hope that this workshop will help to further promote LRI research in JCIA, ACC, and Cefic.
- I think that on-site discussion is mandatory.
- I would like to enter the poster session more easily.
- I would like to hear more from ECHA and EPA researchers.
- If more speaker could be present at the venue, it would be better.
- I'm already satisfied enough, but if I may say so, it would be easier to discuss if overseas members were invited to the site.
- I'm satisfied with this event.
- In person
- Interactivity
- It is better to include a luncheon session for FTF participants.
- It was a very well organized hybrid workshop- thanks!, I had some trouble to enter the box.
- It was better to have QA in Day1 presentations
- It was unfortunate that the audio was sometimes disrupted.
- It will be interesting if the discussion time and the question-and-answer time are a little longer. I entered a sentence in the translation application
- It would be nice if Experts from outside the country could patriciate in person. though I understand the COVID situation make it impossible.
- I don't think there are enough LRI studies published.
- May need to have session on ecological risk assessment
- Maybe it is possible to have a longer plenary session with discussions
- More discussion
- More interactive communication among panelists and government authorities to seek future possibilities
- Nothing really, except maybe to have a small round-table near the end to discuss where they think the "world is going" with respect to the science associated with chemical risk assessments with
- On the first day, interpretation might be useful.
- On the other hand of the above comment, we have to reconsider how to compromise the time difference between three major LRI regions. I do not have any good solution but considering on demand broad casting would be one possible way.
- Online connection for web conferencing

- Paper handouts would be better.
- Providing a place for lively discussions under travel restrictions.
- Simultaneous translation is not reasonably activated, when question is in Japanese and speaker uses English. Some kind of interruptions for translation should be notified from chair.
- Slides or recording should be shared for better understanding the content
- Unfortunately, some of the posters presented information too close to the bottom of the poster board. We should have provided more specific instructions for the layout of the posters so the information would be at or near eye level
- Video of speakers would be satisfactory.
- With more participants or speakers from different countries and regions
- Pursuit of Hybrid Holding Technology

What other topics on Hazard Assessment would you like to see in future scientific discussions or workshop sessions focused on using NAMs to predict repeat-dose and complex apical endpoints?

- Ecotoxicology
- Environmental Hazard Assessment, interspecies correlation
- *In silico* approaches for hazard assessment
- More databases to consider using
- NAM applications in food risk assessments
- No suggestions, the talks were good, and Rusty Thomas' talk was fantastic!

What other topics on Exposure Assessment would you like to see in future scientific discussions or workshop sessions focused on integrating exposure assessment NAMs into risk evaluations?

- Differences between occupational exposure compliance monitoring and conducting occupational exposure monitoring for use in quantitative risk assessments
- Environmental Exposure Assessment, release factors
- PBPK
- Update on ongoing developments
- Lobbying for administrative use
- Assessment by modeling the exposure environment

What other topics or areas would you like to see included in future conferences?

- Collaborative research between the US, EU, and Japan, if possible
- Environmental fate and exposure of chemicals
- More on databases to consider using.
- Pros and Cons for Generic approach to risk management.
- Capacity for building NAMs
- Update on regulatory acceptance.