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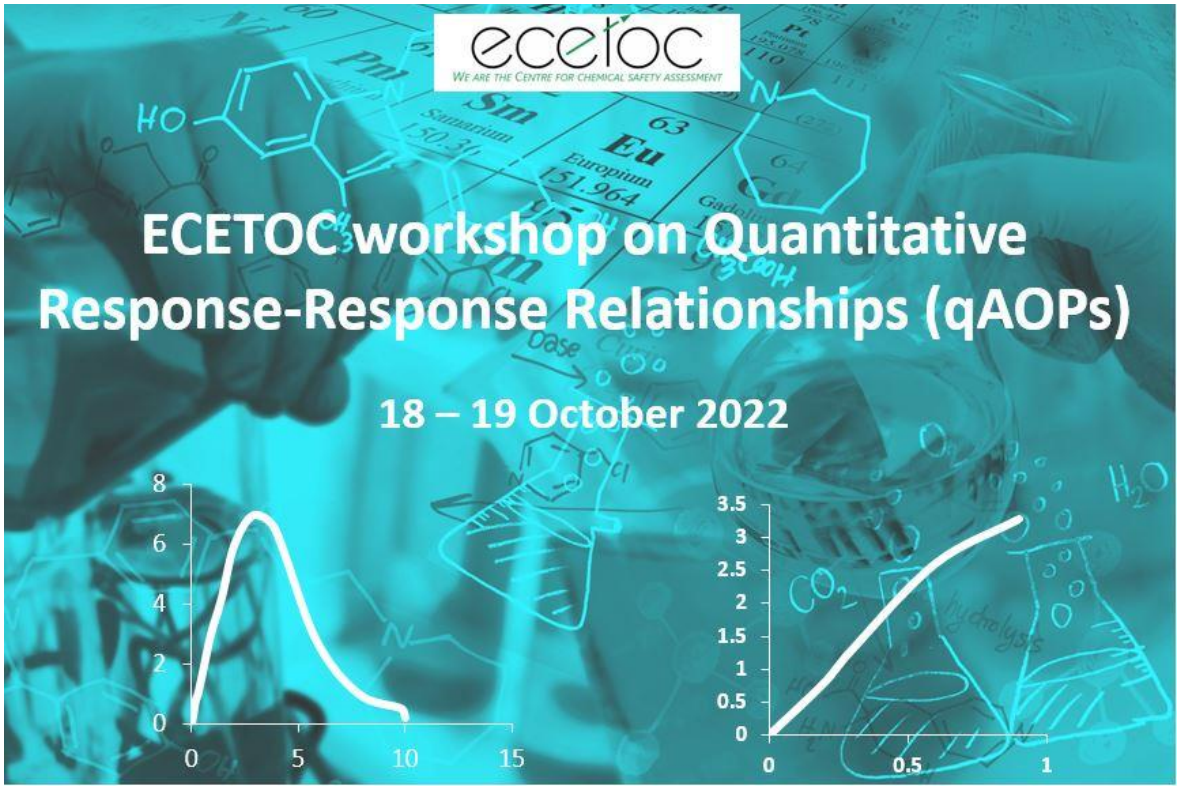

WORKSHOP BACKGROUND

Adverse Outcome Pathways (AOPs) provide a framework for curating and organising toxicological & ecotoxicological knowledge with associated measurements of key events and the dose & temporal concordance of relationships between these events. AOPs have been the topic of many conferences and workshops since the concept was first proposed in 2010. To date these qualitative AOPs have made little impact in regulatory toxicology beyond simple chemical reactivity-driven endpoints. However, quantitative AOPs are likely to be significantly more impactful in next generation risk assessments using new approach methods without the need to conduct animal studies. There is, though little guidance on best practices for quantitative AOP generation.

There are several published tiered frameworks for conducting next generation risk assessments using human-focused in vitro experimentation and in silico modelling of both biological effects and pharmacokinetics to provide estimates of human bioactive doses. A minimally toxic in vitro exposure is derived from this perturbation of human biological effects using a panel of in vitro assays. This is then converted into a human effective dose estimate using in silico physiologically based pharmacokinetic models, which can also be parameterized by in vitro assays. This combination thus provides an estimate of a point of departure for human effects that can be used as an endpoint in a risk assessment and derivation of a health-based guidance value. This is often presented, using the premise that biological responses occur at lower doses than adverse responses, as a protective conservative first tier and is performed without regard for whether the biological perturbation is large enough to cause, or even can cause, an adverse outcome.

If there isn't sufficient margin of exposure to ensure safety, even after risk management to reduce exposures, higher tier refinements are recommended to identify adverse points of departure. However, performing such higher tier risk assessments critically depends on having knowledge of the pathway between exposure and adverse outcome to ensure this pathway is adequately modelled within the in vitro and in silico approaches. The development of a quantitative Adverse Outcome Pathways (AOPs) from these data could bridge this gap.

However, there are several issues with using the existing AOP framework as the underpinning to a higher tier risk assessment:

- Existing AOPs provide incomplete coverage of all potential toxicities, and our knowledge of all toxic mechanisms is also incomplete. Therefore, in these circumstances it isn't clear how to address prediction of adversity using only in vitro and in silico methods.
- Current and emerging regulatory concerns (such as for developmental neurotoxicity) often require us to predict effects in humans where there are no established in vivo model systems, but panels of potentially mechanistically relevant human in vitro assays have or are being developed. However, the relationship of these in vitro assays to human effects is not clear.
- Perhaps most importantly, existing AOPs are not quantitative. The Molecular Initiating Event (MIE) is the foundational event, but for most MIEs the quantitative threshold triggering the downstream key biological events leading to adversity are unknown. Equally, the ability to describe a quantitative AOP from MIE to subsequent key biological events using only in vitro and in silico approaches is unproven.

WORKSHOP GOAL

- To bring together different stakeholders to build on the knowledge gained from existing experience of qAOP generation.
- To make recommendations that will enable the design, interpretation and application of Quantitative Response-Response Relationships based on qualitative/descriptive AOPs that are trusted to provide confidence in use in decision making.
- Emphasis will be placed on the development of open standards to support the FAIRification of qAOP.

Programme – Day 1		
12.00 – 13.00	Lunch Welcome	 Blanca Ramon Serrano (ECETOC)
13.00 – 14.20 (Moderator: Blanca Ramon Serrano)	Introduction, brief recap of ECETOC IVIVE and OMICS PoD workshops	Ben van Ravenzwaay (ECETOC)
	Considerations for increasing quantitative AOP (qAOP) regulatory uptake An Evolving View of Quantitative Adverse Outcome Pathways and Considerations for Application	Magda Sachana (OECD) Dan Villeneuve (US EPA)
	Supporting Regulatory Application of AOPs. The Pivotal Role of Weight of Evidence in Systematic Development and Quantitation	Bette Meek (University of Ottawa)
14.20 – 16.20 (Moderator: Richard Currie)	Development of qAOPs Within the RISK-HUNT3R Project of the ASPIS Cluster Quantitative systems modeling of qAOP and its data integration – ONTOX perspective Identifying Molecular Biomarkers of a Chemical Hazard using New Approach Methodologies	Mark Cronin (Liverpool John Moores University) Huan Yang (esqLABS GmbH) Albert Zhou (University of Birmingham)
	Acute-to-chronic extrapolation in vitro. Implications for the development of KERs.	Peter Macko (EC JRC)
	Quantitative adverse outcome pathway (qAOP) models for toxicity prediction	Nicoleta Spînu (Liverpool John Moores University)
	A Machine-Readable AOP Evidence Data Model: Enhanced data input and retrieval from the AOPwiki	Jason O'Brien (Environment and Climate Change Canada)
16.20 – 16.40	**Break**	
16.40 – 18.00 (Moderator: Alicia Paini)	Case study 1: The state of the art in AgChem – an example using HPPD inhibitors	Steven Webb (Syngenta)
	Case study 2: Moving from detection of cardiovascular liabilities to quantitative translational understanding: challenges and opportunities	Linda Starnes (Astra Zeneca)
	Case study 3: Identifying thyroid hormone disruptors by establishing qAOPs integrating cross-species extrapolations and thresholds	Stephanie Melching-Kollmuss (BASF)
18.00 – 18.10	Closing session day 1	Ben van Ravenzwaay (ECETOC)
19.00 – 21.00	**Dinner**	

Programme – Day 2		
12.00 – 12.30	Lunch	
12.30 - 12.45	Welcome to Day 2 – Short recap of Day 1	Richard Currie (Syngenta)
12.45 - 14.30	Breakout groups (4 groups): • Q1 • Q2 • Q3	Moderators: Phil Botham Richard Currie Cecilia Tan Ben van Ranvenzwaay
14.30 – 15.15	**Break**	
15.15 - 16.00	Plenary session – discuss break-out groups' outcomes	Rapporteurs
16.00 - 17.00	General discussion incl. thoughts on what else is needed to promote trust/confide in qAOPs in support to draft recommendations (outlines of WS report)	All
17.00 – 17.15	Closing remarks and end of workshop	Richard Currie

QUESTIONS TO BE DISCUSSED IN THE BREAK-OUT SESSION:

Q1: How would you choose the most appropriate level of biological detail to include in your qAOP? For example:

- What data should we get and how to organise it?
- How do you judge how complete (in term of the number of key events required to describe the toxicological response) a qAOP needs to be?
- How do you judge the level of complexity (in terms of detailed biochemical/physiological/biological mechanisms and feedbacks) that is required?

Q2: How and why would you choose the most appropriate modelling approach? For example:

- What are the pros and cons of different modelling approaches?
- What are the methods for extrapolating from short-term to longer term exposures?
- How faithful should the model structure be to the qualitative AOP?

Q3: How do we ensure the quality assurance and accessibility of qAOP models and their predictions?

- What would be the quality assurance criteria for the underlying data?
- Which open standards support qAOP development? Are there any gaps?
- How do we ensure the FAIRification of qAOP models and underling data?

RESOURCES

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- Spinu et al, 2022: https://doi.org/10.1016/j.comtox.2021.100206

ORGANISING COMMITTEE

Phil Botham, Syngenta
Mark Cronin, Liverpool John Moores University
Richard Currie, Syngenta
Tim Gant, Imperial College; Faculty of Medicine, School of Public Health
Alicia Paini, esqLABS GmbH
Ben van Ravenzwaay, BASF
Carl Westmoreland, Unilever
Andrew Worth, European Commission Joint Research Centre

Andreea Cuciureanu, ECETOC
Francesca Uguccioni, ECETOC

VENUE:

Renaissance Brussels Hotel
Rue du Parnasse 19
1050 Brussels
Phone: +32 2 505 2929
Hotel website

CONTACT

Francesca Uguccioni, ECETOC
francesca.uguccioni@ecetoc.org

PRESENTATION ABSTRACTS

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Introduction

Bennard van Ravenzwaay – University of Wageningen

	Ben received his degree as doctor of Environmental Sciences/Toxicology at University of Wageningen in 1988, following the preparation of doctoral thesis at the German Cancer Research Centre (DKFZ) Heidelberg (Germany); 1979 – 1985 Study of Environmental Hygiene/Toxicology, University of Wageningen, Netherlands. He worked for 34 years at BASF SE, Ludwigshafen, the last 20 as Senior Vice President of the Department for Experimental Toxicology and Ecology, which included as of 2019 BASF Metabolome Solutions, former Metanomics GmbH. He is an associate professor for Reproduction Toxicity of the University of Wageningen. He is Chairman of the scientific committee of the European Centre for Ecotoxicology and Toxicology (ECETOC). Moreover, he is member of editorial boards of "Archives of Toxicology", "Chemical Biological Interactions" and "Toxicology Letters. He was member of the board of trustees of Health and Environment Science Institute (HESI) from 2012 – 2018. He is a Member of the German Society for Pharmacology and Toxicology, a European registered toxicologist and SOT-Member. He has a teaching assignment at the University of Kaiserslautern (for industrial toxicology) since 2006. He is an author of more than 240 peer reviewed papers. Since January 2022 he is an independent consultant for environmental sciences.

Abstract 1: Considerations for increasing quantitative AOP (qAOP) regulatory uptake

Magda Sachana – OECD

In June 2022, the OECD Adverse Outcome (AOP) programme celebrated its ten-year anniversary, a milestone representing a decade of effort to advance and standardise qualitative AOP development and reviewing. However, during the same time period only fragmented and limited efforts were made towards the development of guidance for quantitative AOPs (qAOPs). It is notable that not a single qAOP was submitted to the OECD for review within these ten years and no any in depth discussions took place in this global policy forum, although from the early years a tool to develop qAOPs was developed and is available to download here. This presentation is aiming to highlight the considerations that should be taken into account when developing qAOPs building on experience gained through other OECD activities with respect to 1) qualitative AOPs, 2) data reporting formats, 3) standardisation of test methods and 4) mathematical model reporting. This talk will further discuss potential needs/criteria for qAOP and how to establish them to address their regulatory uptake.	
	Dr Magda Sachana is a Policy Analyst within the Environment Health and Safety Division of the OECD's Environmental Directorate since 2015. She manages the development and implementation of policies in the field of chemical safety and contributes to the OECD Test Guidelines, Pesticide and Hazard Assessment Programmes. Dr Sachana among other projects is assisting in the coordination of the OECD AOP programme.

Abstract 2: An Evolving View of Quantitative Adverse Outcome Pathways and Considerations for Application

Dan Villeneuve – US EPA

Quantitative adverse outcome pathways (qAOPs) have been broadly defined as AOPs for which the quantitative understanding of the key event relationships is sufficient to allow for estimation of the probability or severity of the adverse outcome occurring based on measured or modeled changes in an early key event in the pathway. This presentation will report on testing and evaluation of an early example of a qAOP based on a system of interconnected, mechanistically-based, computational models. Recognizing that the pace of similar qAOP development has not been sufficient to support the growing interest in application of new approach methodologies in chemical safety decision-making, we consider alternative ways to develop qAOPs. For example, anchoring AOP development to prototypical stressors for which there is a strong database of concentration-response data across key events may provide an alternative approach. However, applying this strategy will involve testing and evaluation of several key assumptions to evaluate whether relationships developed for the prototypical stressor and domain of empirical evidence can be extended to the plausible domain of applicability for the AOP. *The contents of this abstract neither constitute, nor necessarily reflect, US EPA policy.*

Daniel L. Villeneuve is a research toxicologist with the United States Environmental Protection Agency's Office of Research and Development (ORD) in the Center for Computational Toxicology and Exposure (CCTE). He received a BS in Water Resources and Zoology from the University of Wisconsin-Stevens Point and a Ph.D. in Zoology and Environmental Toxicology from Michigan State University. He has over 20 years of experience conducting freshwater ecotoxicology research. His present research is focused on the use of new approach methodologies and adverse outcome pathways to characterize and evaluate hazards organic contaminants pose to fish and wildlife. Dr. Villeneuve has published over 200 peer-reviewed papers in the field of ecotoxicology and his work has been recognized with over 20 US EPA Scientific and Technical Achievement awards.

Abstract 3: Supporting Regulatory Application of AOPs. The Pivotal Role of Weight of Evidence in Systematic Development and Quantitation

Bette Meek- University of Ottawa

Selected Bradford Hill (B/H) considerations form the basis for assessment of the extent of supporting evidence in formalized descriptions of Adverse Outcome Pathways (AOPs) in the Organization for Cooperation and Development (OECD) publically accessible electronic Knowledge Base. These considerations, modified from their original characterization to assess causality in epidemiological studies have evolved through experience in regulatory application in Mode of Action (MOA) analysis and through application in the OECD AOP development program.

The relevant subset of considerations includes biological plausibility, essentiality and empirical support. The considerations, defined to address aspects critical in regulatory acceptance, are also rank ordered to reflect their relative importance in assessing the extent of supporting mechanistic data. Criteria and examples are provided in OECD Guidance to enable developers to consider relative confidence (i.e., high, moderate or low) in the supporting evidence for the Key Event Relationships (KERs) and the AOP overall. This facilitates judgment of the robustness of AOPs for various applications by the risk assessment/regulatory community (for example, in the development of testing strategies and/or as a component of priority setting or hazard characterization). The considerations also inform developers on the nature of studies which provide optimal support for confidence in regulatory application.

One of the considerations, empirical support, relates to the extent to which available data support the expected patterns of quantitative relationships (dose-response and temporal concordance) across KERs. The nature of these expected patterns is based on temporal relationships in AOPs (i.e., that early key events precede later ones) and the expected patterns of relative incidence/abundance and severity of effect across different levels of biological organization. Discernment of these patterns is optimally informed by studies with protocols to determine comparable measures of increased incidence (such as Benchmark Doses) and/or the severity of Key Events (KEs) at multiple levels of biological organization, following challenge by a specified dose of a stressor.

KERs with high and/or moderate confidence determinations for empirical support, are those most likely to support development of quantitative models and higher tier applications. The extent of development and required accuracy of such models is necessarily dependent upon that required for envisaged purpose-specific application, based on objectives framed normally in problem formulation. Principles and guidance for the description and application of purpose-specific quantitative models for AOPs are likely to be similar to those outlined in previous initiatives on physiologically based kinetic (PBK) models and will be addressed.

The significant and rather pressing need for quantification of AOPs as a basis to support testing strategies and higher tier hazard characterization and risk assessment applications has important implications also for the efficient, systematic identification and assimilation of critical evidence for both AOP development and quantitation. This aspect will also be addressed.

Dr. Meek is the Associate Director of Chemical Risk Assessment at the McLaughlin Centre for Population Health Risk Assessment, and Adjunct Professor in the School of Epidemiology and Public Health in the Faculty of Medicine, University of Ottawa. Previously, she contributed to and managed several chemical risk assessment programs within Health Canada. With colleagues internationally, she has contributed to or led initiatives in developing methodology in chemical risk assessment, including mode of action, chemical specific adjustment factors, physiologically-based pharmacokinetic modeling, combined exposures and predictive modeling. These initiatives have involved collaborations with a range of international

	organizations and national Agencies, including the World Health Organization International Programme on Chemical Safety, the Organization for Economic Cooperation and Development, the U.S. Environmental Protection Agency, the European Joint Research Centre and the Agency for Food, Environmental and Occupational Health and Safety of France (ANSES). She has authored over 200 publications in this area and received several awards for contribution in this domain. Dr. Meek has a background in toxicology receiving her M.Sc. in Toxicology (with distinction) from the University of Surrey, U.K. and her Ph.D. in risk assessment from the University of Utrecht, the Netherlands.

Abstract 4: Development of qAOPs within the RISK-HUNT3R Project of the ASPIS Cluster

Marc Cronin – Liverpool John Moores University

The RISK-HUNT3R Project (https://www.risk-hunt3r.eu) aims to develop a new modular framework for animal-free next generation risk assessment. In order to support Next Generation Risk Assessment (NGRA) RISK-HUNT3R will develop quantitative Adverse Outcome Pathways (qAOPs). The qAOPs will allow for the translation of data from New Approach Methodology (NAM) assays into usable outputs for risk assessors. Further, the aim is to integrate toxicokinetic and toxicodynamic outputs into frameworks for Quantitative Systems Toxicology. Suitable AOPs for quantification will be selected based on a number of criteria including the completeness of the AOP itself, availability of data and existing models for the AOP or Key Event Relationships. The development of qAOPs will be supported through the use of NAMs within the Project. The RISK-HUNT3R Project is also collaborating with the ASPIS Cluster on the development of a qAOP for liver steatosis. Acknowledgments: The contributions of the partners in WP8 of the RISK-HUNT3R Project are gratefully acknowledged. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 964537 (RISK-HUNT3R).

	Mark Cronin is Professor of Predictive Toxicology at the School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, UK. He has over 30 years' experience in the application of in silico approaches to predict the toxicity and fate of chemicals; in addition to development of strategies to develop alternatives to whole animal testing for toxicity. His current research includes the application of chemical grouping and read-across to assess human health and environmental endpoints, particularly the quantification of Adverse Outcome Pathways (qAOPs) to inform safety decisions. He has worked in numerous projects in this area including more than fifteen EU framework projects, as well as assisting in the uptake of in silico methods for regulatory purposes. Cronin currently co-leads the Work Package in the RISK-HUNT3R Project which will develop qAOPs to support Next Generation Risk Assessment.

Abstract 5: Quantitative systems modelling of qAOP and its data integration - ONTOX perspective

Huan Yang - esqLABS GmbH

The European funded project called "ONTOX: ontology-driven and artificial intelligence-based repeated dose toxicity testing of chemicals for next generation risk assessment" envisions providing a functional and sustainable solution for advancing human risk assessment of chemicals without the use of animals (https://ontox-project.eu/). This is in line with the principles of 21st century toxicity testing and Next Generation Risk Assessment (NGRA), (PMID: 34216698). As an important component, ONTOX's quantitative Adverse Outcome Pathways (qAOPs) modeling task is to develop qAOP networks for systemic repeated dose toxicity effects in the liver, kidney and developing brain. To develop these qAOP networks, we are exploring various systems modeling frameworks including deterministic ones (like differential equations modeling) and probabilistic ones (like Bayesian approaches). These systems modeling frameworks will integrate data from various biological organizations (including molecular, cellular, tissue, organ, and organism). Towards the NGRA, ONTOX's frameworks will also integrate toxicokinetic modeling to offer an open-source tool (implemented in Open Systems Pharmacology Suite; www.open-systems-pharmacology.org) to predict response-response and exposure-effect relationship. To better assess the confidence about model prediction, we will also develop advanced computational approaches to quantify uncertainty in qAOPs models. During the talk, we will illustrate with some preliminary results.

Acknowledgements: ONTOX has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreements number 963845.

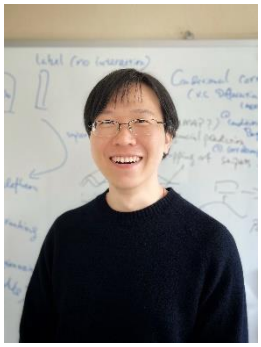
Huan Yang obtained his PhD in applied mathematics at University of Twente, the Netherlands, in 2015. His PhD research focus on computational modeling of nociceptive systems to better understand neural mechanisms underlying chronic pain development, and to assess therapeutic effect and safety of pain medicine like capsaicin patch. From 2016 to 2020, Huan did his postdoc at Leiden University, the Netherlands. He developed quantitative systems approaches to gain insights into how biological systems (ranging from in-vitro PK to signaling pathways) respond to chemical/drug exposure. Being involved in EU-ToxRisk and other public-private partnership projects, his modeling work incorporated various types of data from high-content microscopy imaging, sequencing, and mass spectrometry generated by academic and industrial collaborators. These mathematical models also quantitatively characterized several key events and their relationship in AOPs. Since 2021, Huan has been working as senior scientist in systems toxicology at esqLABS GmbH, Saterland, Germany. He is currently leading the qAOP task in ONTOX of the ASPIS cluster and cochairing the qAOP working group in ASPIS.

Abstract 6: Identifying Molecular Biomarkers of a Chemical Hazard using New Approach Methodologies

Jiarui (Albert) Zhou – University of Birmingham

Environmental pollution has been identified as the largest environmental cause of the premature death of an estimated nine million people. However, hazard assessment of environmental chemicals remains insufficient, limited to hundreds of well-studied compounds generated by a few surrogate models. Traditional toxicity testing remains expensive, time-consuming, and typically demands a large number of mammals. The trending of the 3Rs paradigm encourages replacing traditional mammalian surrogate species with non-sentient species, such as fruit flies, nematodes, water fleas, and embryos of zebrafish and frog. Advancement in non-targeted, high-throughput omics assays further promises data-rich and unbiased biomolecular profiling in the testing species. The multi-omics assays (genomics, transcriptomics, metabolomics, lipidomics etc.) assist a systematic and holistic understanding of the biological responses to the chemical exposure, facilitating the discovery of molecular signatures that are reflective of chemical exposure or even indicative of toxic outcomes. By studying the functional conserveness of the multi-omics signatures, molecular biomarkers of chemical hazards that are rooted in the phylogenetic tree may be revealed to account for better cross-species extrapolation in the animal Tree of Life.

PrecisionTox project aims to improve chemical safety assessment to better protect human health and the environment by using powerful computational approaches to model untargeted multi-omics data collected from non-sentinel species to predict chemical toxicity and understand the molecular mechanisms. We develop new approach methodologies (NAMs) that adopt advanced artificial intelligence and machine learning paradigms to detect and identify molecular key events (mKEs), which in PrecisionTox are defined as a sparse network of interacting genes and their metabolic products that are a necessary element of the adverse outcome pathway (AOP) critical to the outcome. Eventually, the mKE biomarkers are discovered as quantifiable molecular indicators of a toxicological response that are predictive of chemically induced adversity. In this presentation, I'll introduce the structure of the PrecisionTox project, including the progress, challenges, and opportunities. I'll also demonstrate the computational framework for mKE biomarkers identification followed by a case study.



Dr Jiarui Zhou is a Lecturer in Environmental Bioinformatics at the School of Biosciences, University of Birmingham. He is a member of the Centre for Environmental Research and Advocacy (CERA) and the Centre for Computational Biology (CCB). He is a Co-I of the EU H2020 PrecisionTox project and the Chair of the AI/Machine Learning Working Group for the project. His research focuses on using explainable artificial intelligence (XAI), multimodal machine learning, and graph/network modelling for the integrated analysis of multi-omics big data, aiming to bridge the gap between computational and biological fields to tackle the emerging challenges in toxicology and ecotoxicology.

Abstract 7: Acute-to-chronic extrapolation in vitro. Implications for the development of KERs

Peter Macko – European Commission, Joint Research Centre (JRC)

In vitro methods, as alternatives to animal testing, are key elements of new approach methodologies for toxicological hazard and risk assessment. Typical in vitro experiments are conducted over short durations with measurements of response at a single time point, with a focus on providing effect and concentration-response information as input to this assessment. This limits the usefulness of such data since potential chronic effects that cumulate over time are not usually considered. To address this, an experimental design is presented to characterise the toxicodynamics of a response not only in terms of concentration but also as a function of time. Generation of concentration-time-effect responses allows both the extrapolation of points of departure from an acute to chronic exposure and the determination of a chronicity index that provides a quantitative measure of a chemical's potential to cause cumulative effects over time. In addition, the approach provides a means to characterise the dynamics of key event relationships for the development of quantitative adverse outcome pathways.

Peter Macko graduated in physics from Comenius University in Bratislava, he completed his Ph.D. in laser spectroscopy at Joseph Fourier University in Grenoble. His early carrier research was in experimental and computational physics, focusing on highly sensitive spectroscopic techniques applied in atmospheric, interstellar, and plasma physics and chemistry. He gained extensive experience with laser detection techniques, optical systems and microscopy, and computational skills with modelling optical systems, rate equations of photon-molecule interactions, and the dynamics and kinetics of chemical, transport, and diffusion processes. He later oriented his research towards biomolecular imaging and for more than 10 years has been working at EURL ECVAM with high content imaging platforms and in vitro methods, the alternatives to animal testing for toxicological hazard and risk assessment.

Abstract 8: Quantitative adverse outcome pathway (qAOP) models for toxicity prediction

Nicoleta Spînu – Liverpool John Moores University

The concept of quantitative Adverse Outcome Pathways (qAOP) has gained interest over the past decade. This is because of its ability to use *in silico* computational techniques that integrate different data modalities, including New Approach Methodologies (NAMs), and translate mechanistic understanding of toxicity into safety testing strategies and estimates of risks, i.e., the magnitude of exposure to elicit an adverse effect. This presentation will address how the qAOP concept has advanced over the past decade including methodologies and applications of these models. 23 qAOP models were identified in the scientific literature and were assessed for several criteria including type of input data, key elements, the applicability domain in the context of chemical risk assessment. Various stressors triggered the biological paths such as nanoparticles, chemicals, mixtures and environmental factors. Both linear and network of AOPs served as the causal construction for the computational modelling. *In silico, in vitro*, and *in vivo* data were used to model response-response relationships. The qAOP models were constructed to either inform on the mechanism of action or to derive points of departure and a risk. The findings can guide the development of qAOPs where further efforts are required to achieve validation, harmonisation and regulatory acceptance of qAOP models.

	Nicoleta Spînu has obtained her PhD in computational toxicology in the Lab of Mark Cronin, Chemoinformatics Research Group, at Liverpool John Moores University, Liverpool, United Kingdom. Her PhD focused on the development and applications of quantitative Adverse Outcome Pathway (qAOP) models for toxicity prediction, and was funded by the MSCA ITN in3 Project.

Abstract 9: A Machine-Readable AOP Evidence Data Model: Enhanced data input and retrieval from the AOPwiki

Jason O'Brien (Environment and Climate Change Canada)

The committee that oversees the AOP Knowledgebase (AOP-KB) is considering modifications to the underlying database model that may improve the ease and transparency of how information are collected, organized, and retrieved, particularly with respect to automatable processes. Here, we present a pilot study that tests one of these modifications: a machine-readable model of the AOP evidence structure. For this pilot study, we designed a generalized data model based on the modified Bradford Hill criteria of causality for key event relationships (KERs). A central theme was to ensure that evidence was transparently reported using easily queried structures. Accordingly, each unit of evidence could be explicitly linked to specific descriptions of stressors, measurement methods, biological domains, publication references, as well as quantitative data. We also developed a graphical user interface for manual input while enforcing fixed vocabularies. The model was tested by reconstructing the KER evidence of two OECD-endorsed AOPs (AOP #25: Aromatase inhibition leading to reproductive dysfunction; and AOP #131: Aryl hydrocarbon receptor activation leading to uroporphyria). Our objectives were to 1) evaluate the ease with which users could input information into the data model; 2) determine if coherent KER evidence structures could be represented; 3) test the transparency and accessibility of the resulting database using queries that cannot be conducted in the current AOP-KB; and 4) identify aspects of evidence collection that could potentially be automated. If implemented, this data model has the potential to significantly facilitate both manual and automated data input and retrieval from the AOP wiki.

Affiliations

- Research Scientist in the Ecotoxicology and Wildlife Health Division at Environment Climate Change Canada
- Adjunct Research Professor in the Department of Biology at Carleton University in Ottawa, Canada

Research

- Dr. O'Brien specializes in developing and applying modern molecular and high-throughput technologies, such as genomics and in vitro models, for characterizing the toxicological hazard of ecological pollutants.
- Dr. O'Brien works with Canadian and International regulatory organizations to promote and facilitate the incorporation of modern molecular toxicology data into the chemical risk assessment process.

Abstract 10: The state of the art in AgChem – an example using HPPD inhibitors

Steven Webb - Syngenta

We developed a quantitative multi-scale *in silico* model of mammalian Hydroxyphenylpyruvate dioxygenase (HPPD) inhibition to predict thresholds for toxicity and allow quantitative cross-species extrapolation. HPPD is essential for plant carotenoid biosynthesis; and is also present in mammals where it is involved in the catabolism of tyrosine, an amino acid derived from dietary proteins. However, inhibition of the mammalian orthologs of HPPD can result in accumulation of systemic tyrosine (tyrosinemia), which can result in a spectrum of effects including ocular lesions, liver and kidney weight effects in systemic toxicity studies.

The *in silico* model is presented as a source-to-outcome case study to demonstrate the integration of multiple-scale pharmacodynamic and pharmacokinetic modelling using the Aggregate Exposure Pathway and Adverse Outcome Pathway Frameworks. The pharmacodynamic element consists of a systems-based description of the catabolic pathway for tyrosine, which is coupled with the HPPD inhibitor pharmacokinetics which we then extrapolate from rat to human to allow for an evaluation of potential risks associated with HPPD inhibitor exposures in human populations.

In this AgChem case study, extensive *in vivo* rat data (from over 100 HPPD inhibitor molecules) were used for model development and validation. The challenge is developing and validating this type of *in silico* model in the absence of such animal data. Therefore, we highlight areas where New Approach Methods could have been alternatively employed but also where further development is required.

Dr Steven Webb is a Senior Modelling Expert in Early Stage Research within Product Safety at Syngenta and has over 20 years research experience in mathematical modelling, in particular pharmacokinetic/dynamics, in-vitro to in-vivo extrapolation and quantitative systems toxicology (>60 publications, >1500 citations). Prior to joining Syngenta in March 2020, Dr Webb held the John Anderson Research Lectureship in Mathematical Medicine at the University of Strathclyde (2007-2012), was a Lecturer in Systems Toxicology in the Centre for Drug Safety Science at the University of Liverpool (2012-2015) and a Reader in Applied Mathematics at Liverpool John Moores University (2015-2020).

Abstract 11: Moving from detection of cardiovascular liabilities to quantitative translational understanding: challenges and opportunities

Linda Starnes – Astra Zenaca

Cardiovascular safety findings encompass a range of perturbations covering ECG changes, haemodynamics and cardiac pathology. These changes can occur independently or concomitantly, either directly or indirectly related to PK parameters. Within cardiovascular safety, molecular understanding is key to developing quantitative translational insights and ultimately to predicting quantitative outcomes in patients. The concepts and techniques used depend on whether the molecular mechanism is known or unknown. We will discuss these scenarios using real examples to highlight the difficulties, challenges and impact associated with developing quantitative mechanistic understanding within drug discovery.

Molecular understanding of different cardiovascular effects varies, currently the key ion channels responsible for changes in QT, QRS and PR intervals are well established. In addition to some mechanisms for haemodynamic perturbations are known, for example inhibition of VEGFR2 and blood pressure increases. Such understanding enables the development of quantitative tools. However, cardiovascular effects are often multifactorial, and the mechanisms are largely unknown, presenting bigger challenges. Technological developments in terms of 'omics' technologies, off-target profiling and data mining/bioinformatics have the potential to begin to fill this void. These approaches allow hypotheses to be developed that require further investigations. Application examples include off-target profiling utilising multiple kinase and omics (proteomics, transcriptomics and metabolomics) technology platforms. These approaches are enabling molecular understanding of cardiac pathology. If confirmed, such approaches could be used as the basis for further quantitative mechanistic understanding incorporating systems pharmacology modelling. Success will facilitate quantitative predictive outcomes in patients and informed drug design.

Linda Starnes is currently a Director of Cardiac Safety at AstraZeneca. She has an extensive background in molecular and cellular biology spanning basic disease mechanisms to translational sciences. Linda earned a MSc in Cancer Biology from the University of Calgary, a PhD in Cell Biology from University of Rome La Sapienza, and Postdoctoral research performed at UCSF and the Novo Nordisk Centre for Protein Research in Copenhagen. Linda joined AstraZeneca in 2016 as an Associate Principal Scientist focused on in vitro model and translational assay development across multiple target organs. In 2017 she joined the Cardiovascular Safety team with broad responsibilities ranging from design and execution of safety assessment plans, contribution to the teams strategy and innovation roadmap, leading external collaborations all to push forward AZ Cardiovascular Safety Science.

Abstract 12: Identifying thyroid hormone disruptors by establishing qAOPs integrating cross-species extrapolations and thresholds

Stephanie Melching-Kollmuss – BASF SE

Endocrine disruption (ED) assessments are conducted for agrochemicals in Europe based on the European Commission ED criteria and the ECHA/EFSA Guidance Documents. The endocrine (thyroid hormone) disruption potential of substances, inducing thyroid histopathological and/or thyroid hormone (TH) effects in rodent studies, is to be assessed using Adverse Outcome Pathways (AOPs). The adverse outcome (AO) of neurodevelopmental toxicity, as a consequence of thyroid hormone disturbance, has many uncertainties, including knowledge gaps and missing robust and validated assays/technology to determine MIEs (molecular initiating events), key events (KEs), and AOs. Furthermore, there are no agreed assessment schemes established to estimate the quantitative nature of thyroid AOPs, taking into account thresholds of KEs and species differences.

Extensive literature searches and evaluations have been conducted by the ECETOC Thyroid Task Force (TF) having started with an in-depth evaluation of the human/epidemiological data on TH related neurodevelopmental toxicity and on KEs of relevant adverse outcome pathways (Sauer et al., 2020, Marty et al., 2021). In the recently submitted third publication, 4 case studies grouping data from 14 substances based upon similar MIEs were identified, and the thyroid- and brain-related effects seen in rodent studies after gestational/lactational exposure-, indicative of neurodevelopmental toxicity were investigated. According to this evaluation maternal serum TH levels alone were not sufficient to establish a causal relationship with neurodevelopmental effects in rats. Offspring serum T4, together with T3 and TSH values should be used together. Threshold of approximately. ≥50%/60% offspring serum T4 reduction indicated an increased likelihood for neurodevelopmental effects in rats. Brain TH levels - are likely relevant, too. However, the overall dataset was limited.

In order to address correlations between rat and humans, a physiologically based biokinetic model has been developed to evaluate species differences in TH storage and clearance and to predict the effects of liver enzyme inducers (PXR/CAR activators, leading to thyroid effects in *in vivo* rat studies) on hormone homeostasis in humans. Predicted plasma TH concentrations for euthyroid adult rat / humans were within published data and validated against radiolabeled TH data. Published rat data on effects of the CAR inducer, phenobarbital (PB) on T4 glucuronidation were used to predict the 40% decrease in total plasma T4 in PB-treated rats at 100 mg/kg/day for five days. Successful extrapolation of the rat acute TH model across dosing regimens and species supports its potential for use as a predictive tool for an assessment of the effects of PXR/CAR activators on TH homeostasis in humans. In a follow-up project, modelling of offspring thyroid hormones (rat vs. humans) is targeted, as offspring thyroid hormone levels showed a better correlation to rat DNT outcomes.

The concept of quantitative AOPs (qAOPs) should be better able to assess correlations between MIEs, KEs and AOs for thyroid compounds taking into account thresholds, and cross-species extrapolations and to finally inform about their thyroid hormone disruption potential. In the talk thoughts on hazard/risk assessment of liver enzyme inducers, which have an effect on thyroid hormone concentrations in rodents, by using the concepts of qAOPs will be presented as a basis for regulatory decision making.

	Stephanie Melching-Kollmuss is working in the field of regulatory toxicology for chemicals and plant protection products since over 20 years, since more than 15 years at BASF SE. Her special interest is in mixture toxicity and endocrine disruption. Both areas are of huge public interest, and are scientifically challenging, esp. when it comes to implementation into regulation. Stephanie was involved in two Cefic LRI projects: “Combined low-dose exposures to anti-androgenic substances” and “Developing a quantitative AOP for liver-mediated thyroid modulation after prenatal exposure to a xenobiotic compound in the rat”. Further, she was a member in the ECETOC low-dose interaction and the Thyroid Task Force, which she is chairing since 2020. Stephanie is also a member in endocrine expert groups within industry associations (e.g. Crop Life Europe). Stephanie is coordinating endocrine-related research activities and working on assessment strategies for endocrine disrupting compounds. In this context projects are running to develop PBPK models to estimate thyroid hormone concentrations in blood of rats vs. humans and to assess species differences in thyroid hormone metabolism and clearance.

Closing presentation

Richard A. Currie - Syngenta

	Richard Currie is a Syngenta Fellow based at Syngenta’s Jealotts Hill International Research Centre (Bracknell, UK). He is an internal consultant and intrapreneur for predictive / computational approaches for Product Safety assessment and chemical design. His research interests are in mechanistic toxicology and the use of that knowledge for the development, evaluation, and ultimately the application and deployment of new approaches to toxicity prediction. He has interests in the development and application of SAR, mechanism of toxicity (including MOA/AOPs), systems models for toxicology, ecotoxicology and environmental fate.

LIST OF PARTICIPANTS:

Roman Ashauer, Syngenta
Lola Bajard, Recetox, Masaryk University
Hugh Barlow, Unilever
Joost Beltman, Leiden University
Phil Botham, Syngenta
Mark Cronin, Liverpool John Moores University
Andreea Cuciureanu, ECETOC
Richard Currie, Syngenta
Stephen Edwards, RTI International
Thomas Exner, Seven Past Nine
Filippo Di Tillio, Leiden University
Caroline Gomes, BASF SE
Paul Jennings, Vrije Universiteit Amsterdam
Dustin Kapraun, US EPA
Nynke Kramer, Wageningen University
Sunil Kulkarni, Health Canada
Mirjam Luijten, RIVM
Peter Macko, EC Joint Research Centre (JRC)
Marvin Martens, Maastricht University
Bette Meek, University of Ottawa
Stephanie Melching-Kollmuss, BASF SE
Chander Negi, Recetox, Masaryk University
Jason O'Brien, Environment and Climate Change Canada
Alicia Paini, esqLABS GmbH
David Pamies Aubalat, Lausanne University
Prakash Patel, Cyprotex
Audrey Phan, Recetox, Masaryk University
Magda Sachana, OECD
Stefan Schaller, esqLABS GmbH
Stefan Scholz, Helmholtz Centre for Environmental Research (UFZ)
Blanca Serrano Ramon, ECETOC
Iva Sovadinova, Recetox, Masaryk University
Nicoleta Spînu, Liverpool John Moores University
Linda Starnes, AstraZeneca
Cecilia Tan, US EPA
Marcus Tindall, University of Reading
Knut Erik Tollefsen, Niva
Chun-Wei Tung, National Health Research Institutes
Bennard van Ravenzwaay, Wageningen University
Daniel Villeneuve, US EPA
Steven Webb, Syngenta
Andrew Worth, EC Joint Research Centre (JRC)
Huan Yang, esqLABS GmbH
Elias Zgheib, Certara
Jiarui (Albert) Zhou, University of Birmingham

DAY 1 ONLY (ONLINE PARTICIPATION)
Dennis Becker, Clariant
Dorothee Funk-Weyer, BASF
Thomas Preuss, Bayer
Carlos Rodriguez, P&G

BREAKOUT GROUPS – DAY 2

GROUP 2 (ROOM 2)

Ben	van Ravenzwaay	Moderator
Nynke	Kramer	Rapporteur
Joost	Beltman	
Phil	Botham	
Caroline	Gomes	
Paul	Jennings	
Marvin	Martens	
Prakash	Patel	
Audrey	Phan	
Magda	Sachana	
Steven	Webb	

GROUP 3 (ROOM 3)

Marcus	Tindall	Moderator
Roman	Ashauer	Rapporteur
Mark	Cronin	
Peter	Macko	
Bette	Meek	
Stephanie	Melching-Kollmuss	
David	Pamies Aubalat	
Marcus	Tindall	
Knut Erik	Tollefsen	
Huan	Yang	
Elias	Zgheib	

GROUP 4 (ONLY ONLINE)

Cecilia	Tan	Moderator
Alicia	Paini	Rapporteur
Lola	Bajard	
Hugh	Barlow	
Richard	Currie	
Filippo	Di Tillio	
Steve	Edwards	
Thomas	Exner	

Dustin	Kapraun
Mirjam	Luijten
Jason	O’Brien
Stefan	Scholz
Dan	Villeneuve
John	Ward
Andrew	Worth