7 & 8 November 2023 | Brussels

CHRONOS AND KAIROS

UNDERSTANDING TIME IN BIOLOGY - TIME 4NGRA

NEXT-GENERATION RISK ASSESSMENT

Workshop background and objectives

The aim of this workshop is to explore the need and approaches to study the influence of time and level of biological organisation (population, organism, tissues, cells etc.) in toxicity testing in next generation risk assessment based on new approach methodologies (NAMs). Notably, the aim is to discuss how to integrate the influence of exposure time window, duration, frequency and damage accrual rate in developing and interpreting in vitro models, quantitative adverse outcome pathways (qAOP) and quantitative in vitro to in vivo extrapolation (QIVIVE). While computational models allow us to reduce toxicity readout 'noise' due to exposure and observation timing and visualize and interpret the intricacies of toxicity development.

The workshop will bring together toxicologists, biologists, bioinformaticians, modelers and risk assessors to support the discussion around the concept of time in human toxicology. This interdisciplinary setting is essential to achieve and provide a forum for experimentalists to meet with modelers to map out how the future of chemical safety assessment can exploit knowledge of the effect of time on toxicity. In so doing, we aim to develop a strategy for including 'time - variables' in next-generation risk assessment.

The workshop outcome will be documented in an ECETOC workshop report or a peer reviewed manuscript.

Workshop programme

Day 1: 7 November		
10.30 – 11.00	*Arrival and registration for in-person participants*	
11.00 – 11.20	Welcome, introduction and workshop objectives	**Blanca Serrano** (ECETOC), **Alicia Paini** (esqLABS, DE)
11.20 – 11.40	Studying and comparing the role of time in in vitro and in vivo toxicity tests	**Nynke Kramer** (Wageningen University and Research, NL)
11.40 – 11.50	Systems modelling of quantitative adverse outcome pathways: progress on temporal integration of toxicokinetics and beyond	**Huan Yang** (esqLABS, DE)
11.50 – 12.00	General discussion/Q&A	
11.50 – 12.10	Time variables and exposure in in vitro testing strategies	**Peter Macko** (JRC, IT)
12.10 – 12.20	General discussion/Q&A	
12.20 –12.40	*Title TBC* Topic: integrating the effect of exposure time and time window in (next generation) risk assessment	**Harvey Clewell** (Ramboll, US)
12.40 – 12.50	General discussion/Q&A	
12.50 – 13.50	**Lunch**	
13.50 – 14.10	Bringing the pieces of the puzzle together: considering time and biological scale with new approach methodologies	**Gladys Ouedraogo** (L'Oréal, FR)
14.10 – 14.20	General discussion/Q&A	
14.20 – 14.40	Dose and Time Responses using in vitro Metabolomics	**Ben van Ravenzwaay** (Wageningen University and Research, NL)
14.40 – 14.50	General discussion/Q&A	
14.50 - 15.20	**Coffee break**	
15.20 – 15.40	Integration of time-related factors in dose-response analysis and exposure assessment	**Cecilia Tan** (US EPA, US)
15.40 – 15.50	General discussion/Q&A	
15.50 – 16.10	*Title TBC* Topic: Ecotox modelling acute to chronic	**Aaron Redman** (ExxonMobil, US)
16.10 – 16.20	General discussion/Q&A	
16.20 – 16.40	Brief summary/overview of presentations, discussion & last remarks/notes	**Nynke Kramer**
16.40 – 17.00	Introduction to day 2	**Alicia Paini**
17.00 – 18.30	**Aperitivo**	

Day 2: 8 November			
09.30 – 09.45	Welcome and introduction to Day 2		
09.45 – 12.30	Breakout groups on case studies, guided by charge questions and a matrix		
	Breakout group 1	*Skin Sensitisation AOP (e.g. AOP 40)*	Moderator: **Daniela Holland** (ExxonMobil, BE) Rapporteur: **Petra Kern** (Procter & Gamble, BE)
	Breakout group 2	*Neurodegenerative diseases AOP (e.g AOP 3)*	Moderator: **Alicia Paini** (EsqLABS, DE) Rapporteur: **Susana Proenca** (Wageningen University and Research, NL)
	Breakout group 3	*Carcinogenicity AOP (e.g. AOP)*	Moderator: **David Rouquie** *(online)* (Bayer, FR) Rapporteur: TBC
	Breakout group 4	*Liver toxicity cholestasis AOP (e.g. AOP 27)*	Moderator: **Nynke Kramer** (Wageningen University and Research, NL) Rapporteur: **René Geci** (esqLabs GmbH/ University Hospital Aachen, DE)
	Breakout group 5	*ED-mediated DART AOP (e.g. AOP 23)*	Moderator: **Ben van Ravenzwaay** (Wageningen University and Research, NL) Rapporteur: **Tim Gant** (Imperial College London, UK)
12.30 – 13.30	**Lunch**		
13.30 – 15.30	Plenary feedback from breakout groups		**Rapporteurs from breakout groups**
15.30 – 16.00	Summarise and close		**Ben van Ravenzwaay**

Organising Committee

Paolo Boffetta, Bologna University
Tim Gant, Imperial College London
Daniela Holland, ExxonMobil
Nynke Kramer, Wageningen University and Research
Philippe Lemaire, TotalEnergies
Alastair Middleton, Unilever
Alicia Paini, esqLABS
David Rouquie, Bayer
Kees van Leeuwen, KWR Water Research Institute
Ben van Ravenzwaay, Wageningen University and Research

Blanca Serrano, ECETOC
Andrea Salvadori, ECETOC
Lucy Wilmot, ECETOC

Venue

ECETOC Offices
Rue Belliard 40 – 10th floor,
Brussels, Belgium

Contact

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Speakers' bios and abstracts

Studying and comparing the role of time in in vitro and in vivo toxicity tests

Nynke Kramer, Wageningen University and Research, NL

In risk assessment, the role of time on the toxic potential of a chemical is generally assessed using a suite of toxicity assays on animals which are exposed and observed for a defined period of time. These different tests result in different toxic endpoints and potencies. With the shift in paradigm towards the use of non-animal testing methods for toxicity testing and risk assessment, new challenges arise aligning in vitro-derived toxicity data to the different in vivo toxicity tests with defined exposure durations. Toxicokinetic-toxicodynamic (TK-TD) modelling from ecotoxicity studies may be able to help overcome these challenges and provide a mechanistic approach to understanding the role that time plays in toxicology. In this presentation, studies illustrating the application of TK-TD modelling in in vitro toxicology and quantitative in vitro-in vivo extrapolation (QIVIVE) will be highlighted. These include two repeat-dose in vitro studies integrating TK-TD modelling to assess the neurotoxic and hepatotoxic potential of amiodarone, a highly lipophilic drug to treat arrythmia.

Nynke Kramer is associate professor in toxicology in the Toxicology Division of Wageningen University and Research. Her research focusses on enhancing the uptake of in vitro models in toxicological risk assessment by developing models extrapolating effect concentrations obtained from in vitro cell assays to toxic doses relevant to humans and animals. She teaches pharmacokinetics and (eco)toxicological risk assessment at undergraduate, graduate, and postgraduate level. Her teaching and research neatly integrate the skills she acquired as an assistant professor and post-doctoral fellow at the Institute for Risk Assessment Sciences of Utrecht University, as well as during her PhD in toxicology at Utrecht University, her MSc in Environmental Change and Management at Oxford University, and her BSc in Life Sciences at University College Utrecht.

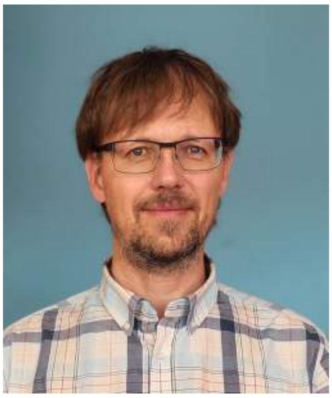
Systems modelling of quantitative adverse outcome pathways: progress on temporal integration of toxicokinetics and beyond

Huan Yang, esqLABs, DE

Time variables and exposure in in vitro testing strategies

Peter Macko, Joint Research Center (JRC), European Commission

In vitro methodologies serve as valuable alternatives to animal testing, forming integral components of novel approach methodologies for toxicological hazard and risk assessments. However, in vitro experiments often have limitations in terms of their duration, measurements of responses, and rarely consider more time points, which may result in the disregard of potential cumulative chronic effects over time. To address this issue, we propose an experimental design that not only characterizes the toxicodynamics of a response in relation to concentration but also incorporates the dimension of time. The concentration-time responses are modelled using a set of ordinary differential equations (ODEs). This approach enables the characterization of the dynamics of key events and their relationships, thus facilitating the development of quantitative adverse outcome pathways.



Peter Macko received a degree in physics from Comenius University in Bratislava before obtaining his PhD in laser spectroscopy from Joseph Fourier University in Grenoble. During the early years of his career, he focused on experimental and computational physics, primarily utilizing highly sensitive spectroscopic techniques to investigate atmospheric, interstellar, and plasma physics and chemistry. He possesses a wealth of experience in laser detection techniques, optical systems, microscopy, and computational skills, including the modelling of optical systems, and the dynamics and kinetics of chemical, transport, and diffusion processes. Later on, his research shifted towards biomolecular imaging. He has spent over a decade working at EURL ECVAM with high-throughput and high-content imaging platforms for in vitro methods, and with computational toxicology.

TBC

Harvey Clewell, Ramboll, US

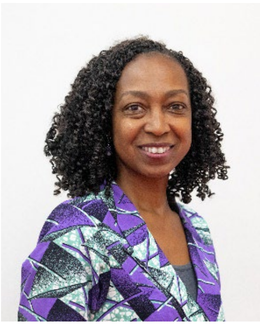
Bringing the pieces of the puzzle together: considering time and biological scale with new approach methodologies

Gladys Ouedraogo, L'Oréal, FR

There is a growing need of new approach methodologies for addressing hazard and risk assessment. Some regulations like the European Cosmetics' one banned animal testing.
Several initiatives are trying to address this need. For local effects like skin and eye irritation, there are some strategies to addressing them with new approach methodologies -NAMs-. When it comes to complex endpoints like systemic/reproductive toxicity more effort is needed to establish tools and approaches allowing safety assessment and the likelihood of causing adversity which is a requirement for chemical registration. It is now commonly accepted that no one to one replacement nor a "one size fits all" approaches are suitable.
Characterizing adversity with new approach methodologies is challenging for many reasons:

- There are multiple ways to cause systemic toxicity and most of the underlying mechanisms leading to adversity are unknown.
- The temporality aspect between exposure and when toxicity occurs.
- Scaling from molecular, cellular effects to organs or organisms/populations.
- A pragmatic approach allowing proposer biological coverage of key pathways related to human health.

Here, examples of using NAMs to address long term effects like general repeated dose systemic toxicity and carcinogenesis will be presented. They will feed into the discussion of establishing relevant experimental conditions when developing NAMs.

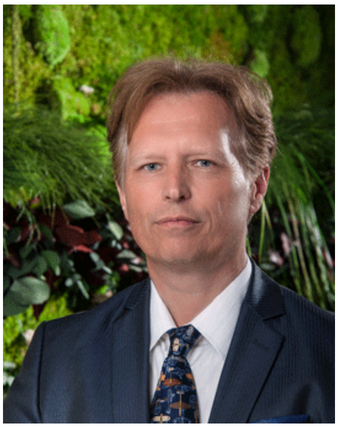


Gladys Ouédraogo has extensive experience in the development of New Approach Methodologies.
She joined L'Oréal R&I in 2003 to establish a unit for predicting cancer without animal testing. During her career, she created and led various research projects on technologies and emerging topics in the field of toxicity assessment. In doing so, she worked on genotoxicity, molecular modeling, systemic toxicity and endocrine modulation. In 2013, after leading teams working on alternative methods for predicting toxicity and efficacy for three years, she has been managing several collaborations and activities in areas such as repeat-dose systematic toxicity – an area that she is also actively developing within L'Oréal R&I.

Dose and time responses using in vitro metabolomics

Ben van Ravenzwaay, Wageningen University and Research, NL

In regulatory toxicity testing the duration of exposure has an influence on both the quality (which organs are affected and to which extend) of the toxicity observed as well as the quantity (dose without an effect). Depending also on the kinetics, time may only have a moderate aggravating effect or can be even more important than dose (complete carcinogens). How can we account for such time dependent properties in in vitro studies? Using in vitro metabolomics dose and time dependent responses of 256 intracellular metabolites were investigated following 3, 6, 24 and 48h exposure to various concentrations of nitrofurantoin. Increasing the dose and exposure duration were observed enhance the metabolic response. For the high concentration a non-linear response was seen for some metabolites, most likely related to the occurrence of cytotoxicity at the later time points. For the low concentrations this was not the case. Analysis of such dynamics may help to clarify if a time related change in the quality of the toxicity response may occur for a particular compound. What might happen beyond 48h would require further investigation.



Bennard van Ravenzwaay is a doctor of Environmental Sciences/Toxicology from Wageningen University, Netherlands in collaboration with the German Cancer Research Centre in Heidelberg, Germany. He worked for 34 years at BASF SE, Ludwigshafen, the last 20 as Senior Vice President of the Department for Experimental Toxicology and Ecology and BASF Metabolome Solutions.

He is an associate professor for Reproduction Toxicity of the University of Wageningen and had a teaching assignment at the University of Kaiserslautern until 2021.

He is Chairman of the Scientific Committee of the European Centre for Ecotoxicology and Toxicology (ECETOC) and a member of editorial boards of “Archives of Toxicology”, “Chemical Biological Interactions” and “Toxicology Letters.

He was member of the board of trustees of the 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 is an author more than 250 peer reviewed publications.

Since 2022 he is an independent consultant for environmental sciences.

Integration of time-related factors in dose-response analysis and exposure assessment

Cecilia Tan, U.S. Environmental Protection Agency, Durham, NC

In conventional chemical safety testing, animals are exposed to varying durations to simulate scenarios relevant to human exposure. For instance, acute toxicity tests aim to replicate one-time, high-dose accidental exposure, while chronic studies attempt to emulate a lifetime of continuous exposure. Nevertheless, it remains challenging for animal studies to cover the full range of potential human exposure scenarios, encompassing time-related factors such as exposure duration and frequency, and critical exposure windows. Instead, a pragmatic approach is taken, where dose-response analysis estimates a reference dose; exposure assessment predicts potential exposure ranges; and comparing the reference dose with exposure estimates to assess risk. Time-related factors are integrated into both dose-response analysis and exposure assessment, yielding estimates of “doses”. Such a pragmatic approach also applies to in vitro testing, which can be used to identify doses that trigger molecular initial events within adverse outcome pathways. To bridge the gap between the dose of interest from in vitro assay, the dose within the target tissue, and the dose being exposed, physiologically based kinetic (PBK) models can be a powerful tool. In addition, PBK models possess the capability to integrate time-related factors into exposure-relevant or response-specific doses in risk assessment.

Disclaimer: The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency

Dr. Cecilia Tan is a senior science advisor at the Health Effects Division at the US. Environmental Protection Agency's Office of Pesticide Programs. Her main role is to review and apply pharmacokinetic data and models to improve the scientific basis for inter- and intra-species extrapolations in pesticide risk assessment. Before joining the Office of Pesticide Programs in 2018, Dr. Tan was a researcher at the EPA's Office of Research & Development. Her research involved using computational modeling to understand the quantitative relationships between external exposure, internal doses, and adverse outcomes. She is actively involved in several physiologically based kinetic (PBK) modeling-related committees to facilitate more applications of PBK modeling in regulatory risk assessment. Dr. Tan has a MS degree in Environmental Health Sciences from the Harvard School of Public Health, Ph.D. in Environmental Engineering and Sciences from the University of North Carolina, Chapel Hill, and MBA from North Carolina State University.

TBC

Aaron Redman, ExxonMobil, US