Exploring best practices in building quantitative AOPs (qAOPs)

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EUROPEAN CENTRE FOR ECOTOXICOLOGY AND TOXICOLOGY OF CHEMICALS

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DISCLAIMER:

Representatives of the European Commission, ECHA and Member State competent authorities expressed no view or position on the scientific matters discussed during the breakout sessions, hence their participation cannot be taken as agreement/disagreement to the views and positions expressed during the breakout sessions. Representatives of the OECD also attended - the opinions expressed, and arguments employed herein are those of the authors and do not necessarily reflect the official views of the OECD or of the governments of its member countries.

This workshop report is intended to accurately reflect the workshop discussions and conclusions, but it is noted that not all workshop participants provided written input.

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SUMMARY

The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) held a workshop in October 2022 on best practices in building quantitative adverse outcome pathways (qAOPs) to support their development and use in next generation risk assessment.
qAOPs can be used to interpret mechanistic data, for example from in vitro studies, to derive a point of departure for an organism or population for use in chemical safety assessment.
Workshop participants highlighted the importance of following the FAIR principles, including good modelling practices and quality assurance, as well as documenting assumptions, when developing qAOPs to support their application in chemical safety assessment.
They also identified the need to develop international harmonised guidance on the quality assurance, validation and reporting of qAOPs. It was also recommended to use a tiered strategy when developing qAOPs, so that they are easily updateable when new data becomes available and/or the research question changes. Lastly, case studies integrating qAOPs in chemical risk assessment are needed to provide confidence in their application.

INTRODUCTION

The adverse outcome pathway (AOP) is a conceptual construct that facilitates organisation and interpretation of mechanistic data, obtained from in silico, in vitro and in vivo assays, across various levels of biological complexity (Ankley et al. 2010; Vinken et al. 2017; Knapen et al. 2018; Perkins et al. 2019). AOPs are composed of a series of key events (KEs), which are the measurable steps in the pathway that link a stressor-generated molecular initiating event (MIE) to an adverse health outcome. AOPs are useful for developing and integrating new approach methodologies (NAMs), such as in vitro toxicity assays measuring molecular perturbations after exposure to a stressor. Additionally, AOPs contribute to improving chemical safety assessment by serving as a roadmap to identify data gaps and prioritise research (Tollefsen et al. 2014; Kleinstreuer et al. 2016; Wittwehr et al. 2017).

Next generation risk assessments incorporating AOPs and using NAMs, including human-focused in vitro experimentation and in silico modelling of both biological effects and kinetics, typically follow published tiered frameworks to estimate human bioactive doses (Dent et al. 2018; Krewski et al. 2020). In these frameworks, a minimally toxic in vitro exposure is derived from this perturbation of human biological effects using a panel of in vitro assays. This exposure is then converted into a human effective dose estimate, i.e., point of departure (POD), using in silico physiologically based kinetic (PBK) models, which often need to be parameterised by in vitro assays and quantitative structure activity relationships (QSARs). This estimated human effective dose can then be used in a risk assessment to support the derivation of a health-based guidance value. Such an approach is often considered protective based on the premise that biological responses occur at lower doses than adverse responses (Friedman et al. 2020).

The use of the existing AOP framework as the basis for a higher tier risk assessment also presents several issues, including:

- From a qualitative point of view, existing AOPs provide incomplete coverage of common toxicities, and our knowledge of toxic mechanisms is also incomplete.
- Current and emerging regulatory concerns, such as those related to developmental neurotoxicity, often require making predictions about human effects where there are no established in vivo model systems. While panels of potentially mechanistically relevant human in vitro assays have or are being developed, the qualitative and quantitative relationships of these in vitro assays to in vivo effects are not always clear.
- Most of the existing AOPs are not quantitative. Theoretically, in vitro toxicity assays can be used to estimate a dose that triggers an MIE, the foundational event, but the threshold triggering the downstream KEs leading to adversity is difficult to quantify.

Indeed, the majority of the AOPs developed thus far are based on qualitative relationships between KEs and adverse outcomes, which do not relate the severity or likelihood of an adverse outcome to a level of perturbation of MIEs. For use in chemical risk assessment, quantitative AOPs (qAOPs) can further allow for quantifying and predicting dose-response relationships. While definitions differ (Spinu et al. 2020), for the purpose of this workshop report, a qAOP is considered to be a type of AOP that incorporates quantitative data and models to predict the likelihood and/or severity of downstream KEs and adverse outcomes associated with a given level of perturbation of a MIE upon exposure to a (chemical) stressor (Conolly et al. 2017; Perkins et al. 2019; Spinu et al. 2020). In essence, qAOPs identify key event relationships (KERs) that quantitatively describe the nature of the causal relationship between KEs. qAOP development requires the identification and extraction of reliable data and information, and guidance is needed on the development, practical application, and validation of qAOPs (Spinu et al. 2019; Spinu et al. 2020; Paini et al. 2022).

Quantitative AOPs can be viewed as quantitative response-response relationships based on qualitative or descriptive AOPs. To facilitate the design, interpretation and application of qAOPs, and to promote a better understanding of their strengths and limitations, the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) invited experts from different sectors for a two-day workshop (18-19 October 2022, Brussels (F2F and online)). The workshop aimed to bring together AOP experts, toxicologists, biologists, and computational modellers to share and build on the collective knowledge gained from their experience in developing and using qAOPs. The discussions in the workshop focused on human health aspects of qAOPs.

This report summarises the outcome of the workshop discussions on the following questions (also in Table 1):

I. What is the most appropriate modelling approach?
II. What level of biological detail is necessary to include in a qAOP?
III. How do we ensure the quality and accessibility of qAOP models and their predictions?

The workshop programme, organising committee, participants, contributors to this report and a list of speakers' abstracts and reflections can be found in Appendices A-F of this report.

Table 1: Questions addressed by the breakout groups

Questions	Q1: How and why would you choose the most appropriate modelling approach?	Q2: How would you choose the most appropriate level of biological detail to include in your qAOP?	Q3: How do we ensure the quality assurance and accessibility of qAOP models and their predictions?
Prompts	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?	What data should we get and how to organise it? How do you judge how complete (in terms 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?	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[1] of qAOP models and underling data?

BREAKOUT GROUP DISCUSSIONS

The following subsections summarise the workshop breakout group discussions, which seek to provide answers and suggestions to those questions posed in Table 1.

I. qAOP modelling approaches

qAOPs provide quantitative dose-response and time-course predictions to support regulatory decision-making. Over the past five years, examples of qAOPs have been published, and can be classified into three categories based on the approach used to develop them (Spinu et al. 2020). They include: (semi-)quantitative weight of evidence qAOPs that use quantitative weighting of lines of evidence to rank the confidence in KERs (Perkins et al. 2019); probabilistic qAOPs that incorporate statistical or probabilistic approaches, such as Bayesian networks, to establish predictive relationships between KEs (Zgheib et al. 2019); and mechanistic qAOPs, which are developed based on detailed knowledge of the biological mechanisms leading to the adverse outcome (Conolly et al. 2017; Hassan et al. 2017). The models are deterministic and use mathematical functions of the KER to predict the likelihood of a later KE occurring given a change in an earlier KE. The mathematical expression for each KER includes for example regression equations or ordinary differential

[1] FAIR data = Findable, Accessible, Interoperable, Reusable

equations (ODE) (see Spinu et al. (2020) for review), resulting in linear, sigmoidal and Gaussian-type plots. Mechanistic qAOPs provide greater detail, but can be challenging to develop, especially when the biological mechanisms involved are not well understood; and/or quantitative, time-resolved dose-response data are lacking. On the other hand, unlike mechanistic qAOPs, the qAOPs in the first two categories are easier to develop but are largely restricted to the chemical and in vitro/in vivo models used to fit the qAOP.

II. Level of biological detail

The choice of modelling approach for developing qAOPs depends on the available data, the complexity of the underlying biological mechanism, as well as the question at hand based on the specific research or regulatory question being addressed. It is important to distinguish between data that inform the KERs and data that describe the underlying biological system relevant to the toxicology question. Data can provide qualitative and/or quantitative information, available at different biological scales, and represent snapshots of the system at key time points or over time (time course data). Whilst a KER describes the causal relationship between two KEs, the biological processes (subcellular to whole organ scale) which may be affected by a compound constitute highly nonlinear, interconnected, multiscale (both biologically and spatiotemporally) activity. For example, a downstream KE will occur at a later time point, be at a higher level of biological organisation, and the magnitude of change is likely dependent on several earlier sequences of events.

Similar to the battery of models involved in a QSAR model, multiple modelling approaches may be used in one qAOP, depending on the completeness of the AOP and data available for individual KEs (Conolly et al. 2017). As with any computational model that approximates the system it represents, no qAOP describes every level of biological organisation in an AOP and quantifies every KER (Spinu et al. 2020). Due to data paucity, existing qAOPs generally do not include kinetic considerations, details about compensatory mechanisms, or feedback loops, although some consider modulating factors such as environmental conditions (Chu 2018). However, for qAOPs to be used in risk assessment, not every KER needs quantification. Intermediate steps may be skipped when knowledge of the rate limiting step or when a simple qAOP model answers the question set (e.g. Stadnicka-Michalak et al. (2015)). The level of detail and completeness required is dependent on the research question, and it also determines the applicability domain of the qAOP.

A tiered strategy should be considered when developing qAOPs, where they are easily updateable when new data becomes available and/or the application requires greater detail, precision, and accuracy. The modelling of a qAOP needs to encompass both the scientific question of interest and the knowledge and data on relevant KEs and the associated underlying biological processes for parameterising the model. Models should include enough explanatory variables relevant to explain the known system outcomes, while seeking to expand their ability to address more questions as knowledge regarding the system grows. At the same time, one strength of mathematical modelling is its ability to test plausible hypotheses by using the discrepancies between model predictions and observed data to inform potential sources of variation, such as missing KEs or KERs that are not properly described in a qAOP. Indeed, differences between models that are not able to reproduce known KE outcome behaviour can be equally as informative as those that are.

Thus, the modelling approach to develop a qAOP is a crucial factor, however, as crucial is our confidence in the data going into developing the model and defining the applicability domain. Evaluation of a qAOP involves

assessment of the confidence in the underlying AOP. Low confidence in the AOP, based on its biological plausibility, essentiality, and empirical support, is likely to limit the applicability of the qAOP model. Every mathematical model is a simplified representation of a biological system, so a model is not expected to make predictions about all elements within the system. Rather, a model is developed to make predictions, which can be used to fill knowledge gaps or enhance understanding of the system for a specific purpose. The intended purpose of a model determines its structure and the type of data that are relevant for a model. Relevant data may comprise those data that are necessary for model parameterisation as well as data that are necessary for model evaluation, validation or refinement. Thus, the most important aspect of qAOP model development is that the mathematical model needs to be "fit-for-purpose". Both the specific purpose and the applicability domain of the qAOP need to be defined upfront. A qAOP could be further refined as more information becomes available. Ideally, data collection and model refinement should be an iterative process to improve a qAOP's predictive capability (Perkins et al. 2019). Different questions require different models, therefore a qAOP may represent only a subset of the AOP taken as starting point and the resulting model can include parts and data that are not within the AOP (Perkins et al. 2019).

III. qAOP data requirements

The data required at each stage of the modelling process (formulation, parameterisation and testing) may differ to some degree in its form. For instance, data for model formulation can consist of diagrammatic details that illustrate how KEs or pathways within the biological system are linked (i.e. the underlying AOP construct). Data for parameterising and testing the formulated models often takes the form of singly reported values, such as reaction rate constants, concentrations, or time course data. Data may also be specific to the biological/physiological aspects of the system of focus, such as certain cell types or combined data from varying cell types. Sufficiently good quality data are needed to test and validate the model predictions, ideally from different experiments/sources used to parameterise the model to assess the identifiability of model parameters and predictive ability of the model.

For KER modelling, data relevant to the key outcomes is needed to inform how events proceed and to ensure the model accurately captures the known behaviour. Whilst the quantity of publicly available data for informing biological modelling at different scales continues to grow and is available to qAOP researchers, questions remain about how qAOP data from prior toxicology studies should be organised for easy access. Should data be organised according to specific compounds and their respective adverse outcomes in certain organs and tissues? Should data be archived by organs to enable extracting data for a range of compounds? Ideally both approaches should be made available to researchers to enable them to design, inform, and subsequently test the development of qAOPs.

To achieve this goal, it is recommended to identify existing data sources and examine the reusability of existing data. In addition, the adoption of the Findability, Accessibility, Interoperability, and Reusability (FAIR) principles when generating data is strongly encouraged by the experts (Wilkinson et al. 2016). The Grading of Recommendations, Assessment, Development and Evaluation (GRADE) has also proposed a framework for selecting models (Brozek et al. 2021). Assessing uncertainty associated with models, input data, and the qualitative information in an AOP itself is essential; as well as integrating these uncertainties in the risk assessment process, for example by using probabilistic risk assessment (Maertens et al. 2022).

The FAIR principles, which focus on improving scientific output, were first published by Wilkinson et al. (2016). These principles were initially applied to experimental data but can also be extended to 'any digital object', including mathematical models, software, and experimental and computational workflows. Various recommendations, guidelines, and FAIRification tools have been proposed to support the implementation of the FAIR principles, with many ongoing activities (GO FAIR, ELIXIR FAIR Cookbook and RDMkit, etc.). When applying the FAIR principles to qAOP models, specific aspects become relevant. The FAIRness of a qAOP model is dependent not only on the model itself, but also on the underlying AOP (Cronin et al. 2023).

AOPs that have been entered into the AOP-Wiki meet the **Findability** criteria regarding the data, and efforts are underway to enhance the findability of the metadata. To facilitate the identification of qAOP models based on selected features (e.g. stressor, species, biological assays, modelling technique), an interactive qAOP visualisation tool[2] has been developed, leveraging a review of published qAOP models and their characteristics (Spinu et al. 2019). A good strategy is to create a catalogue of model components retrieved from the literature. In the future, linking qAOP models directly from the AOP-Wiki would be an ideal solution, and should be feasible by building upon the collaborative efforts within the qAOP community.

The **Accessibility** principle focuses on ensuring that the AOP and associated qAOP model are freely and openly accessible. AOPs stored in the AOP-Wiki are available in both human-readable form via web pages and computer-readable form via both XML and JSON formats. All content is freely available with no restrictions on use except for citation of the original work. Ideally, the qAOP models would be accessible in a similar manner to allow for replicating results and promote confidence via transparency by end users from regulatory bodies. While open-source software is the preferred solution, models built using a licensed/ proprietary environment can meet the minimum criteria if the code is available for users with a license. Sharing model code can be done through general options, such as GitHub[3], or specialised systems like ELIXIR's BioModels[4]. A final consideration regarding accessibility is how readily the model can be understood by users with varying levels of expertise (e.g. model experts versus others that do not have that knowledge). A user-friendly interface that allows easy accessibility by non-experts coupled with a transparent codebase available for review by other modelling experts is ideal.

Interoperability is primarily driven using standardised ontologies, terminologies, and vocabularies. For qAOP models, the semantic descriptions used to describe the underlying AOP are heavily dependent on standardised terms. While the AOP-Wiki currently stores most of the information in free text form, there are options available to authors wherein they can provide a more computable description based upon commonly used biological ontologies. Future plans for the AOP-Wiki include reducing the free-text descriptions and requiring authors to provide the information in a computer-readable form to promote interoperability. At that stage, qAOP models should incorporate these entities and the associated ontological terms to promote interoperability. For full interoperability, the assumptions built into the model and the units associated with all inputs and outputs must also be clearly stated. Ideally, the model should require units associated with all

[2] https://public.flourish.studio/visualisation/11342208/

[3] https://github.com/

[4] https://elixir-europe.org/services/tag/elixir-deposition-databases & https://www.ebi.ac.uk/biomodels/

input data be specified by the user and provide the units for the outputs in computer-readable form. This requirement would allow crosstalk between models without the need for data conversion scripts in the middle. The model assumptions are more difficult to codify, but they are equally important. If one model produces outputs based on the maximum or minimum values from a range and the subsequent model assumes those outputs were based upon the central tendency, then all results from the combined models will be completely wrong. Ideally in this scenario, both inputs and outputs from both models would represent the full distribution curve, but this is often not practical. Thus, a clear documentation of the assumptions is the minimum requirement.

To make the qAOP **Reusable,** good modelling practices and quality assurance (QA) criteria should be followed, and the mathematical code should be published so that model outputs can be reproduced. The development and use of a qAOP is constrained by both the availability and quality of data used to formulate, parameterise and test the model. The quality of a qAOP is dependent on the reliability and relevance of the data, such as the reproducibility of measurements and how the in vitro data represent the in vivo system. To prevent the issue of 'garbage in, garbage out', especially in cases where model predictions cannot be evaluated against data, a QA process should be implemented. The QA process should examine the following aspects: a) the underlying modelling theory and assumptions; b) the mathematical model formulation and (in silico) implementation; c) the integration of high-quality data used to simulate KERs; and d) calibration and evaluation/validation informed by sensitivity and uncertainty analysis. The exact nature of a QA process involved in qAOP development depends on the qAOP, which is represented by one or more KE/KERs, and the level of detail required to achieve the intended purpose. The QA process should also identify data gaps and their impact on model outputs, chemical applicability domain, and the strength of causality described by the model. Therefore, a qAOP can be considered as a quality check of the qualitative AOP and its application domain. Additionally, the qAOP model development needs to be fit-for-purpose'; with both the specific purpose and the applicability domain of the qAOP defined upfront. Here again, a qAOP could be further refined as more information becomes available, and ideally, data collection and model refinement are iterative processes.

An example of a QA process to assess qAOP input data and model assumptions is to assess whether the studies used to collect the data followed established US Environmental Protection Agency (EPA) or Organisation for Economic Co-operation and Development (OECD) test guidelines (TGs), guidance documents (such as Good In vitro Method Practices (GIVIMP) and PBPK guidance (EPA 2006; WHO 2010; EPA 2020; Tan et al. 2020a; OECD 2021)) or represented a specific endpoint, such as the OECD developmental neurotoxicity (DNT) guidance (OECD 2023). However, following a test guideline or guidance as criteria may be overly restrictive. At present, relatively few NAM-based TGs and reporting templates for testing and reporting KEs are available but are in development.

Because TGs may be overly restrictive, it would be relevant to provide criteria for conducting non-TG experiments to show the quality of the data, such as clear data provenance trails, proper documentation of the quality control steps, description of the underlying assumptions and measurement principles, and data processing and avoidance of unintentional/fraudulent data manipulation. Integrating new, innovative methods with the potential to improve the qAOP could be considered without first fulfilling all requirements of existing TGs and good laboratory practice (GLP). For example, conducting a comparative study (i.e. comparing outputs from a series of different NAMs) can increase the confidence of data quality, and NAMs

can also be integrated into qAOPs first to demonstrate their relevance before going through the time-consuming task of TG development (e.g. (OECD 2017). It is also very important to assess the readiness of the specific test battery, which can be assessed by specific score limits as proposed by Crouzet and co-workers (Crouzet et al. 2023), and was first proposed by Bal-Price et al. (2018). The European Commission started in early 2023 a new project at the OECD to develop guidance[5] on how to best use academic data for regulatory purposes. This will cover guidance on quality and reporting standards that scientists can use, as well as guidance on how regulators can find, extract, and evaluate such data. A proposed next step is to query and compile relevant criteria from publications and existing guidance (Table A1 in Appendix F of this report), such as those for exposure, PBK and QSAR modelling approaches, to establish a qAOP guideline for academic and regulatory applications.

As aforementioned as part of good modelling practice, documentation of the QA process is essential (Loizou et al. 2008; Paini et al. 2019), and depending on the application and context a peer review is recommended. It is imperative to document how the quality and relevance of the data were determined following available standards. Since 2002, the OECD has developed OECD Harmonised Templates (OHTs) for the reporting of chemical test summaries and data generated based on OECD TGs. OHTs provide standard data formats designed for reporting information used for chemical risk assessment, including physico-chemical properties, and effects on human health (e.g., toxicokinetics, skin irritation, repeated dose toxicity) and effects and fate in the environment (e.g., toxicity to wildlife, biodegradation in soil, metabolism of residues in crops). Another series of OHTs describes chemical use and related exposure of workers, consumers, and the environment. In 2021, the OECD published the OHT No. 201[6] to report intermediate effects from in vitro and in silico methodologies without a TG (Carnesecchi et al. 2023). These standards are useful to chemical database developers and maintainers of databases (Carnesecchi et al. 2023). Providing data in OHTs also enhances the OECD mutual acceptance of data (MAD) decision, where data from one member state can be used by another member country. Data management guided by these formats facilitates the use of the results for registration of chemicals in an EU context or in qAOP development. However, the requirements for data transfer might be different for different purposes. Thus, an independent system can be used to transform the data into any data transfer format that is best suited for the specific use case.

When developing a qAOP, every step of model development should be recorded (e.g. in a research notebook or journal) to track the different versions. The use of platforms, such as GitHub[7], that are based upon a version control system can increase transparency. In addition to detailed provenance on the model development, the steps used to validate the model along with the results and a clearly defined applicability domain, should be reported with the model code. AOPs are in principle chemical agnostic. However, it is not clear if qAOP models should also be chemical agnostic because qAOP models are system and chemical dependent to some extent. These dependencies must be clearly documented for reproducibility. The final consideration for use is a clear and accessible data use agreement that includes an explicit license governing the use of the model and/or

[5] Evidence-informed policymaking: a new document to foster discussion on a better use of scientific knowledge in policy (europa.eu)

[6] https://search.oecd.org/ehs/templates/harmonised-templates-intermediate-effects.htm

[7] https://github.com/

associated data. All models should clearly state any restrictions on use, and those models with the least restrictions will be much more valuable to the scientific and regulatory community.

General workshop recommendations for qAOP implementation

The workshop experts agreed that qAOPs are important tools for next generation risk assessment. qAOPs provide risk assessors with a means to assess the relevance of molecular and cellular perturbation observed in NAM studies for an organism or population. qAOPs can link the level of early KE perturbation to the type and severity of a downstream KE perturbation or adverse outcome. In combination with quantitative in vitro-in vivo extrapolation (QIVIVE) using physiologically based kinetic (PBK) modelling, the resulting in vivo relevant dose-response curve can then be used to derive a point of departure for risk assessment.

Despite their potential, no qAOPs have been used yet to derive points of departure in NAM-based risk assessment. Case studies integrating qAOPs in chemical risk assessment are needed to provide confidence in their relevance. These case studies should ideally be put forward by and/or reviewed by the regulatory community. This will ensure the specific needs of the regulatory community are considered. Case studies may include systemic adverse outcomes with well-defined AOP, for which in vitro and in silico NAMs are readily available and well characterised.

The workshop experts also noted that there are currently no published standards available to characterise and validate qAOPs, although efforts are underway to develop model evaluation guidance through a qAOP working group within the EU Horizon 2020 funded Animal-free Safety assessment of chemicals (ASPIS[8]) project cluster. The group aims to develop guidance on how to evaluate these models. Since the quality and type of qAOP depends on the quality of the AOP and input data (e.g. time-resolved dose-response data), a reporting template for qAOP should be developed and applied to case studies. In parallel, it was also discussed the need for a specific guidance to identify the best modelling approach for qAOP development, given the intended purpose. Where possible, existing standards, such as the QSAR Model Reporting Format (QMRF, required by the EU's Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation, and others), MOdelling DAta generalisation (MODA, endorsed by the European Materials Modelling Council) and PBK model reporting template (Tan et al. 2020b) should be cited.

Finally, it is important to provide educational programmes and tools for stakeholders, including researchers, modellers, regulators, and risk assessors, to understand the modelling approaches, input data and evaluation techniques, given the complexity of the suggested mixed-model, tiered approach to qAOP modelling.

[8] https://aspis-cluster.eu/

CONCLUSIONS

The October 2022 ECETOC workshop on qAOP was setup to help guide qAOP development and use in next generation risk assessment. To do so, it aimed to answer the following questions:

I. What is the most appropriate modelling approach?
II. What level of biological detail is necessary to include in a qAOP?
III. How do we ensure the quality and accessibility of qAOP models and their prediction?

It was concluded that there is not a single appropriate modelling approach, but that the modelling approach is dependent on the completeness, complexity and quality of the underlying AOP as well as the data used to derive and evaluate KERs. The level of biological detail and completeness required is dependent on the research question, and it also determines the applicability domain of the qAOP. A tiered strategy should be considered when developing qAOPs, where they are easily updateable when new data becomes available and/or the application requires greater detail, precision, and accuracy. It was also concluded that for the use of qAOPs for chemical risk assessment, the findability, accessibility, interoperability, and reusability (FAIR) of the qAOP models and data, and assumptions used to develop and evaluate the model, are important. A quality assurance (QA) process should be implemented and may make use of existing good modelling practice guidelines and reporting templates. It was specifically indicated that there is a need to develop international harmonised guidance, e.g. via OECD, on the quality assurance, validation and reporting of qAOP models. In parallel training and education should be explored ad hoc or during conferences (Federation of European Toxicologists & European Societies of Toxicology (EUROTOX), Society of Environmental Toxicology and Chemistry (SETAC), Society of Toxicology (SOT), among others). In conclusion, when ensuring data availability, a QA process and training are in place, it was highlighted how AOP and translation to quantitative dose responses using measured data and in silico models will be beneficial to set POD for specific key events to inform chemical risk assessment.

Since this workshop on qAOPs in October 2022, ECETOC held a second workshop in November 2023 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 (q)AOP and NAMs. The workshop outcome will be published during 2024.

APPENDIX A: WORKSHOP PROGRAMME

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, Syngenta)	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)

esqLABS GmbH)	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 (Syngenta) Richard Currie (Syngenta) Cecilia Tan (US EPA) Ben van Ranvenzwaay (BASF)
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

APPENDIX B: WORKSHOP ORGANISING COMMITTEE

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APPENDIX E: SPEAKER ABSTRACTS AND REFLECTIONS

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 not 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.

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. Recognising 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.*

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 formalised 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 characterisation 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 characterisation). 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 organisation. 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 organisation, 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 characterisation 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.

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

Mark 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).

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) (Vinken et al. 2021). 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 organisations (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.

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.

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.

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.

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, organised, 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 generalised 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.

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.

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.

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.

Speakers' reflections

Mark Cronin – Liverpool John Moores University

The RISK-HUNT3R project is developing qAOPs as part of the implementation of quantitative systems toxicology (QST) (https://www.risk-hunt3r.eu/; Pallocca et al. (2022)). As part of the work into qAOPs, the RISK-HUNT3R project has developed a strategy to create qAOPs, which is described as part of a document to support development. The document provides a source of reference including a summary of resources and guidance across a whole range of topics relevant to qAOPs. The key aspects of qAOPs identified for their development are the completeness of the underlying AOP, the availability of suitable data for quantification and the availability of suitable data for modelling. As part of the implementation of qAOPs, a framework to characterise uncertainty of qAOPs has been developed.
The RISK-HUNT3R project is part of the ASPIS Cluster of projects (https://aspis-cluster.eu/) which aims utilise resources optimally from across the three projects involved. Current activities within the ASPIS Cluster include the development of a qAOP for liver steatosis based on publicly available data and a framework to validate qAOPs.
Overall conclusions from the experience of RISK-HUNT3R to quantify AOPs are that there is no ideal AOP to quantify in terms of completeness, data and the availability of a model. Further, it is the availability of data and a suitable model that is key to the development of qAOPs. Many challenges exist in the development and use of qAOPs, including the demonstration of their use in current risk assessment as well as NGRA.
Acknowledgment:
In preparing his contribution to this workshop, Mark Cronin received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 964537 (RISK-HUNT3R).
Reference:
Pallocca, G., Moné, M. J., Kamp, H., Luijten, M., van de Water, B. and Leist, M. (2022) "Next-generation risk assessment of chemicals – Rolling out a human-centric testing strategy to drive 3R implementation: The RISK-

HUNT3R project perspective", ALTEX - Alternatives to animal experimentation, 39(3), pp. 419–426. doi: https://doi.org/10.14573/altex.2204051

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

Quantifying the KERs using the inverse functions of dose-responses can be used if following conditions are met:

- the MIE and KEs are reversible/transient events, so they follow C_{max} scenario and the steady state of the events is achieved quickly under constant exposure,
- the dose responses were measured once the steady state was achieved.

If output of some of the events is permanent, so it follows the 'area under curve' like scenario, and the steady state (at low but toxic dose) is not achieved quickly, the differential equation calculus needs to be used to take into account the changes over time. Moreover, the differential equation calculus allows the model to take into account a possible occurrence of feedback processes in a rigorous way. As a consequence, the model may become not only descriptive but also predictive.

Therefore, my recommendation for the in vitro method developers would be to focus more on providing measurements of concentration responses of events over time to assess their dynamics characteristics. The key parameters to determine would be the average residence time of the stressor on its target(s) for MIE, and the average recovery time for KEs.

Bayesian approach has the potential to be used in situations where other approaches might fail, e.g. when the experimental data are very heterogeneous (e.g. not complete/noisy responses; the MIE and KEs are measured using in vitro assays from different biological sources, which have different sensitivity to stressors - cells/tissues from different donors, laying on different sides of the distributions in terms of individual sensitivity, cells with overexpress receptors making the assay much more sensitive), where subjective judgment of experts can be introduced and handle the data in a mathematically correct/reproductive way.

Bette Meek - University of Ottawa

The development of AOPs as outlined in the Developers' Handbook for the OECD AOP program and associated knowledge base (KB) focus early attention on patterns and data relevant to quantitation of key event relationships (KERs) critical to supporting testing strategies and higher tier hazard characterisation and risk assessment applications. One of the formal weight of evidence considerations considers the extent of available data addressing expected patterns of quantitative relationships (dose-response and temporal concordance) across KERs.

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 organisation, following challenge by specified doses of stressors known to initiate the pathway. Formal consideration of the extent of the evidence including empirical support promotes, then, common understanding of developers and stakeholders concerning the elements and types of data or study design which increase confidence for regulatory application of quantitative AOPs/MOA.

For cases where there is a preponderance of high and/or moderate confidence determinations for empirical support, it's likely that data are sufficient 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, which take into account the recognised value of common, consistent, simple "metrics" concerning relative confidence in the extent of the evidence which supports the model. Key considerations to facilitate regulatory acceptance include documentation sufficient to enable reproduction of the input–output relationships and adequate mathematical description, computer implementation and verification, parameter estimation and analysis and model verification.

The quality of the data supporting description and quantitation is also being addressed in a systematic methods initiative in the OECD AOP program to focus data identification and assimilation critical to application. In order to optimise investment to support application, it will be important to focus on relevant "trip points" in the pathway for quantitation (i.e., those past which the pathway is irreversible with continued exposure), rather than all KERs.

Engagement of the regulatory community in development and description of quantitative AOP models is likely to be critical also in their acceptance.

Magda Sachana - OECD

Until now, no qAOPs have been submitted for reviewing at OECD level. The SARA model is currently in the OECD Test Guidelines Programme and one can argue that this is a qAOP. That brought the issue of qAOP definition that appeared not to be consistent among the participants. Clearly defining qAOPs and having examples reviewed by regulators could accelerate their regulatory utility and uptake.

The issue of agnosticity of qAOPs was brought up many times and concluded that qAOPs are not entirely chemical agnostic as they require specific chemical data to build the models. How to understand the quality of data used for modelling was also discussed aa well as the need to build best practices. The existence of OECD standardised reporting templates not only for data but also for models was acknowledge as a good starting point for reporting qAOPs, but the lack of databases with curated models was brought up as a good thing to have to build trust and confidence on models.

APPENDIX F: AVAILABLE GUIDANCE ON IN SILICO MODELS FOR CHEMICAL SAFETY ASSESSMENT

Table A1: List of available guidance for exposure/QSAR/PBK models with a short description and link that could be used to build a guidance document for qAOP characterisation, evaluation and reporting.

Note: All websites were accessed in December 2023

Organisation, year	Type of model	Short description	Link
OECD, 2004	Structure-activity relationship (SAR) and quantitative structure-activity relationship (QSAR) models	Lay down 5 key principles for validating (Q)SAR models for their use in regulatory assessment of chemical safety.	OECD Validation of (Q)SAR Models
EPA, 2006	PBK models	Application and evaluation of PBPK models for risk assessment purposes. These models represent an important class of dosimetry models that are useful for predicting internal dose at target organs for risk assessment applications.	Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment (Final Report). National Center for Environmental Assessment, Washington, DC. EPA/600/R- 05/043F
WHO, 2010	PBK models	Covers the use of tissue dosimetry in risk assessment, the characterisation and documentation of PBPK models, and the application of these models to risk assessment.	WHO/IPCS GUIDANCE ON PBPK MODELLING FOR RISK ASSESSMENT
EFSA, 2014	Environmental models	as a stepwise analysis of issues relevant to both the development and the evaluation of models to assess ecological effects of pesticides. The regulatory model should be selected or developed to address the relevant specific protection goal.	Scientific Opinion on good modelling practice in the context of mechanistic effect models for risk assessment of plant protection products
CEN, 2016	Large exposure models	Key components of the documentation of chemical exposure models are presented, and a way to structure the communication of the information is proposed.	CEN/WS MERLIN-EXPO - Standard documentation of large chemical exposure models (iteh.ai)
EPA, 2020	PBK model	Umbrella quality assurance project plan (QAPP) for dosimetry and mechanism-based models	Umbrella quality assurance project plan (QAPP) for dosimetry and mechanism-based models | Health & Environmental Research Online (HERO) | US EPA
FDA, 2020	PBK model	Provides general recommendations regarding the development, evaluation,	The Use of Physiologically Based Pharmacokinetic

Organisation, year	Type of model	Short description	Link
		and use of PBPK analyses for biopharmaceutics applications employed by sponsors of investigational new drug applications, or drug product development, manufacturing changes, and controls.	Analyses — Biopharmaceutics Applications for Oral Drug Product Development, Manufacturing Changes, and Controls
EMA, 2019	PBK mode	Reporting PBPK models and simulations in support of submission of dossier to register drug at EMA.	Guideline on the reporting of physiologically based pharmacokinetic (PBPK) modelling and simulation
OECD, 2021	PBK model	Provides insights into how the data generated by in vitro and in silico (non-animal) methods can be applied to construct physiologically based kinetic (PBK) models and how these models can be validated.	OECD Series on Testing and Assessment No. 331; Environment, Health and Safety, Environment Directorate

REFERENCES

Note: All websites were accessed in December 2023

Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrrano JA, Tietge JE, Villeneuve DL. 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ Toxicol Chem. 29(3):730-741. eng.

Bal-Price A, Hogberg HT, Crofton KM, Daneshian M, FitzGerald RE, Fritsche E, Heinonen T, Hougaard Bennekou S, Klima S, Piersma AH, Sachana M, Shafer TJ, Terron A, Monnet-Tschudi F, Viviani B, Waldmann T, Westerink RHS, Wilks MF, Witters H, Zurich M-G, Leist M. 2018. Recommendation on test readiness criteria for new approach methods in toxicology: Exemplified for developmental neurotoxicity. ALTEX - Alternatives to animal experimentation. 35(3):306-352.

Brozek JL, Canelo-Aybar C, Akl EA, Bowen JM, Bucher J, Chiu WA, Cronin M, Djulbegovic B, Falavigna M, Guyatt GH, Gordon AA, Hilton Boon M, Hutubessy RCW, Joore MA, Katikireddi V, LaKind J, Langendam M, Manja V, Magnuson K, Mathioudakis AG, Meerpohl J, Mertz D, Mezencev R, Morgan R, Morgano GP, Mustafa R, O'Flaherty M, Patlewicz G, Riva JJ, Posso M, Rooney A, Schlosser PM, Schwartz L, Shemilt I, Tarride JE, Thayer KA, Tsaioun K, Vale L, Wambaugh J, Wignall J, Williams A, Xie F, Zhang Y, Schünemann HJ. 2021. GRADE Guidelines 30: the GRADE approach to assessing the certainty of modeled evidence-An overview in the context of health decision-making. J Clin Epidemiol. 129:138-150. eng.

Carnesecchi E, Langezaal I, Browne P, Batista-Leite S, Campia I, Coecke S, Dagallier B, Deceuninck P, Dorne JLCM, Tarazona JV, Le Goff F, Leinala E, Morath S, Munn S, Richardson J, Paini A, Wittwehr C. 2023. OECD harmonised template 201: Structuring and reporting mechanistic information to foster the integration of new approach methodologies for hazard and risk assessment of chemicals. Regulatory Toxicology and Pharmacology. 142:105426.

Chu VR. 2018. Assessing the Effects of Chemical Mixtures using a Bayesian Network-Relative Risk Model (BN-RRM) Integrating Adverse Outcome Pathways (AOPs) in Four Watersheds. WWU Graduate School Collection 699.

Conolly RB, Ankley GT, Cheng W, Mayo ML, Miller DH, Perkins EJ, Villeneuve DL, Watanabe KH. 2017. Quantitative Adverse Outcome Pathways and Their Application to Predictive Toxicology. Environmental Science & Technology. 51(8):4661-4672.

Cronin MTD, Belfield SJ, Briggs KA, Enoch SJ, Firman JW, Frericks M, Garrard C, Maccallum PH, Madden JC, Pastor M, Sanz F, Soininen I, Sousoni D. 2023. Making in silico predictive models for toxicology FAIR. Regulatory Toxicology and Pharmacology. 140:105385.

Crouzet T, Grignard E, Brion F, Blanc EB, Podechard N, Langouet S, Alonso-Magdalena P, Hubert P, Kim MJ, Audouze K. 2023. ReadEDTest: A tool to assess the readiness of in vitro test methods under development for identifying endocrine disruptors. Environment International. 174:107910.

Dent M, Amaral RT, Da Silva PA, Ansell J, Boisleve F, Hatao M, Hirose A, Kasai Y, Kern P, Kreiling R, Milstein S, Montemayor B, Oliveira J, Richarz A, Taalman R, Vaillancourt E, Verma R, Posada NVORC, Weiss C, Kojima H. 2018. Principles underpinning the use of new methodologies in the risk assessment of cosmetic ingredients. Computational Toxicology. 7:20-26.

EPA. 2006. Approaches For the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data In Risk Assessment (Final Report). US Environmental Protection Agency, Washington, DC. EPA/600/R-05/043F.

EPA. 2020. Umbrella quality assurance project plan (QAPP) for dosimetry and mechanism-based models US Environmental Protection Agency, Washington, DC.

Friedman PK, Gagne M, Loo LH, Karamertzanis P, Netzeva T, Sobanski T, Franzosa JA, Richard AM, Lougee RR, Gissi A, Lee JJ, Angrish M, Dorne JL, Foster S, Raffaele K, Bahadori T, Gwinn MR, Lambert J, Whelan M, Rasenberg M, Barton-Maclaren T, Thomas RS. 2020. Utility of In Vitro Bioactivity as a Lower Bound Estimate of In Vivo Adverse Effect Levels and in Risk-Based Prioritization. Toxicol Sci. 173(1):202-225. eng.

Hassan I, El-Masri H, Kosian PA, Ford J, Degitz SJ, Gilbert ME. 2017. Neurodevelopment and Thyroid Hormone Synthesis Inhibition in the Rat: Quantitative Understanding Within the Adverse Outcome Pathway Framework. Toxicological Sciences. 160(1):57-73.

Kleinstreuer NC, Sullivan K, Allen D, Edwards S, Mendrick DL, Embry M, Matheson J, Rowlands JC, Munn S, Maull E, Casey W. 2016. Adverse outcome pathways: From research to regulation scientific workshop report. Regulatory Toxicology and Pharmacology. 76:39-50.

Knapen D, Angrish MM, Fortin MC, Katsiadaki I, Leonard M, Margiotta-Casaluci L, Munn S, O'Brien JM, Pollesch N, Smith LC, Zhang X, Villeneuve DL. 2018. Adverse outcome pathway networks I: Development and applications. Environ Toxicol Chem. 37(6):1723-1733. eng.

Krewski D, Andersen ME, Tyshenko MG, Krishnan K, Hartung T, Boekelheide K, Wambaugh JF, Jones D, Whelan M, Thomas R, Yauk C, Barton-Maclaren T, Cote I. 2020. Toxicity testing in the 21st century: progress in the past decade and future perspectives. Archives of Toxicology. 94(1):1-58.

Loizou G, Spendiff M, Barton HA, Bessems J, Bois FY, d'Yvoire MB, Buist H, Clewell HJ, Meek B, Gundert-Remy U, Goerlitz G, Schmitt W. 2008. Development of good modelling practice for physiologically based pharmacokinetic models for use in risk assessment: The first steps. Regulatory Toxicology and Pharmacology. 50(3):400-411.

Maertens A, Golden E, Luechtefeld TH, Hoffmann S, Tsaioun K, Hartung T. 2022. Probabilistic risk assessment – the keystone for the future of toxicology. ALTEX - Alternatives to animal experimentation. 39(1):3-29.

OECD. 2017. Guidance Document for the Use of Adverse Outcome Pathways in Developing Integrated Approaches to Testing and Assessment (IATA), OECD Series on Testing and Assessment, No. 260, OECD Publishing, Paris, .

OECD. 2021. Guidance document on the characterisation, validation and reporting of Physiologically Based Kinetic (PBK) models for regulatory purposes. No. 331.

OECD. 2023. Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In-Vitro Testing Battery. Series on Testing and Assessment No. 377. ENV/CBC/MONO(2023)13.

Paini A, Campia I, Cronin MTD, Asturiol D, Ceriani L, Exner TE, Gao W, Gomes C, Kruisselbrink J, Martens M, Meek MEB, Pamies D, Pletz J, Scholz S, Schüttler A, Spînu N, Villeneuve DL, Wittwehr C, Worth A, Luijten M.

2022. Towards a qAOP framework for predictive toxicology - Linking data to decisions. Computational Toxicology. 21:100195.

Paini A, Leonard JA, Joossens E, Bessems JGM, Desalegn A, Dorne JL, Gosling JP, Heringa MB, Klaric M, Kliment T, Kramer NI, Loizou G, Louisse J, Lumen A, Madden JC, Patterson EA, Proença S, Punt A, Setzer RW, Suciu N, Troutman J, Yoon M, Worth A, Tan YM. 2019. Next generation physiologically based kinetic (NG-PBK) models in support of regulatory decision making. Comput Toxicol. 9:61-72. eng.

Pallocca G, Moné MJ, Kamp H, Luijten M, Van de Water B, Leist M. 2022. Next-generation risk assessment of chemicals - Rolling out a human-centric testing strategy to drive 3R implementation: The RISK-HUNT3R project perspective. Altex. 39(3):419–426. eng.

Perkins EJ, Gayen K, Shoemaker JE, Antczak P, Burgoon L, Falciani F, Gutsell S, Hodges G, Kienzler A, Knapen D, McBride M, Willett C, Doyle FJ, Garcia-Reyero N. 2019. Chemical hazard prediction and hypothesis testing using quantitative adverse outcome pathways. Altex. 36(1):91-102. eng.

Spinu N, Bal-Price A, Cronin MTD, Enoch SJ, Madden JC, Worth AP. 2019. Development and analysis of an adverse outcome pathway network for human neurotoxicity. Archives of Toxicology. 93(10):2759-2772.

Spinu N, Cronin MTD, Enoch SJ, Madden JC, Worth AP. 2020. Quantitative adverse outcome pathway (qAOP) models for toxicity prediction. Archives of Toxicology. 94(5):1497-1510.

Stadnicka-Michalak J, Schirmer K, Ashauer R. 2015. Toxicology across scales: Cell population growth in vitro predicts reduced fish growth. Science Advances. 1(7):e1500302.

Tan Y-M, Chan M, Chukwudebe A, Domoradzki J, Fisher J, Hack CE, Hinderliter P, Hirasawa K, Leonard J, Lumen A, Paini A, Qian H, Ruiz P, Wambaugh J, Zhang F, Embry M. 2020a. PBPK model reporting template for chemical risk assessment applications. Regulatory Toxicology and Pharmacology. 115:104691.

Tan Y-M, Chan M, Chukwudebe A, Domoradzki J, Fisher J, Hack CE, Hinderliter P, Hirasawa K, Leonard J, Lumen A, Paini A, Qian H, Ruiz P, Wambaugh J, Zhang F, Embry M. 2020b. PBPK model reporting template for chemical risk assessment applications. Regul Toxicol Pharmacol. 115:104691. eng.

Tollefsen KE, Scholz S, Cronin MT, Edwards SW, de Knecht J, Crofton K, Garcia-Reyero N, Hartung T, Worth A, Patlewicz G. 2014. Applying Adverse Outcome Pathways (AOPs) to support Integrated Approaches to Testing and Assessment (IATA). Regul Toxicol Pharmacol. 70(3):629-640. eng.

Vinken M, Benfenati E, Busquet F, Castell J, Clevert DA, de Kok TM, Dirven H, Fritsche E, Geris L, Gozalbes R, Hartung T, Jennen D, Jover R, Kandarova H, Kramer N, Krul C, Luechtefeld T, Masereeuw R, Roggen E, Schaller S, Vanhaecke T, Yang C, Piersma AH. 2021. Safer chemicals using less animals: kick-off of the European ONTOX project. Toxicology. 458:152846. eng.

Vinken M, Knapen D, Vergauwen L, Hengstler JG, Angrish M, Whelan M. 2017. Adverse outcome pathways: a concise introduction for toxicologists. Arch Toxicol. 91(11):3697-3707. eng.

WHO. 2010. Characterization and application of physiologically based pharmacokinetic models in risk assessment. International Programme on Chemical Safety. Harmonization Project Document No. 9. World Health Organization Available at:

http://www.inchem.org/documents/harmproj/harmproj/harmproj9.pdf

Wilkinson MD, Dumontier M, Aalbersberg IJ, Appleton G, Axton M, Baak A, Blomberg N, Boiten J-W, da Silva Santos LB, Bourne PE, Bouwman J, Brookes AJ, Clark T, Crosas M, Dillo I, Dumon O, Edmunds S, Evelo CT, Finkers R, Gonzalez-Beltran A, Gray AJG, Groth P, Goble C, Grethe JS, Heringa J, 't Hoen PAC, Hooft R, Kuhn T, Kok R, Kok J, Lusher SJ, Martone ME, Mons A, Packer AL, Persson B, Rocca-Serra P, Roos M, van Schaik R, Sansone S-A, Schultes E, Sengstag T, Slater T, Strawn G, Swertz MA, Thompson M, van der Lei J, van Mulligen E, Velterop J, Waagmeester A, Wittenburg P, Wolstencroft K, Zhao J, Mons B. 2016. The FAIR Guiding Principles for scientific data management and stewardship. Scientific Data. 3(1):160018.

Wittwehr C, Aladjov H, Ankley G, Byrne HJ, de Knecht J, Heinzle E, Klambauer G, Landesmann B, Luijten M, MacKay C, Maxwell G, Meek ME, Paini A, Perkins E, Sobanski T, Villeneuve D, Waters KM, Whelan M. 2017. How Adverse Outcome Pathways Can Aid the Development and Use of Computational Prediction Models for Regulatory Toxicology. Toxicol Sci. 155(2):326-336. eng.

Zgheib E, Gao W, Limonciel A, Aladjov H, Yang H, Tebby C, Gayraud G, Jennings P, Sachana M, Beltman JB, Bois FY. 2019. Application of three approaches for quantitative AOP development to renal toxicity. Computational Toxicology. 11:1-13.

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