Presentations and panel discussions - 2020 Workshop Report

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The workshop was divided into different area sessions: New Approach Methodologies & Special Scenarios; Approaches fit for purpose: Validation of methodologies; PBPK modelling; and Future Methodologies (micro-physiological environment) and consisted of presentations accompanied by roundtable discussions of case studies with feedback and a further discussion about future research needs. The presentations were delivered by invited experts and had been designed to provide relevant information to inform the later discussions and case studies.

Presentations

In the session **New Approach Methodologies & Special Scenarios**, Professor Mark Cronin (Liverpool John Moores University) introduced the topic of “New Approach Methodologies (NAMs): Application in Risk Assessment”. The future short-term aims of NAMs in risk assessment include filling in data gaps and provision of relevant information for regulatory submissions. Longer term, NAMs aim to support the mechanistic and exposure profiling of chemicals and will provide the data to support the new paradigm in non-animal safety assessment of chemicals. Highlighting the challenges faced when using NAMs in risk assessment, are their translation from theory to practice; the development of robust, reliable, and reproducible methods; integration into schemes for risk/safety assessment; and their global harmonisation with regard to regulatory acceptance.

Dr Camilla Alexander-White (Royal Society of Chemistry) discussed recent case studies of regulatory use (or not) for risk assessment. This included chemical grouping; human biomonitoring 4 EU programme (HBM4EU) in Europe); an example of how a PBPK model was accepted by EU regulators built on a plethora of *in vivo* (Bernauer et al., 2016) data and an example of a case study using quantitative *in vitro* to *in vivo* extrapolation for environmental esters (Campbell et al., 2015).

Dr Fiona Sewell (NC3Rs) presented on using in silico approaches to support Replacement, Reduction and Refinement (3Rs) in safety assessment by the UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs). NC3Rs is a science-led and evidence-based organisation established in 2004 to accelerate the development and uptake of new models and tools that replace, reduce, or refine the use of animals in research. The presentation described how these new approaches can be incorporated to improve decision-making. Though the ultimate aim is to work towards replacement, ‘alternatives’ are unlikely to offer a direct 1:1 solution and a tiered/combinatory approach may be necessary.

Dr Carl Westmoreland (Unilever) highlighted recent publications in the area of *in vitro* and *in silico* risk assessment, a tiered approach to be used (highlighting the processes and current methodologies available) and the principles of Next Generation Risk Assessment (NGRA) according to the International Cooperation on Cosmetics Regulation. A case study method was presented, which was used to test the NGRA tiered approach assuming that there were no traditional toxicology

data for a commonly used ingredient (coumarin).

In the session for **Approach that is fit for purpose: Validation of methodologies** Professor Gary Hutchison (Edinburgh Napier University) presented on alternative testing and exposure strategies for nanomaterials (NM) outlining the various Horizon 2020 projects such as Grouping, Read-across, Characterisation and classification framework for regulatory risk assessment of manufactured nanomaterials and Safer design of nano (GRACIOUS). It also considered the investigation and verification of current testing methods for engineered NMs and their applicability for use with nanobiomaterials (NBMs) and how these could be used in proposed Integrated Approaches to Testing and Assessment (IATA) for Developmental and Reproductive Toxicology (DaRT). Finally, the talk outlined key challenges for the future, highlighting the move to complex 3D cell models and microfluidic systems and how we ascertain dose may be challenging; agreement on definitions and measurement of dose (mass, surface area); stability of (Bio) nanomaterials in solution; corona assessment; assessment of complex 3rd generation bio nanomaterial, within possible matrices, will challenge traditional approaches and understanding the implications of endotoxin contamination in production lines are all key areas that need to be worked through to support the safe development of the technology.

Dr Judith Madden (Liverpool John Moores University) presented on establishing the Credibility of Model including using credibility criteria. These alternative methods include leveraging existing data, in silico modelling and the use of (human relevant) *in vitro* models. The Joint Research Centre (JRC) EU Reference Laboratory for alternatives to animal testing (EURL ECVAM) report (2017) established four criteria for achieving model credibility: (i) understanding the model; (ii) understanding the data underpinning the model; (iii) clearly stating assumptions and hypothesis encoded and; (iv) considering the gap between the model and reality. Credibility of PBK models can be visualised using a matrix that characterises the degree of confidence in the components of the model: i.e., its biological plausibility, how well it simulates known data and its overall reliability considering uncertainty and sensitivity. *In vitro* assays can be used to generate new data, these should be conducted in accordance with Organisation for Economic Co-operation and Development (OECD) Technical Guidance documents or the OECD Guidance Document on Good *In Vitro* Method Practices (GIVIMP, 2018). Model reporting needs to adequately justify and document both the model structure and the parameters used, to ensure reproducibility and confidence in the model. It was concluded that to advance PBK modelling, in the context of supporting chemical safety assessment, it is essential that there was an ongoing

dialogue between model developers and (regulatory) users.

In the session **PBPK modelling**, Dr George Loizou (Health and Safety Executive) presented a software tool called RVis which is a prototype application for the analysis of structure and performance of physiologically PBPK and other models. The input parameters comprise anatomical, physiological, metabolic and physicochemical values and the calculated outputs are the rates of uptake, elimination and organ and tissue concentrations (i.e., the internal dose). The advantages of utilising RVis as a tool for probabilistic PBPK is that it accounts for human inter-individual variability, has the ability to determine a credible interval for BMD lower bound values, and also offers a fully quantified measure of uncertainty for quantitative *in vitro* to *in vivo* extrapolation.

Dr Sheila Annie Peters (Merck) discussed establishing confidence in PBPK models without human toxicokinetic data. This was done by introducing the barriers to establishing mechanistic credibility of PBPK models in bottom-up and top-down approaches. A workflow to verify and validate the predictive performance of a PBPK model was presented, in addition to the utility and role of sensitivity analysis. Food and Drug Administration (FDA) recent white paper published a framework that can be used by industry and regulatory agencies to assess the credibility of computational models. There are five key concepts that can be used to establish model credibility (namely, the question of interest, defining the context of use, assessing model risk, establishing risk-informed credibility, and assessing model credibility). However, it was noted that there is a lack of consensus on best practices for determining if a model is fit-for purpose (with reference to validation, performance/sensitivity metrics, and platform independence). It was concluded that knowledge gaps and uncertainties in predicted human pharmacokinetics cannot be overcome by any level of sophistication in pharmacokinetic modelling. Furthermore, the number of model assumptions tends to be proportionate to model complexity to a point that a complex model could become too distant to whatever is being modelled. Transparent communication of underlying assumptions and knowledge gaps is needed. Although PBPK offers valuable opportunities for data integration, mechanistic basis and route extrapolation, value addition of PBPK needs to be objectively evaluated, demonstrated, and understood before it is adopted.

In the session **Future Methodologies: Micro-physiological environment**, Professor Ian Wilson (Imperial College London) addressed the potential use of organ-on-a-chip technology, *in silico* modelling and the gut microbiome in exploring dose response. A particular challenge for the future highlighted is the

role of the gut microbiota. The micro-organisms resident in the gut represent a major and highly variable component of metabolism and prospects for the use of *in vitro* systems to aid in its modelling were detailed. However, variability in the composition of the gut microflora complicates modelling as it results in, sometimes significant, interindividual differences in the metabolism, pharmacology and toxicity of dietary components and xenobiotics. gut microflora can have an array of effects on the following: drugs and their metabolites, bioavailability of dietary constituents, expression of host drug metabolising enzymes, and toxicity. Ultimately, future *in vitro* and *in silico* models will have to take into account gut wall metabolism for oral exposures. In addition, such models should benefit from the increased *in vitro* assessment of gut microbial activity and the highly targeted use of both gut microflora and organ-based humanized *in vivo* models.

Dr Tim Allen (University of Cambridge) discussed AI, machine learning and big data in risk assessment. The talk first gave an overview on the AOP, after which structural alerts was discussed. Dr Allen presented on one his projects using 2D structural alerts to define chemical categories for molecular initiating events (Allen et al., 2018) It was discussed how molecular-initiating events (MIEs) are important concepts for *in silico* predictions. They can be used to link chemical characteristics to biological activity through an AOP. Furthermore, the project explained how the tool provides the first step in an AOP-based risk assessment, linking chemical structure to toxicity endpoint. Neural networks and quantitative predictions were also introduced. In biologically inspired neural networks, mathematical relationships link artificial neurons in layers leading to a prediction in the output layer. There was also description of another project by Wedlake et al using 90 biological targets representing important human MIEs, structural alert-based models which have been constructed with an automated procedure that uses Bayesian statistics to iteratively select substructures. These networks can be used as both binary predictors and quantitative predictors, which are more suitable for a risk assessment procedure.