Session III

In this guide

Adopters of change Innovation vs Adoption vs Compatibility

Dr Fiona Sewell (NC3Rs) presented on “International regulatory acceptance of non-animal methodology in safety assessment”.

66. The National Centre for 3Rs has a large toxicology and regulatory sciences programme. The NC3Rs have a vision to apply the 3Rs in the current regulatory framework and provide funding including through CRACK IT Challenges .

67. Lessons learnt included the need to exploit the latest science, existing data, collaboration, safe-harbour approaches, and regulator buy-in.

68. Global harmonisation will be key, especially with different regulatory requirements that can cause 3Rs issues regarding testing needs. Inherently, there will be variation in interpretation of the guidance. OECD test guidelines do exist to help align this and more importantly some regulators are open to alternatives. However, requirements and needs can be confusing, often a risk averse approach.

69. There was discussion on work that needs to be done including exploring how animals do not always provide the best models.

70. There needs to be a bigger focus on drivers and incentives with a need for international buy-in. This will be a stepwise process to build confidence in NAMs and provide evidence and be clear about what questions need to be answered.

71. Science and technological innovation are crowded and moving fast. Which NAMs should be invested in? Which NAMs need to be included in decision making? Time pressures and funding are challenges with implementing methods.

72. Now is the time for a culture change to allow regulators to move forward from a reliance on animal testing.

Dr Ans Punt (Wageningen University) presented on “Moving the paradigm shift in toxicology towards an animal-free chemical risk assessment”.

73. Dr Punt introduced NAMs at Wageningen Food Safety Research (WFSR) and how Quantitative *in vitro* to *in vivo* Extrapolation (QIVIVE) and *in vitro* PBPK are used for predicting safety of chemicals in in feed and food.

74. Regulatory acceptance of these models may be easier than other areas such as PBPK models. It is better to have some information rather than none at all.

75. The challenges of regulatory acceptance can be seen for example when EFSA evaluated coumarin and established the TDI. Was this risk assessment too strict or is coumarin in cinnamon a real concern?

76. For example, the bioactivation route and detoxification routes were assessed *in vitro* including the measurement of the conversion of coumarin to its metabolites. However, rats do not have a detoxification route, but humans do. Humans are less sensitive to this than rats. EFSA was enthusiastic about the model but had not accepted it because it was not validated as no human data were available. If we do not have data available to validate these models, it will be difficult to get a model accepted. In order to get a model accepted there would need to be a refinement of the risk assessment. The key point is how are models going to get to a point whereby they are accepted for more involved and important decisions.

77. The 2017 Lorentz Centre Workshop was an important output (Punt et al., 2020). It discussed the OECD guidance documents and established OECD guidelines. However, there is no risk assessment framework that requires the submission of quantitative *in vitro* kinetic data.

78. It was suggested that there should be a survey of experts in food safety to discuss the important drivers/barriers of the use 3Rs in safety evaluations and their predictability.

79. One of the ways to gain confidence in PK predictions without 1:1 *in vivo* data is to determine the key aspects is to determine what is good quality of data. If we study the quality of *in vitro* data, the values for a range of chemicals including clearance measurement and complex kinetic processes will therefore reduce a large variation in output.

80. Evaluating PBPK model predictions can show large variations in the Cmax values because of different input approaches. Therefore, it is important to know about the characteristics of the chemicals that are over-predicted.

81. Finally, PBPK modelling will play a crucial role in the validation and acceptance of NAMs.

Dr Patience Browne (OECD) presented on “Regulatory use and acceptance of alternative methods for chemical risk assessment”.

82. Dr Browne introduced the OECD Chemical Programme and various initiatives develop to increase the uptake of New Approach Methods (NAMs) and reduce *in vivo* testing.

83. The OECD Test Guidelines Programme publishes internationally harmonised method for evaluating chemical hazards, the results of which, when

conducted following the principles of Good Laboratory Practice (GLP) are covered by the agreement on Mutual Acceptance of Data (MAD). MAD is a legally binding agreement between OECD countries which requires all countries to accept the results from the OECD Test Guidelines if they have the requirement for such data. Thus, MAD vastly reduces duplicative chemical safety testing and saves tens of thousands of animals and hundreds of millions of euros each year. There are more than 35 internationally harmonised NAMs include in OECD Test Guidelines.

84. The OECD was an early adopter of the Adverse Outcome Pathway (AOP) framework and has heavily invested in the AOP Knowledge Base and various Guidance Documents on the development and review of AOPs. When initially developed, AOPs were intended to be a tool to develop NAMs including Integrated Approaches to Testing and Assessment (IATAs). OECD Guidance Document number 260 provides information on the use of AOPs for developing IATAs. The publication of this document was coincident with the OECD Hazard Assessment Programme's IATA Case Studies Project that uses innovative approaches to address various endpoints for a specific regulatory context. Case Studies are submitted, peer-reviewed, and revised on an annual cycle and final versions are publicly available through OECD websites. In addition to the case studies, a "Considerations" document is updated annual and evaluates the lessons learned for all case studies in the annual cycle and submitted. The IATA project and it is now in its 7^{th} review cycle. Eight case studies in the review process are focus on developmental neurotoxicity, inhalation toxicity, and the use of transcriptomics to evaluate endocrine disruptors.

85. The IATA is intended to be flexible and use expert judgement. As decisions become less flexible, IATAs move towards "Defined Approaches", which are rules-based, quite structured and do not use expert judgment. Different individuals using a Defined Approach should come to the same conclusion. Defined Approaches may be suitable for including in OECD Test Guidelines, for example, Test Guideline 496 describing Defined Approaches for Skin Sensitisation, which was published in 2021.

86. The OECD Chemical Safety Programme is continuing to evaluate how NAMs and IATAs can be used for regulatory decisions on the safety of chemicals. The results of regulatory assessment methods must be reproducible; the test system must be relevant to the target species (e.g., humans), and performance of the NAM must be as good or better than the current, usually in vivo, method. Consolidation of toxicological data in large databases have allowed for meta-analyses and led to increasing recognition on the limitation of the reproducibility

of the animal test methods and the relevance of animal models to human health outcomes.

87. The OECD is working to prove non-animal methods are just as useful, by using a global datasphere that incorporates 90% of the data in the world that was generated in the last 2 years.

88. The OECD is also developing tools to facilitate sharing of toxicological data. OECD Harmonised Templates are designed to capture chemical hazard data and are compatible with housing information in structured databases. This database is the centre of an electronic ecosystem, and compatible with a variety of internal tools such as the AOP Knowledgebase and OECD QSAR Toolbox, and interoperable with third-party electronic tools. As we move forward, the intention is to ensure the OECD electronic ecosystem can facilitate data sharing, help to develop new NAMs, and reduce the need for generation of new animal data on chemical hazards.

Dr Harvey Clewell presented on "Development of methods for in vitro to in vivo extrapolation of cell-based toxicity assays to inform risk assessment".

89. *In Vitro* to *In Vivo* Extrapolation (IVIVE): *In vitro* toxicity assay results expressed in terms of media concentrations converted to the equivalent *in vivo* doses using *in vitro* metabolism data to estimate *in vivo* clearance of the tested chemical (Yoon et al., 2012). In a typical tiered risk assessment approach, three different IVIVE approaches are required, consistent with the experimental data being extrapolated (Andersen et al., 2019):

- Tier 0 – exposure estimation, QSAR, read across.
- Tier 1 - high throughput assays (HT-IVIVE).
- Tier 2 - fit for purpose assays (Q-IVIVE).
- Tier 3 – targeted animal testing (PBPK-IVIVE).

90. High-throughput IVIVE uses *in vitro* data on the rate of metabolism and blood binding of the compound at low-concentrations to estimate the equivalent dose based solely on hepatic and renal clearance. Quantitative IVIVE attempts to incorporate more complete information on the dose-dependence of metabolism and binding, both in the *in vitro* toxicity assay and *in vivo*. Finally, PBPK-IVIVE models can be used to incorporate differences in physiology and metabolism in order to extrapolate accurately from animal to human.

91. In the early days of *in vitro* toxicity testing, only the *in vitro* bioactivity (effective concentration) was considered when trying to predict relative potency *in vivo*. Unfortunately, the most potent compounds in the *in vitro* studies were not the most potent compounds *in vivo*. This discrepancy was largely due to a failure to consider differences in the *in vivo* clearance; for example, if a compound that had a higher intrinsic potency (in terms of cellular concentration) was eliminated from the body more rapidly, it might not have affects at a lower dose (mg/kg/d) as expected. A joint study by The Hamner Institutes for Health Sciences and the US EPA ToxCast program first demonstrated the necessity of including IVIVE in chemical prioritization (Rotroff et al., 2014). This study demonstrated that the equivalent *in vivo* doses for two compounds producing effects at the same *in vitro* concentrations could differ by several orders of magnitude due to differences in their in vivo clearance. The US EPA has since always used the IVIVE approach for high-throughput screening in the 2nd tier, using a conservative approach. For example, there are two common assumptions made concerning hepatic clearance, restrictive or non-restrictive, that make a big difference for some chemicals (Yoon et al., 2012); the non-restrictive clearance assumption typically works better for environmental chemicals, but the US EPA assumes restrictive clearance because it results in a lower (more health conservative) Human Equivalent Dose (HED).

92. In PBPK models, IVIVE can be used calculate the *in vivo* model metabolism parameters based on the protein content of the metabolizing cells and number of cells. One example of a PBPK platform that uses IVIVE is Simcyp. This platform is well accepted by the pharmaceutical industry and regulatory agencies. Simcyp was developed for the pharmaceutical industry and is proprietary software, but the open-source platform, PK-Sim, provides similar capabilities and is also suitable for some environmental or personal care compounds. However, it is important to recognize that environmental chemicals typically do not possess the characteristics of a “druggable” compound, which include poorly metabolised, non-volatile, and not highly lipophilic. They also often are associated with more complex metabolism, particularly by cytochrome P450s (CYPs). Clearly, a lot of work needs to be done in developing PBPK modelling platforms for broader application to environmental chemicals (Moreau et al., 2022). *In silico* predictions of intrinsic clearance for environmental compounds are not yet reliable, and more development is needed for *in vitro* assays of metabolism for slowly cleared chemicals, volatiles, and lipophilic compounds.

93. Ultimately it will be possible to use IVIVE at all stages of the regulatory decision-making process, but active participation by regulators to help develop

the necessary methods and modelling platforms will be required. The EU Joint Research Centre and the US EPA Center for Computational Toxicology and Exposure have been leading this effort to date.

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94. A participant raised the point that the OECD is made up of 36 countries but what about other countries? What is their viewpoint? and what does this mean?

95. Rather than measure the variability in kinetics it might be better to simulate it. Simcyp does a good job of this. If using *in vitro* approaches which are not primary cells, then expression in the cells should be considered. A continuum, more of a tiered approach was suggested. For a lower tier decision, it may be possible to use a simple PK model. It is possible to increase the complexity to match the uncertainty which would be allowed within the constraints of the model. It should be remembered that there are other contributors besides genetics and the type of cells e.g., lifestyles/diets.

96. A participant raised whether the UK's appetite for risk is poor. Should risk managers be asking about a level that is safe, or should they be asking where it becomes dangerous or what level of protection should there be?

97. Even though the public have a diverse view, the usual consensus moves away from animal testing usage, if it can be helped, without compromising high level safety. Making the public aware of the uncertainty in animal data could help towards the acceptance of uncertainty in NAMs. The discussions that have taken place over COVID vaccines and the science/public media reactions to this should be considered. Linking back to the issue of: How much confidence is there that what there is, is fit for purpose and how can confidence be improved in approaches that are going to replace animals. There will be a reluctance to increasing TDIs.

98. There has already been marshalling of resources and generally there is a reluctance to say that chemicals are safer than originally thought. Examples included trying to use chemical-specific adjustment factors (CSAFs). With human data, there is a lot of resistance to removing the factor of 100. However, if human specific in reduction safety factors would be appropriate. Would there be resistance to it not being a factor of 100?

99. Problem formulation is about asking the right questions. Is it safe? When does it become problematic? And what impact have case studies had on acceptability of these approaches.

100. The need for a roadmap for the UK is timely and fundamental to start getting NAMs accepted by regulators. It needs a clear vision and steps, stakeholders, funding, timeline, and an assessment of what needs to change in law to meet the data requirements. What does validation mean or what are meaningful results? NAMS need to be accepted by regulators by getting to break down any barriers, through funding and the inception to translation.