Session IV

In this guide

From Basic to Applied- Science &Technology Distil, Review, Validate

Professor Mark Viant (University of Birmingham) presented on “The use of case studies, best practice and reporting standards for metabolomics in regulatory toxicology”.

113. Developing and applying metabolomics in toxicology (and more recently in regulatory toxicology), NAMs need to demonstrate their relevance and reliability (including laboratory reproducibility), with transparent reporting. At

EUROTOX 2021, there was emphasis on strengthening read-across using omics derived evidence from an invertebrate model.

114. The **relevance** of metabolomics in the context of toxicology is fundamental when looking at metabolites that are key event biomarkers of adverse effects, e.g., changes in glutathione concentration due to oxidative stress and ornithine/cystine ratio for predicting *in vitro* developmental toxicology. The importance of targeted measurements of toxicologically relevant metabolites was highlighted, along with the detection and reporting of unknown 'features' within mass spectrometry data sets to grow new knowledge of metabolic key event biomarkers.

115. There is a need to define which metabolites should be routinely targeted when using metabolomics in a toxicology context. For example, there are advantages in using a smaller library 'panel' where both the toxicological relevance of the metabolites is known as well as their analytical characteristics (e.g., chromatography retention times, MS-MS conditions, linearity of response), rather than a solely untargeted approach. An example of such a strategy was provided from the transcriptomic area: S1500+, in which a library panel of ca. 2000 genes has been determined to show high toxicological relevance.

116. Inspired by the S1500+ study, and by the urgent need to defragment information sources describing metabolic key event biomarkers, a recent collaboration between Professor Viant's group and ECHA was presented in which a 'panel' of 722 metabolites ('MToX700+') has been developed (Sostare et al., 2022. These metabolic biomarkers are all known, through previous studies, to be associated with disease outcomes, toxicity and/or adverse effects.

117. The important issue of **reporting** metabolomics results consistently was raised. A paper from Nature Communications was introduced that described the outputs from MERIT (Metabolomics Standards Initiative in Toxicology), a 2-year project with industry, government agencies and academia (Viant *et al*., 2022). Additionally, recent progress by the OECD was described, towards developing both metabolomics and transcriptomics reporting frameworks. This was a good example of where two communities worked together to provide a single framework – the OECD Omics Reporting Framework (Harrill et al., 2021). Consistent reporting is key and has proven a significant roadblock to being able to move omics forwards within the NAM space.

118. An on-going project that is addressing the **reproducibility** question in metabolomics is the MetAbolomics Trial for CHemical Grouping (MATCHING)

project. This is an international ring trial involving 7 institutes (from academia, government, and the private sector), where each partner is measuring, analysing, and drawing conclusions from a single batch of biological samples from a chemical grouping study. The aim of the project is to determine the reproducibility of metabolomics, from a toxicology perspective by assessing the extent to which partners derive the same chemical groups using only metabolomics data.

119. Finally, the “inadvertent” advantage of using full scan mass spectrometry techniques for metabolomics, was discussed, specifically that in addition to the intended measurement of endogenous metabolites, this approach can also detect xenobiotic compounds (e.g., a drug or chemical contaminant exposure) within the same analysis. This includes biotransformation products of the parent xenobiotic compounds. While research to more fully evaluate this is on-going, early findings suggest it is possible to extract information for ADME and TK purposes as well as obtaining the ‘classical’ endogenous metabolomics information from the same sample using the same assay.

120. In conclusion, Professor Viant’s overall thoughts were that it is no longer an if, but when, these NAMs approaches are taken up more routinely for toxicology regulatory purposes.

121. At the end of the presentation Arthur de Carvalho e Silva was introduced as a new 4-year postdoctoral research fellow in computational toxicology working jointly with the University of Birmingham, HSE and FSA in the NAMs area.

Professor John Colbourne (University of Birmingham) presented on “ Precision Tox Project”.

122. The Precision Tox project is a new approach, that is UK led, and involves investigators from 15 organisations across 8 countries, and it is supported by the EU’s Horizon 2020 programme. The objective is to show that it can deliver biomolecular key events and their biomarkers, which are necessary for AOPs. The purpose is to attempt to repair the division between human and environmental risk assessment, showing that a unified approach is possible.

123. Current toxicological models are based on analogy, whereas the homology approach is based on similarities shared by evolutionary history in genomes. The project is testing the idea of toxicity by descent, and conserved toxicity pathways, using a suite of species. These include the arthropods and

nematodes representing the invertebrates and Xenopus embryos, zebrafish embryos and human cells representing the vertebrates. Seventy-one percent of gene-disease families evolved from ancestors of both invertebrates and vertebrates.

124. The proof of principle was tested using a PPAR agonist. Mice have many of the human genes except one, and all of the nuclear receptors. *Xenopus* have one nuclear receptor missing. Zebrafish have all the nuclear receptors but miss a few proteins and genes. The PPAR agonist causes liver fibrosis in all these species. *Daphnia* have only one of the nuclear receptors, a few genes, and no liver. However, they showed a similar gene response to mice, so despite having no liver the same pathway was activated.

125. Another aspect is genetic sources of variation in humans resulting in differences in susceptibility. The project is mapping genetic variation in the human population in genes which affect susceptibility levels.

126. The project is conducting case studies with the EU's Joint Research Centre (JRC) and EU/UK regulatory agencies. The approach can help industry to innovate responsibly. Stakeholders have input into the roadmap. The project will hopefully lead to fewer toxic chemicals in the environment.

Dr Stuart Creton (Food Standards Australia New Zealand) presented on "NAMs in food chemical risk assessment: small agency perspectives".

127. Food Standards Australia New Zealand (FSANZ) develops standards that regulate the use of ingredients, processing aids, colourings, additives, vitamins, and minerals. They also cover food composition, novel foods and new food technologies (e.g., GM) and labelling requirements.

128. In toxicology, FSANZ follows Codex guidelines for risk assessment purposes in line with EHC240. They look to harmonise proposed HBGVs with a JECFA opinion where appropriate. They use local consumption dietary survey data (national nutrition surveys) for their own country specific risk assessments. Dr Creton's team also provide risk assessments for imported food.

129. The use of NAMs in toxicology and the FSANZ risk assessment processes are still very limited. If used, then examples have been in low-risk issues or use as a screening/prioritisation tool.

130. Examples of the use of NAMs by FSANZ have included the use of the Threshold of Toxicological Concern (TTC) (in the absence of chemical-specific

toxicity data) in food contact material assessments and using PBPK modelling for PFAS for predicting human equivalent doses from animal data.

131. Another example was looking at the food additive potassium polyaspartate in wine. NAMs were used to decrease the number of animal tests required for carcinogenicity and developmental and reproductive toxicology (DART) assessments.

132. Dr Creton described his team and available resource as small with a high volume of assessments that need to be completed within statutory time frames. FSANZ has limited capacity to work on NAMs research themselves. However, they were keen to track and keep pace in this area.

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133. Participants discussed hypothetically if they had a new chemical, and wanted to know if it will be safe to use as a food ingredient, what would be done first?

134. It was suggested that use should be made of omics approaches (more for molecular changes), and phenotypic profiling approaches (i.e., '*in vitro* pathology'). Scientists that work with partners in TOX21 ask 'what complement of different cell types represents the broadest diversity of biological space'?

135. There are also a series of reference chemicals being assayed that are selective against different (known) biological targets (e.g., receptors, enzymes), and building a database of profiles that are indicative of specific MIEs (molecular initiating events). Therefore, when a screen is conducted with unknown compounds, it is possible to see whether the compound is 1) a selective/specific toxicant, or 2) a non-selective/broad toxicant.

136. A participant said it was more of a challenge for metabolomics (less so for transcriptomics). When mass spectrometry is applied to a biological sample, there are lots of unknown peaks, but presumably they represent endogenous metabolites.

137. At Badische Anilin- und SodaFabrik (BASF), unknown features are included (<10% of total features) because they are still predictive. But how does the regulator view this information, particularly if peaks change in a dose-response manner?

138. Therefore, levels of confidence in metabolite identification need to be considered. What level of confidence should be used? Is it possible that the unknown peaks could be used in regulatory decision making? But then does the mass spectrometry approach become more targeted?

139. The Regulator’s perception tends to be that lots of uncertainty is involved in the application of omics data. The OECD’s reporting framework helps to make sure it is clear what has been done. There needs to be more guidance documentation, e.g., making sure that data are reproducible. Regulators are looking for more case studies for additional confidence for when using omics data.

140. From a scientific perspective, the technical challenges of omics technologies are being addressed. However, there is also the need to characterise uncertainties in the current model. There also needs to be evaluation of the approaches within the community. Are we using these approaches for health protection, or prediction? Someone needs to start applying this in a proper decision-making context.

141. There will be major computational challenges from a regulator’s perspective.