Toxicity testing in the 21st Century: Taking a Pathway-led Approach

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In 2007, the US National Research Council's publication 'Toxicity Testing in the Twenty-First Century (TT21C): A Vision and a Strategy' (Krewski *et al.* 2010) outlined an approach to safety assessment that 'could transform toxicity testing from a system based on whole-animal testing to one founded primarily on *in vitro* methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin'. At the core of this vision is an understanding of key biological pathways (termed 'toxicity pathways' in the original report) which, if sufficiently perturbed by a chemical, would result in an adverse outcome for the exposed human/environment. More recently, this concept of pathways-based approaches to risk assessment has been built upon by the description of 'Adverse Outcome Pathways' (AOPs). Each AOP begins with a Molecular Initiating Event (MIE) in which the chemical interacts with a biological target leading to a sequence of events across different levels of biological organisation (Ankley *et al.*, 2010). The OECD has begun to formalize the use of this framework based on AOPs to capture and peer review the mechanistic understanding of specific toxic effects for the evaluation of non-animal methods (OECD, 2011).

Our goal has been to take the above strategy and vision from the conceptual, to the practical; developing the necessary steps to use the TT21C/AOP foresight as the basis for the safety assessment of new ingredients in consumer products, without animal testing. Recently, members of a work-team in the Transatlantic Think Tank of Toxicology (t^4) produced a workflow on how to implement TT21C for a given ingredient (Blaauboer *et al.* ATLA in preparation). The workflow begins with the chemical of concern and some initial assessment of the route of, and the extent of exposure. However, the toxicity pathways of relevance must be identified for that particular agent. This will require a combination of both *in vitro* high-throughput screening (HTS) for pathway inference, and chemical profiling through chemico-informatics. HTS methods involve screening to identify particular stress/toxicity pathways activated in potentially adverse responses. Additionally, specific receptor screening methods may be needed, as well as faster-throughput transcriptomics, bioinformatics and connectivity mapping of biologically relevant networks associated with the chemical agent or its analogues. The concurrent chemical profiling can be at the level of 'expert knowledge' on the potential chemical impacts and putative molecular initiating events (MIE) associated with the parent chemical and expected metabolic products, plus quantitative structure-activity relationship (QSAR) alerts. The resulting output of these steps is a decision on the stress/toxicity pathways that are most likely to be affected by the chemical and/or any specific agonist- or antagonist-ligand events at G protein, tyrosine kinase, ion-channel or nuclear receptors. These outputs will therefore guide the selection, development and use of appropriate *in vitro* assays needed to adequately characterise the *in vitro* concentration response.

It is important to note that *in vitro* biokinetics will be required to understand, measure or model the free concentration of the test compound in the *in vitro* system(s), so that meaningful dose response curves can be constructed. It is anticipated, however, that these assays will not be sufficient in their own right to define the required *in vitro* adversity

point of departure (POD) concentration; they will be used in conjunction with computational systems biology models that will more completely overview the network/pathways of concern. An iterative synergy between the two will be created where the shortcomings of the *in vitro* systems/assays will be compensated for by the *in silico* network and, in turn, the assay outputs will feed and improve the model network. A decision will then be made on the most sensitive and appropriate POD concentration that can factor into a biokinetic model that has been previously populated with appropriate physicochemical parameters. A quantitative *in vitro* to *in vivo* extrapolation (QIVIVE) can then be performed to make a decision on the *in vivo* human safety estimation.
See: www.TT21C.org

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