Day 2

In this guide

An overview was given to remind participants what had been covered on Day 1.

Professor Rusty Thomas (EPA) presented on “The Changing Toxicology Landscape: Challenges and Innovations to Adapt”.

101. The nature of the discussion has changed over time since the release of Toxicity testing in the 21st century: a vision and a strategy (National Research Council, 2007).

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103. There have been different roadmaps, and these are a good tool to force prospective thinking as an organisation and can help implement NAMs in the regulatory space. At the EPA, they have developed several roadmaps that cover the development and implementation of NAMs in multiple contexts. These include the development and application of NAMs at the Agency level (EPA NAMs Work Plan), application under specific regulatory statutes such as TSCA (TSCA Alternatives Strategic Plan), and strategic research on NAMs (CompTox Blueprint).

104. The landscape of toxicology is changing in multiple ways in order to apply NAMs in regulatory decision making. These changes include:

- Systematically addressing the limitations of current NAMs.
- Accepting that there is likely not a primary mechanism/mode of action for most environmental/industrial chemicals.
- Working through how to assemble NAMs in a coherent, practical, fit for purpose testing framework.
- Understanding how to benchmark new approaches.
- Evaluating protection vs. prediction in our current and future approaches.
- Developing a flexible and fit for purpose validation/confidence framework to evaluating new approaches.
- Quantifying public health and economic trade-offs of testing more chemicals/faster.

105. In the first area of change, there needs to be an acknowledgement that while there are technical challenges associated with NAMs (e.g., black-box predictions and limited chemical domain applicability), there needs to be a concerted and systematic research initiatives to overcome them. For example, many in vitro systems have limited coverage of important cellular and intracellular processes compared to a whole animal system. To address this limitation, high content profiling systems such as transcriptomics and imaging systems are being developed and applied across multiple cell types to comprehensively evaluate chemical interactions over broad biological space. In another example, there have been a number of improvements in in vitro exposure systems allow high-throughput testing of volatile chemicals and aerosols in concentration response.

106. In the second area of change, the community needs to acknowledge that most chemicals interact with biological systems in a non-selective manner where multiple biological pathways and processes are impacted in a narrow dose/concentration range. The non-selective nature of chemicals on biological systems impacts how we think about mode of action and test chemicals using NAMs.

107. The third area of change follows on the last one. We need to develop a fit-for-purpose toxicological testing framework that is consistent with the lack of biological selectivity of chemicals. For most chemicals we may not be able to identify specific mechanisms of action due to the lack of selectivity and we would need to use general biological activity to derive a point-of-departure. For those few that are selective, we can identify their primary molecular and cellular targets and link them to key events associated with their toxicity using the AOP framework to predict potential organ and tissue effects more accurately.

108. In the fourth area, we need to better understand how to benchmark NAMs. Comparing with existing models may not always be appropriate if the current models don't predict human toxicity; however, historically we have compared results with NAMs with our traditional animal studies. But, in order to do that, we need to characterize the variability of our existing models. Using databases of curated legacy toxicity studies, we have estimated that LOAEL values from repeat dose studies can vary +/- 10-fold. Similarly, we have curated time course in vivo toxicokinetic studies to help set expectations for *in silico* and *in vitro*-to-*in vivo* extrapolation methods.

109. In the fifth area of change, the community needs to further discuss the issue of protection versus prediction for NAMs as well as our existing practices. In evaluating the concordance of rodent and human toxicological in pre-clinical and clinical pharmaceutical studies, rodents show limited ability to accurately predict the specific toxicological response in humans, but they are able to more robustly predict the absence of toxicological response. Current risk assessment practices are generally consistent with these observations given that we typically identify the most sensitive response that is considered adverse and use that to calculate a point-of-departure and derive a protective toxicity value. For NAMs, a similar approach could be taken, and this was highlighted in a case study performed by Katie Paul-Friedman where she compared the results from bioactivity in the ToxCast battery with *in vivo* points-of-departure from repeat dose animal studies. For ~90% of the chemicals, *in vitro* bioactivity was generally protective of *in vivo* toxicological responses. On average, it was 100-fold

protective. In addition to the ToxCast battery of assays, similar results have been demonstrated using biological activity in high-content imaging assays.

110. In the sixth area of change, we need to develop fit-for-purpose validation and scientific confidence frameworks that allow the community to develop the appropriate confidence in NAMs for regulatory application in a more timely and sustainable way. The EPA are planning to deliver an EPA scientific confidence framework to evaluate the quality, reliability, and relevance of NAMs for our decision making in 2024.

111. In the seventh area of change, the community needs to evaluate the public health trade-offs of uncertainty, timeliness, and costs associated with different toxicity testing methods. If there are two toxicity testing methodologies available and one is more uncertain but gets the answer 5 times faster, what is the trade-off? In our analysis, the timeliness of toxicity testing results has at least as big, and in many cases bigger, impact on public health than uncertainty. In other words, knowing the results of a toxicity test sooner is more important than the degree of certainty in those test results. This has important implications for NAMs as many of them are quicker but are perceived to be more uncertainty compared to the slower traditional animal tests.

112. Finally, one of the biggest challenges facing the toxicological landscape is organisational inertia. In many regulatory agencies, if the wrong decision is made using a new method there is a significant downside, but if a new method is used, it will not always widely publicised. For environmental and industrial chemicals, the right answer is not always known immediately, if at all.