

## Title: Perspectives on pathway perturbation: focused research to enhance 3R objectives

In vitro high-throughput screening (HTS) and in silico technologies are emerging as 21<sup>st</sup> century tools for hazard identification. Computational methods that strategically examine cross-species conservation of protein sequence/structural information for chemical molecular targets (or molecular initiating events [MIEs]) provide a non-destructive means to identify the range of species likely to be adversely effected by chemical perturbation. Libraries of MIEs could be readily identified from U.S. Environmental Protection Agency's ToxCast HTS data and, when linked to adverse outcome pathways (AOPs), can form a basis for hazard identification. With the development of AOPs, that anchor the chemical-biomolecule interaction (MIE) to key events along the toxicity pathway leading to an adverse outcome at the individual or population level, experiments can be refined to examine earlier upstream molecular events predictive of the apical response, therefore improving animal welfare. With key endpoints elucidated, tools such as the U.S. EPA's Sequence Alignment to Predict Across Species Susceptibility algorithm (LaLone et al. 2013) can be used to identify likely susceptible species, further focusing testing on specific taxa and potentially reducing the need for testing others. Overall, these methods allow for more efficient and targeted toxicity test designs that support efforts to reduce, refine, and replace animal testing.