TU054 Cross-species assay validation using the AOP "deiodinase inhibition leading to impaired posterior chamber inflation"E. Stinckens, University of Antwerp; L. Vergauwen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences SPHERE; J.E. Cavallin, U.S. EPA / US EPA MidContinent Ecology Division; A. Schroeder, University of Minnesota-Crookston / Math Science and Technology; B.R. Blackwell, ORISE / National Health and Environmental Effects Research Laboratory; H. Witters, VITO / Applied Bio & molecular Systems; R. Blust, University of Antwerp; G.T. Ankley, U.S. EPA / National Health and Environmental Effects Research Laboratory; D.L. Villeneuve, U.S. Environmental Protection Agency / National Health and Environmental Effects Research Laboratory; D. Knapen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences. The Adverse Outcome Pathway (AOP) concept is increasingly being recognized as a promising conceptual framework for describing toxicity pathways, which contains information that is sufficient to predict an adverse outcome of regulatory importance. Previously, we assessed the feasibility of developing an alternative, mechanistically informative testing strategy to replace the chronic Fish Early-Life Stage test (FELS, OECD TG 210), using an AOP-based approach. We developed an AOP encompassing deiodinase (DIO) inhibition resulting in decreased T3 concentrations leading to impaired swim bladder inflation in fish. *In vitro* assays were used to measure DIO enzyme activity of 51 relevant compounds. Using these results, *in vivo* effects on swim bladder inflation were predicted. These predictions were biologically validated for a set of 14 compounds, with the exception of only 2 false positives and 1 false negative, using zebrafish as model organism. Our results were in line with our AOP and illustrate how AOP-derived information can be used for assay development and refinement. In a next step, we assessed the cross-species applicability of our AOP-based assays. In order to predict an AO based on a molecular initiating event (MIE) or key event, one needs to take into account the fact that the affinity of certain compounds to interact with receptors, enzymes etc. can differ among species. As DIO1 inhibition is the MIE of our AOP on which an *in vitro* assay was based, we investigated whether the use of porcine, rat or fish liver as starting material would result in similar *in vitro* DIO1 inhibition patterns for a set of 22 compounds, and thus whether predictions in one species can be made based on assays using tissues of other species as the starting material. Results show that the DIO1 inhibitory potential compared to a reference compound is nearly identical between the three selected species. However, a set of bisphenol A derivatives showed lower inhibition potential in fish and rat compared to pig. A reassessment of the validation experiments based on zebrafish inhibition data shows that TCBPA is no longer a false positive prediction, while BPA appears to become a false negative prediction. These results show that for most compounds, tissue originating from different species can be used in our DIO1 assay to predict apical outcomes in fish. Furthermore, it demonstrates that AOPs can support cross-species extrapolation after investigating their taxonomic applicability.