MO256 Cross-species applicability of the adverse outcome pathway "deiodinase inhibition leading to impaired swim bladder inflation in zebrafish" E. Stinckens, University of Antwerp; L. Vergauwen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences SPHERE; H. Witters, VITO / Applied Bio & molecular Systems; R. Blust, University of Antwerp / Department of Biology (SPHERE Research Group); G.T. Ankley, D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research Laboratory; D. Knapen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences. The adverse outcome pathway (AOP) framework can be used to help support the development of alternative testing strategies aimed at predicting adverse outcomes caused by triggering specific toxicity pathways. Previously, we developed an AOP describing how inhibition of deiodinase (DIO) enzyme activity leads to impaired swim bladder inflation in fish. Next we assessed the feasibility of selecting alternative *in chemico* assays targeting specific key events along the AOP and evaluated the potential of these *in chemico* data for predicting higher biological *in vivo* endpoints. We were able to demonstrate that the *in chemico* dataset can be used to effectively predict effects on swim bladder inflation. For a limited number of compounds however, zebrafish responded differently than what was expected. In this presentation, we will assess these outliers by examining (1) the cross-species applicability of our AOP-based assays, (2) toxicological mechanisms other than thyroid disruption that could result in effects on swim bladder inflation. We performed *in vitro* DIO assays for 20 compounds using porcine, rat and fish liver homogenates to characterize similarities and differences among species. Results show that the DIO1 inhibitory potential is nearly identical between the selected species. However, a set of bisphenol A derivatives showed lower inhibition in fish and rat compared to pig. In addition, we performed qPCR analysis of a set of 29 genes related to thyroid metabolism and swim bladder inflation after exposing zebrafish to 4 compounds for which false negative predictions were observed. These results suggest that PFOS affects surfactant properties which could impact swim bladder inflation. SMX affected genes related to the development of the 3 cell layers of the swim bladder, suggesting that this compound inhibits swim bladder development and subsequent inflation. Our results suggest that for most compounds, tissue originating from different vertebrate species can be used in the DIO assay to predict apical outcomes in fish. However, it is expected that any predictive model based on measuring only few molecular initiating events could be refined as knowledge on the involvement of other specific thyroid related processes in swim bladder inflation grows. In addition to the fact that differences in predicted effects may be observed as a result of cross-species differences, many different toxicological mechanisms can lead to swim bladder inflation effects as well.