232 Assay development based on the AOP network "thyroperoxidase and deiodinase inhibition leading to impaired swim bladder inflation" E. Stinckens, University of Antwerp; L. Vergauwen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences; J.E. Cavallin, U.S. EPA / US EPA MidContinent Ecology Division; A. Schroeder, University of Minnesota-Crookston / Math Science and Technology; W. Maho, University of Antwerp Toxicological Center / Toxicological Centre Dept Pharmaceutical Sciences; B.R. Blackwell, ORISE / National Health and Environmental Effects Research Laboratory; H. Witters, VITO / Applied Bio molecular Systems; R. Blust, Systemic Physiological and Ecotoxicological Research group University of Antwerp; G.T. Ankley, U.S. EPA / National Health and Environmental Effects Research Laboratory; A. Covaci, University of Antwerp / Toxicological Center; D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research Laboratory; D. Knapen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences. The vast number of industrial chemicals has generated a strong focus on alternative test development for ecological risk assessment. Therefore, we are developing a non-animal testing strategy for the prediction of chronic aquatic toxicity including *in vitro* tests and *in vivo* ZFET (Zebrafish Embryo Acute Toxicity Test, OECD TG 236) assays. Our assay development process is based on using the adverse outcome pathway (AOP) framework to identify key events (KEs) that could be used to predict chronic toxicity. We developed an AOP network, encompassing thyroperoxidase (TPO) and deiodinase (DIO) activity inhibition, leading to decreased T4 (prohormone) and/or T3 (biologically active hormone) concentrations, impacting swim bladder development and inflation. The latter is considered an ecologically relevant adverse outcome as it affects feeding behaviour and predator avoidance, resulting in lower survival probability. For the assessment of AOP-specific KEs, we optimized *in vitro* assays to characterize the effects of a battery of environmental chemicals with suspected thyroid disrupting activity on TPO/DIO activity. Results were used to predict the impact on swim bladder inflation and validation was accomplished using 120 hours post fertilization (hpf) ZFET and 32 days post fertilization (dpf) FELS (Fish Early-life Stage Toxicity Test, OECD TG 210) experiments. Mercaptobenzothiazole (MBT), a compound identified as a TPO inhibitor, does not directly impair posterior chamber inflation at 120 hpf, while iopanoic acid, a DIO inhibitor, and propylthiouracil, which inhibits TPO and DIO, do. These results affirm our AOP network stating that embryonic TPO activity is not essential for posterior chamber inflation, because of the presence of maternal T4, while DIO activity is needed to activate maternal T4 into T3. However, both enzymes are needed to synthesize and activate THs at later developmental stages, so both TPO and DIO inhibitors were predicted to impair anterior chamber inflation. A FELS MBT exposure indeed resulted in impaired anterior chamber inflation at 21 dpf. Moreover, a relationship between T4 levels and anterior chamber surface further suggests an influence by THs on anterior chamber inflation. *In vitro* assays measuring the TPO/DIO inhibitory potential of compounds were optimized and used to screen a battery of relevant compounds. The results were successfully used to predict the impact on swim bladder inflation, which was validated using ZFET and FELS tests.