TU004 Development of an alternative testing strategy for the fish early life-stage test using 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 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.C. Volz, University of California, Riverside / Environmental Sciences; 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 prediction of reproductive and early developmental toxicity has largely relied on the use of animals. Currently, the fish early-life stage (FELS) test (OECD TG 210) is one of the primary tests used to estimate chronic toxicity of chemicals in fish to support risk assessment. However, there is widespread agreement regarding the need to develop alternative testing strategies. We assessed the feasibility of using an AOP-based approach for developing such an alternative, mechanistically informative testing strategy by investigating whether the AOP framework forms a basis for (1) the development of new non-animal test methods, (2) the prioritization of assay development and (3) obtaining biological context for mechanistic information. We developed an AOP network, with two main pathways leading to effects on the swim bladder of zebrafish and fathead minnow. The swim bladder consists of a posterior and an anterior chamber, which inflate during a Fish Embryo Acute Toxicity (FET, OECD TG 236, early development) and FELS (late development) timeframe, respectively. We defined underlying key events leading to the adverse outcomes and postulated that embryonic thyroperoxidase (TPO) activity is not essential to posterior inflation, while deiodinase (DIO) activity is needed to activate maternal T4 into T3. However, both enzymes are needed at later developmental stages, and inhibition of either enzyme results in impaired anterior inflation. In order to validate this proposed mechanism, we optimized *in vitro* tools for the assessment of the *in vitro* TPO/DIO inhibitory potential of 51 relevant contaminants. Predictions regarding the *in vivo* impact on swim bladder inflation were made, which were validated using 168 hours post fertilization FET and 32 days post fertilization FELS experiments. Results show that only DIO inhibitors decrease posterior chamber surface area at low concentrations and completely inhibit posterior inflation at higher concentrations. The posterior surface area appears to be more sensitive compared to the binary observation of posterior inflation. Finally, FELS exposures with two TPO inhibitors resulted in impaired anterior inflation. Our results are in line with, and increase confidence in, our AOP network. We can conclude that we successfully used the AOP framework for (1) the selection and prioritization of key events and predictive assays, (2) accurate prediction of acute and chronic toxicity and (3) the development of non-animal test methods.