Validation of the AOP network “Thyroperoxidase and/or deiodinase inhibition leading to impaired swim bladder inflation”

Stinckens Evelyn, University of Antwerp, Zebrafishlab (Presenting author)
Vergauwen Lucia, University of Antwerp, Zebrafishlab
Cavallin Jenna, US EPA, ORD NHEERL, Mid-Continent Ecology Division
Blackwell Brett R., US EPA, ORD NHEERL, Mid-Continent Ecology Division
Witters Hilda, Flemish Institute for Technological Research (VITO)
Blust Ronny, University of Antwerp, Systemic Physiological and Ecotoxicological Research (SPHERE)
Ankley Gerald T., US EPA, ORD NHEERL, Mid-Continent Ecology Division
Volz David C., University of California, Department of Environmental Sciences
Villeneuve Daniel L., US EPA, ORD NHEERL, Mid-Continent Ecology Division
Knapen Dries, University of Antwerp, Zebrafishlab

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 FET (Fish Embryo Acute Toxicity Test, OECD TG 236) assays. Our assay development process has employed 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) inhibition, leading to decreased T4 and/or T3 concentrations, impacting swim bladder inflation and ultimately young of year survival. The swim bladder of zebrafish and fathead minnow, the two species used to construct the network, consists of a posterior and an anterior chamber, which inflate during a FET (early development) and Fish Early-life Stage Toxicity Test (FELS, OECD TG 210, late development) timeframe, respectively.

To assess the selected KEs, we first optimized in vitro assays to screen a battery of 50 environmental contaminants for their TPO/DIO inhibitory potential. Results were used to predict the impact on swim bladder inflation in vivo. Predictions were validated using 120/168 hours post fertilization (hpf) FET and 32 days post fertilization (dpf) FELS tests.

Results show that compounds identified as TPO inhibitors do not directly impair posterior chamber inflation at 168 hpf, while DIO inhibitors do. Compounds without TPO/DIO inhibitory capacities did not affect posterior inflation. An inter-lab validation experiment confirmed these findings. In addition, effects on posterior chamber surface area were found at lower concentrations when posterior inflation was impaired at higher concentrations.

Our results increase confidence in our AOP network-based hypothesis demonstrating that embryonic TPO activity is not essential to posterior inflation, but DIO activity is needed to activate maternal T4 into T3. However, both enzymes are needed at later developmental stages and thus anterior chamber inflation. FELS exposures with methimazole and mercaptobenzothiazole indeed resulted in impaired anterior chamber inflation at 21 dpf.

In conclusion, we successfully used an AOP-based approach to select key events, develop assays, and to correctly predict chronic toxicity.

Keywords: Thyroid disruption, Zebrafish embryo, Swim bladder inflation, Adverse outcome pathway

Contact: Evelyn.Stinckens@uantwerpen.be