Abstract

Validation of the AOP network "thyroperoxidase and/or deiodinase inhibition leading to impaired swim bladder inflation"

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Industry and regulatory bodies have expressed the need for developing alternative chemical toxicity testing strategies focusing on obtaining mechanistic information. We are developing a non-animal testing strategy for the prediction of chronic Fish Early-Life Stage (FELS, OECD TG 210) toxicity based on the adverse outcome pathway (AOP) framework.

We developed an AOP network, which is part of Project 1.35 of the OECD AOP development programme workplan, in several iterations using 4 reference compounds. The adverse outcome concerns effects on the swim bladder of zebrafish and fathead minnow, the two species used to construct this network. 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.

The network postulates that embryonic thyroperoxidase (TPO) activity is not essential to posterior chamber 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 chamber inflation.

Although AOPs can support development of non-animal test methods, newly hypothesized AOPs, and assays developed based on these AOPs, require validation. Therefore, we optimized *in vitro* assays to measure the TPO/DIO inhibitory potential of 50 relevant contaminants. Predictions regarding the *in vivo* impact on swim bladder inflation were made, which were validated using 168 hours post fertilization (hpf) FET and 32 days post fertilization (dpf) FELS experiments.

Results indicate that only DIO inhibitors, and not TPO inhibitors, decrease posterior chamber surface area at low concentrations and completely inhibit posterior inflation at higher concentrations. These findings were confirmed in an inter-laboratory validation experiment. The posterior surface area seems to be a

more sensitive endpoint that could refine our AOP with regard to the binary observation of posterior inflation. Finally, FELS exposures with two TPO inhibitors resulted in impaired anterior inflation at 21 dpf.

In conclusion, our results are in line with, and increase confidence in, our AOP network and we successfully used an AOP-based approach to select key events, develop assays, and correctly predict acute and chronic toxicity.