An AOP-based alternative testing strategy to predict the impact of thyroid hormone disruption on swim bladder inflation in zebrafish

This project involved the development and validation of novel in chemico assays as an alternative to in vivo fish studies for the identification of thyroid hormone disrupting chemicals.

Part of the Cefic-LRI ECO20/ECO20.2 projects entitled “Development of an alternative testing strategy for the fish early life-stage test using the AOP framework”
This project demonstrates the use of *in chemico* assays targeting specific key events of an established Adverse Outcome Pathway (AOP) to predict higher biological endpoints. One of the case studies in this project focused on the role of thyroid hormones in the embryonic development of fish.

An AOP network linking thyroid hormone disruption to impaired swim bladder inflation in fish was developed. This network was used to select alternative *in chemico* assays measuring thyroperoxidase and deiodinase inhibition, key enzymes in the thyroid hormone metabolism. A set of 51 compounds was screened, and data were used to predict acute and chronic effects on swim bladder inflation.

Predictions were validated using acute (FET) and chronic (FELS) *in vivo* experiments in zebrafish and fathead minnow, and *in chemico* to *in vivo* extrapolation thresholds were established. A tiered testing strategy for the identification of thyroid hormone disrupting compounds was then proposed.

**Regulatory relevance**

- **OECD**: Our thyroid hormone disruption AOP network is part of the AOP development programme workplan of the OECD (project 1.35). OECD review is planned for late 2018.
- **EPA**: As part of their endocrine disruptor screening program, the US EPA included our work while assembling a conceptual thyroid AOP network spanning different taxonomic groups (fish, amphibians, mammals) to assist high throughput assay development.

The currently ongoing JRC EURL ECVAM validation effort of *in vitro* assays for thyroid disruption screening, to which VITO is contributing as one of the EU-NETVAL laboratories, is making use of this project’s data to ensure synergies and overlap.