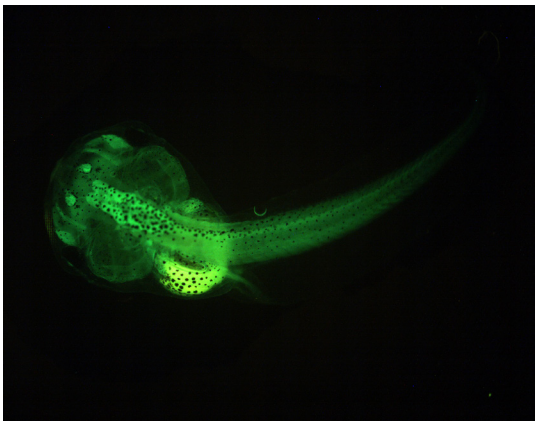
LABORATOIRE WATCHFROG

OECD TG NO. 248 - XETA (*XENOPUS* EMBRYONIC THYROID ASSAY)

// GENERAL DESCRIPTION



Fluorescence induction in a tadpole following exposure to thyroid hormone (T3).

Endocrine disruptors are substances acting at the whole-organism level. Watchfrog offers fluorescent-based screening in miniature translucent aquatic larvae **to reveal physiological key events related to endocrine disruption**.

These tests described in the ECHA/EFSA guidance document to identify endocrine chemicals are progressively implemented in the OECD Conceptual Framework for the Screening and Testing of Endocrine Disrupting Chemicals (Level 3).

Just after hatching, these models represent a **physiological test solution at the** *in vitro* **scale**. This not only guarantees the best sensitivity, but also prevents false positive results. **Fluorescence quantification** allows determination of the disruptive potential expressed **in hormone-equivalent values**.

Another advantage of this approach is the possibility to anticipate **effects of "cocktails"** of chemicals.

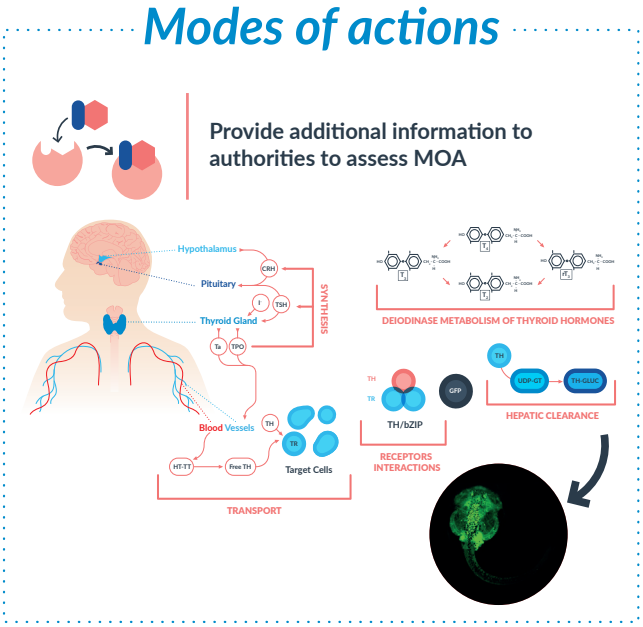
The test protocol could also be adapted for co-treating with substrates – specific inhibitors of mechanisms along the thyroid pathway – to contribute **elucidating which mechanism** could be affected by the sample.

For thyroid disruption assay, the test carried out and the obtained results are based on the physiological criteria of thyroid disruption as defined by the OECD for the **AMA thyroid disruption assay** (Amphibian Metamorphosis Assay, **OECD TG No. 231**).

// PERFORMING THE TEST

To evaluate the presence of thyroid disruptors, the sample is tested on **Xenopus tadpoles TH/bZIP–GFP**. The TH/bZIP gene is implicated in the phenomenon of metamorphosis, a process controlled by thyroid hormones (Furlow et al., 1999, Mol. Endocrinol., 13(12):2076-89). **Therefore, the use of this gene as a biomarker allows the detection of thyroid activity induced by the tested sample**. In this model, the thyroid signalisation is revealed by a modification of the level of fluorescence of the larvae in the brain (increase in the case of **pro-thyroid** effect and decrease in the case of **anti-thyroid** effect).

Larvae are placed into exposure solution. Each sample is tested alone or in co-treatment with reference thyroid hormone. This co-treatment enables activation of the thyroid axis of the larvae and therefore the detection of **synergistic or inhibitor effects** on the axis. All exposure solution and controls are renewed every 24hr. The readout of the test is carried out by a robotised imagery system.



// RESULTS

For regulatory purpose, the result of the test determines the capacity of the sample to act specifically on the thyroid pathway. The test is **quantitative** which facilitates its use also as a **predictive tool**. This analysis allows the identification of the concentration of the **thyroid hormone T3** which gives an equivalent effect to that of the sample. In order to do this, a concentration range of T3 is carried out in parallel to the testing of the product to allow modelling of this T3 standard curve.
The obtained fluorescence value for the sample is then compared to the T3 curve or used to perform statistical analysis.

// BIBLIOGRAPHY

J.B. Fini, S. Lemevel, N. Turque, K. Palmier, B.A. Demeneix. An in vivo multiwell-based fluorescent screen for monitoring vertebrate endocrine disruption. Environmental Science and Technology. 2007 Aug 15;41(16):5908-14.
OECD Guidelines for the Testing of Chemicals, Section 2: Test No. 231: Amphibian Metamorphosis Assay (AMA).
Reference protocol: OECD Guidelines for the Testing of Chemicals, Section 2: Test No. 248: *Xenopus* Eleutheroembryo Thyroid Assay (XETA).

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