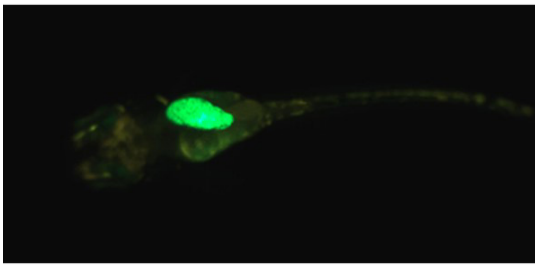
ESTROGENIC DISRUPTION & AROMATASE ACTIVITY

LABORATOIRE WATCHFROG

OECD TG NO. 252 - REACTIV (RAPID ESTROGEN ACTIVITY TEST *IN VIVO*)

// GENERAL DESCRIPTION



Fluorescence induction in a fish fry (liver) following exposure to estrogenic hormone (ethinyl-estradiol, EE2).

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.

To evaluate the presence of estrogenic disruptors, the sample is tested on **medaka ChgH-GFP**. This Asian biological model has many technical advantages. For example, for the evaluation of the sex steroid axes, the medaka is distinguished by a **well-established sexual determination**. In this model, the estrogenic signalisation is revealed by a modification of the level of fluorescence of the larvae in the liver (increase in the case of **pro-estrogenic** effect and decrease in the case of **anti-estrogenic** effect).

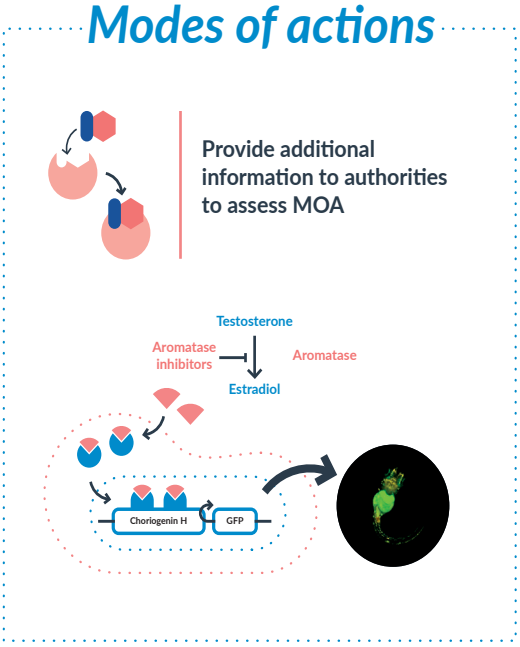
For estrogenic disruption assay, the test carried out and the obtained results are based on the physiological criteria of estrogenic disruption as defined by the OECD in the **OECD Test Guidelines No. 229 and No. 230.**

// PERFORMING THE TEST

The larvae used for assessment of estrogen axis disruption harbour a genetic construct composed of the promoter of the **Choriogenin** (ChgH) gene driving expression of the fluorescent reporter gene. Choriogenin is an egg protein produced in the liver of fish. **Its production is under the control of estrogenic hormones**. Therefore, the use of this gene as a biomarker allows the detection of estrogenic activity induced by the tested sample.

Larvae are placed into exposure solution. In order to take into account the physiological state of the whole organism, each sample is tested **with or without a co-treatment** with the reference hormone **testosterone**. This co-treatment enables activation of the estrogenic axis and detects **effects on the enzyme aromatase**.

Aromatase is an essential **enzyme for the endogenous production of estradiol** from testosterone. It **controls the balance between estrogen and testosterone** in the body and therefore participates in the **sexual identity** of individuals.



// RESULTS

For regulatory purpose, the result of the test determines the capacity of the sample to act specifically on the estrogenic pathway. The test is **quantitative** which facilitates its use also as a **predictive tool**. This analysis allows the identification of the concentration of the synthetic **estrogen hormone ethinyl-estradiol (EE2)** which gives an equivalent effect to that of the sample. In order to do this, a concentration range of EE2 is carried out in parallel to the testing of the sample to allow modelling of this EE2 standard curve. The obtained fluorescence value for the sample is then compared to the EE2 curve or used to perform statistical analysis.

// BIBLIOGRAPHY

Spirhanzlova P, Leleu M, Sébillot A, Lemkine GF, Iguchi T, Demeneix BA, Tindall AJ. Oestrogen reporter transgenic medaka for non-invasive evaluation of aromatase activity. Comp Biochem Physiol C Toxicol Pharmacol. 2016 Jan;179:64-71.
OECD Test Guideline No. 229 : " Fish Short Term Reproduction Assay".
OECD Test Guideline No. 230 : "21-day Fish Assay. A Short term Screening for Oestrogenic and Androgenic Activity, and Aromatase Inhibition".

PHONE: +33 1 69 36 11 15 – EMAIL: INFO@WATCHFROG.FR