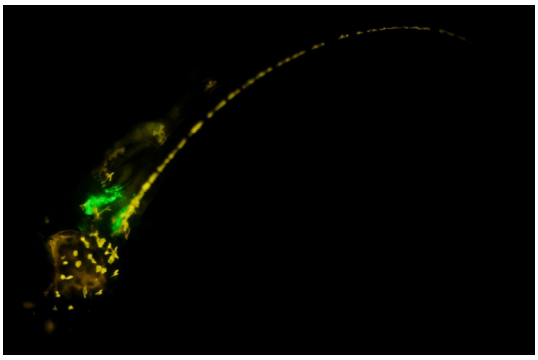
LABORATOIRE WATCHFROG

OECD TG NO. 251 - RADAR (RAPID ANDROGEN DISRUPTION ACTIVITY REPORTER ASSAY)

// GENERAL DESCRIPTION



Fluorescence induction in a fish fry (kidneys) following exposure to androgenic hormone (17 α-methyltestosterone, 17MT).

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 androgen axis disruptors, the sample is tested on **medaka SPG-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 androgenic signalisation is revealed by a modification of the level of fluorescence of the larvae in kidneys (increase in the case of **pro-androgenic** effect / decrease in the case of **anti-androgenic** effect).

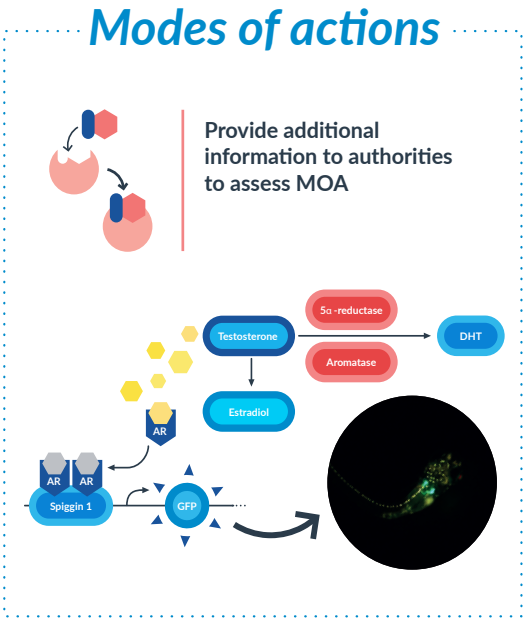
For androgenic disruption assay, the test carried out and the obtained results are based on the physiological criteria of androgen axis disruption as defined by the **OECD Test Guideline No. 128 (AFSS:** Androgenised Female Stickleback Screen).

// PERFORMING THE TEST

The larvae used for assessment of androgen axis disruption harbour a genetic construct composed of the promoter of the **Spiggin** (Spg) gene driving expression of the fluorescent reporter gene.

Spiggin is a protein produced by the kidney. **Its production is under the control of androgenic hormones**. In the breeding season, male stickleback fish (*Gasterosteus aculeatus*) produce Spiggin glue protein in their kidneys in response to elevated circulating androgen to allow the building of nests. **Therefore, the use of this gene as a biomarker allows the detection of androgenic 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 **17 α-methyltestosterone** (17MT). This co-treatment enables activation of the androgen axis of the larvae and therefore the detection of **synergic or inhibition effects on the axis**.



// RESULTS

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

// BIBLIOGRAPHY

Sébillot A, Damdimopoulou P, Ogino Y, Spirhanzlova P, Miyagawa S, Du Pasquier D, Mouatassim N, Iguchi T, Lemkine GF, Demeneix BA, Tindall AJ. Rapid Fluorescent Detection of (Anti)androgens with spiggin-gfp Medaka. Environ Sci Technol. 2014 Aug;48(18):10919–10928.

OECD Test Guideline No. 128: Androgenised Female Stickleback Screen (AFSS), 2009.

Reference protocol: OECD Guidelines for the Testing of Chemicals, Section 2: Test No. 251: Rapid Androgen Disruption Activity Reporter (RADAR) assay.

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