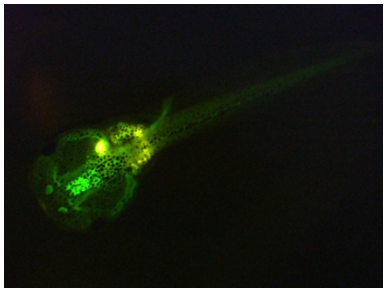
THE *XENOPUS* ELEUTHEROEMBRYO THYROID ASSAY (XETA)

Purpose



Fluorescent Elutherotrophic Embryo (Watchfrog)

The *Xenopus* Eleuthero Thyroid Assay (XETA) provides a short-term screening assay to measure the response of transgenic South African clawed frog (*Xenopus laevis*) eleutheroembryos to potential thyroid active chemicals. Due to the capacity of a chemical to activate or inhibit the transcription of the genetic construct (THb/ZIP-GFP *X.laevis*), chemicals can be classified as potentially thyroid active or inactive by quantifying the resulting fluorescence.

The test takes into account the entire physiological pathway leading to disruptive outcome and is sensitive to the mode of action.

Study Design

Test organisms

The South African clawed frog *Xenopus laevis* is a relevant amphibian model for both, early development and thyroid hormones dependent metamorphosis; and is currently used in two OECD test guidelines: the AMA (amphibian metamorphosis assay, OECD TG 231, OECD 2009) and the LAGDA (larval amphibian growth and development assay, OECD TG 241, OECD 2015).

Due to high genetic homology as well as similar biotransformation systems and homologous endocrine pathways compared to higher vertebrates, test with X. laevis can provide information that might be extrapolated to other taxa.

Test Concentrations

A minimum of three concentration levels, a test medium control (and solvent control, if necessary) should be tested in the absence (“unspiked mode”) and presence (“spiked mode”) of the plasma concentration of thyroid hormone triiodothyronine (T3; 3.25 µg/L). Furthermore, a T3 control and a T4 control (thyroxine; saturation control) are tested. A spacing factor of 3.2 to 10-fold is recommended. One test comprises three independent, valid runs. Each run includes two replicates per treatment groups and controls.

The maximum concentration tested is the limit of solubility, 100 mg/L or the concentration inducing malformations in less than 10% of eleutheroembryo, whichever is lowest.

Course of the test

The transgenic *X. laevis* embryos are obtained from our partner Laboratoire Watchfrog as early as possible in the development and are reared in-house at 21 ± 1 °C without illumination. The test will be conducted according to the new OECD Test Guideline 248 (OECD 2019):

For the duration of the test, eleutheroembryos from a single spawn are exposed from stage NF 45 (approx. 7 days post fertilisation) to stage NF47 (72 hours) in inert 6-well culture plates under semi-static conditions. No feeding occurs during the test.

Assessments and Endpoints

During the test, embryos are checked for abnormal appearance and death after 24, 48 and 72 hours.

At the end of the test, the normally developed eleutheroembryos are assessed for fluorescence using a spectrofluorometer. Data from the three independent runs are pooled and statistically analysed. If the change in fluorescence compared to the test medium control ("unspiked mode") or the T3 control ("spiked mode") is greater than 12 %, the test is considered positive.

Guidelines and Literature

- Niewkoop, P. D., Faber, J. (1994). Normal table of *Xenopus laevis* (Daudin). Garland Publishing Inc, New York ISBN 0-8153-1896-0
- OECD (2019). Test No. 248: *Xenopus* Eleutheroembryo Thyroid Assay (XETA), OECD Giudelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris.

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