FET4Thyroid – A Fish Eleutheroembryo Test for Thyroid Activity

Overall aim

1. The aim of this Challenge is to develop an assay using non-protected life-stages [1] of fish to detect thyroid-active chemicals that is designed to meet OECD principles for use in a regulatory context.

Duration

2. This is a Single-Phase Challenge with funding for up to 18 months.

Budget

3. Up to £100k.

Sponsors

4. Sponsors define the Challenges in collaboration with the NC3Rs to set out the business case and 3Rs benefits, with a view to using the product developed. Sponsors are required to provide in-kind contributions to help solve the Challenge.

5. The Sponsors for this Challenge are Bayer AG and BASF SE.

Co-funders

6. Bayer AG.

Background

7. Environmental Risk Assessment (ERA) evaluates the likelihood that organisms in the environment are impacted due to exposure to chemicals. ERA mainly relies on the relationship between the exposure of natural ecosystems to a chemical (e.g. emissions resulting from consumer use or from industrial processes into water bodies) and the inherent hazard of that chemical (e.g. potential for it to cause harm to relevant species). Traditionally, ERA relies on animal testing to assess toxicological

[1] Fish protected life stages are defined under UK Home Office guidance and the EU Directive 2010/63/EU.

properties of chemicals, including the evaluation of potential endocrine disruption (ED) through interaction with endocrine-related receptors such as the oestrogen, androgen, and thyroid receptors (1). Current regulatory ED assessment for aquatic species relies mostly on data from chronic *in vivo* studies in fish in accordance with US Environmental Protection Agency (EPA) and OECD Test Guidelines (TG). The assessment of thyroid-mediated endocrine-disrupting properties of chemicals is unique in that amphibian rather than fish are almost exclusively used, as the current fish tests do not include endpoints relevant for thyroid disruption. These studies often use large numbers of animals.

8. Global research efforts into the development and application of New Approach Methodologies (NAMs) aim to provide information for chemical hazard and risk assessment and reduce the reliance on *in vivo* studies. An amphibian eleutheroembryonic assay – the XETA assay – (2) has been developed as a NAM to evaluate endocrine activity mediated via the thyroid axis, but it does not currently cover all thyroid-mediated mechanisms, such as thyroperoxidase (TPO) and sodium-iodide symporter (NIS) inhibition. Therefore, testing using amphibians and fish (for other endocrine modes of action) is still conducted to cover these and to address adverse effects on growth and development to fulfil data requirements for regulatory ERA.

9. Ongoing research initiatives such as the European Programme Horizon2020 ERGO project (3) are working on the development and incorporation of thyroid-specific endpoints into existing OECD *in vivo* fish TGs 210, 234 (4). These developments hold promise in terms of reduction of animal testing, as fish TGs that include thyroid-specific endpoints could avoid the need for amphibian studies. However, a NAM based thyroid-specific test using fish eleutheroembryos, that covers an increased range of thyroid-mediated mechanisms and builds on other work in the area (5) has the potential to replace the use of both fish and amphibian protected life-stages for assessing thyroid-mediated ED.

10. The aim of this Challenge is to develop an assay using fish eleutheroembryos to specifically detect thyroid activity for use in ED and ERA.

3Rs benefits

11. Thousands of animal tests are conducted worldwide each year for ERA purposes. Implementing a thyroid-specific fish eleutheroembryo test to identify chemical classes with potential thyroid-mediated endocrine activity has the potential to replace some of these studies. In the short-term, a reliable thyroid-specific fish eleutheroembryo test would preclude the requirement for studies in amphibians. In the longer term, extending the scope and coverage of NAMs for ED and wider ERA could lead to a suite of NAMs that can begin to fully replace the use of fish in regulatory testing.

12. Current European Chemicals Agency (ECHA) and European Food Safety Authority ED guidance for the identification of endocrine disruptors (6,7) includes an annex where the specific conditions for the

use of the NAM-based XETA assay are defined. It is expected that this guidance will evolve to include newly developed eleutheroembryo assays for endocrine modes-of-action other than the thyroid such as the OECD EASZY (8) and RADAR assays (9) over the next few years. This provides a significant opportunity for a successful FET4Thyroid Challenge to be included. Draft ECHA guidance supporting the new regulation (10) on the classification, labelling and packaging of substances and mixtures also includes recommendations on the tests to be conducted for ED assessment, presenting additional opportunity for the inclusion of new NAMs.

Key deliverables

13. A thyroid-specific assay using fish eleutheroembryos (non-protected life stages) to detect thyroid activity for use in ERA.

- The choice of the fish species should be guided by existing eleutheroembryonic assays such as those using fluorescence reporter-based systems with medaka or zebrafish. The fathead minnow, commonly used in OECD level-3 screening assays (11), is not excluded if reporter technology is available for this species.
- All mechanisms of thyroid-mediated activity should be captured in the fish eleutheroembryonic assay. Among the thyroid targets identified in the assay, particular attention should be paid to TPO and the NIS.
- The mechanisms and applicability domain covered by the assay must be well characterised (i.e. to inform the “specified conditions” for the use of data from the test to negate follow-up chronic fish or amphibian *in vivo* tests).
- A pre-validation report and a manuscript ready for submission to a peer-reviewed journal. These should include a detailed test protocol, a precise description of the mechanisms of action that can be detected in the assay (i.e. applicability domain), and a thorough evaluation of both the endpoint specificity and sensitivity to reference thyroid-active chemicals, and the repeatability and reproducibility of the assay.
- The required information for a Standard Project Submission Form for a multi-laboratory validation exercise of the protocol as an OECD TG. This should include partner laboratories and the list of reference chemicals to be used for the validation.
- The selected laboratory should subscribe to OECD Intellectual Property policy for access to the model system used.

Sponsor in-kind contributions

14. The Challenge will be supported through the provision of in-kind support from the Sponsors. The in-kind support offered includes:

- Internal expertise on thyroid-mediated endocrine mechanisms, expected applicability domain and validation procedures.
- Input on selection of reference chemicals.
- Support for analytical measurements, for example, verification of the exposure concentrations in the assay.

References

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4. Kraft M *et al.* (2023) Developmental exposure to triclosan and benzophenone-2 causes morphological alterations in zebrafish (*Danio rerio*) thyroid follicles and eyes. *Environ Sci Pollut Res* **30**, 33711–24. doi: 10.1007/s11356-022-24531-2
5. Project 2.64; Inclusion of thyroid endpoints in OECD fish Test Guidelines Work plan for the Test Guidelines Programme (TGP) [Accessed 4 July 2023]
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8. OECD (2021), *Test No. 250: EASZY assay - Detection of Endocrine Active Substances, acting through estrogen receptors, using transgenic tg(cyp19a1b:GFP) Zebrafish embrYos, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris,* https://doi.org/10.1787/0a39b48b-en.
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10. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 (Text with EEA relevance)
11. OECD (2018), *Test No. 150: Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption, OECD Series on Testing and Assessment, OECD Publishing, Paris*. doi: 10.1787/9789264304741-en