CEFIC Long-range Research Initiative
Request for Proposals (RfP)

Title and Code Number
Integrating the FET into the Weight of Evidence to Inform Acute Fish Toxicity – **LRI-ECO51**

Background
The Fish Embryo Test with zebrafish (*Danio rerio*) was accepted as OECD TG (Test Guideline) 236 in 2013 after a 4-year validation program (Busquet et al., 2014). The multi-sector effort involved academic, private sector, government and NGO scientists and is arguably the most comprehensive OECD environmental TG developed to date. Much of industry embraced the FET to fulfil acute aquatic toxicity endpoints for fish (AFT, acute fish toxicity) in the spirit of improved animal welfare. However, regulatory uptake of the FET at the international level has been slow.

Specific questions have lingered across a range of diverse issues (Scholz et al. 2016) resulting in the convening of an international workshop on FET acceptance in May, 2017 hosted by UBA, Germany, the sponsor nation of the OECD TG 236 and ECHA in Helsinki, Finland (UBA, ECHA 2017). The workshop identified the following remaining concerns with the FET: (1) the lack of universality of FET in being equisensitive to AFT for all compounds – especially for specifically acting compounds (e.g., neurotoxicants); (2) the poor predictive relationships for several narcotic compounds; (3) select scientific queries on roles of physical-chemical properties on uptake across the chorion versus fish gills; and (4) the role of the differences in metabolic capacity of embryos versus more developed fish life stages. Lastly, ECHA requested that ecotoxicologists develop guidance on how to devise a functional weight of evidence (WoE) approach for use of the FET in risk assessment, PBT assessment and Classification and Labelling.

Since the 2017 workshop, fish embryo testing science has continued to develop and progress (Birk and Scholz 2019; Carr et al. 2018; Fischer et al. in press; Rawlings et al. 2019; Stengel et al. 2018) driven by the high interest in commitment to animal alternatives for ecological risk assessment. A recently established work program at OECD has also sought to identify opportunities (Project 2.54) to develop an acute fish intelligent approach to testing and assessment which would be consistent with devising a WoE framework.

Objectives
This project will develop and subsequently recommend a systematic WoE to supporting fish embryo testing to inform on the potential for acute fish toxicity for regulatory purposes including hazard assessment and Classification and Labelling.

The project's objectives are to:

1. Develop an approach that provides improved WoE on acute fish toxicity beyond direct assessment of fish embryo tests as predictors of acute fish toxicity. The approach may be qualitative or quantitative.
2. Develop a means to address non-conformance of FET predictions for specifically acting toxicants.
3. Improve the assessment of acute fish toxicity studies used to establish comparative relationships between the AFT and alternative approaches.
4. Identify additional gap filling needs and develop short- and long-term priorities for addressing these needs.

Scope

Weight of evidence has received a large amount of attention in both regulatory frameworks (https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/weight-of-evidence; SCHEER 2018; USEPA 2016) and scientific literature (Hope and Clarkson 2014; Hall et al. 2018). Flexibility is present in the myriad of approaches, so the scope here will be rather broad. Physical-chemistry, domain of applicability, sources of informative biological and ecotoxicological data and so forth can all independently contribute to improved WoE for the FET. These may be of an *in silico*, *in vitro*, or *in vivo* nature. As the goal is to predict acute fish toxicity there is no fully *a priori* decision as to the breadth and type of information that is expected to be useful and it will be up to the study investigators to assist in defining the inputs to the WoE.

Deliverables

The final report shall contain an executive summary (2 pages max), a main part (max. 50 pages) and a detailed bibliography. It is expected that the findings will be developed into at least one peer reviewed publication, following poster(s) and presentation(s) at suitable scientific conference(s). At least one publication shall be open-access.

Cost and Timing

Start in Q1 2020, duration 2 years
Budget in the order of €250 000

Partnering/Co-funding

Applicants should provide an indication of additional partners and funding opportunities that can be appropriately leveraged as part of their proposal. Partners can include, but are not limited to industry, government/regulatory organizations, research institutes, etc. Statements from potential partners should be included in the proposal package.

Fit with LRI objectives/Possible regulatory and policy impact involvements/ Dissemination

Applicants should provide information on the fit of their proposal with LRI objectives and an indication on how and where they could play a role in the regulatory and policy areas. Dissemination plans of study results should also be outlined.

References

- Birk, A. and S. Scholz. 2019. Zebrafish embryo and acute fish toxicity test show similar sensitivity for narcotic compounds. ALTEX-Alternatives to Animal Experimentation 36:131-135.
- Busquet, F. R. and 26 co-authors. 2014. OECD validation study to assess intra- and inter-laboratory reproducibility of the zebrafish embryo toxicity test for acute aquatic toxicity testing. Reg Toxicol Pharmacol 69:496-511.
- Carr, G. J., J. M. Rawlings, J. Bailer, and S. E. Belanger. 2018. On the effect of sample size in acute fish toxicity testing: Sample size matters. Env Toxicol Chem 37:1565-1578.
- Fischer, M. and 20 co-authors. 2019. Repeatability and reproducibility of the RTgill-W1 cell line assay for predicting fish acute toxicity. Toxicological Science. In Press.
- Hall, T., O. Martin, S. Belanger, M. Galay-Burgos, P. D. Guiney, G. Maack, and W. Stubblefield. 2017. Weight of Evidence for Ecotoxicological Effects Characterization in Regulatory Decision-Making. Int Env Assess Manag 13:573-579.
- Hope B, Clarkson J. 2014. A strategy for using weight-of-evidence methods in ecological risk assessments. Hum Ecol Risk Assess 20(2):290–315.
- OECD (Organization for Economic Co-operation and Development). 2013. Test No. 236: Fish Embryo Acute Toxicity (FET) Test. OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing, Paris, France. doi:10.1787/9789264203709-en
- SCHEER (Scientific Committee on Health, Environmental and Emerging Risks). 2018. Memorandum on weight of evidence and uncertainties (Revision 2018). Opinion adopted by written procedure on 26 June 2018. 48p.
- Rawlings, J. M., S. E. Belanger, K. A. Connors and G. J. Carr. Fish Embryo Tests and Acute Fish Toxicity Tests are Interchangeable in the Application of the Threshold Approach. Env Toxicol Chem 38:671-681.
- Scholz, S. N. Klüver, and R. Kühne. 2016. Analysis of the relevance and adequateness of using Fish Embryo Acute Toxicity (FET) Test Guidance (OECD 236) to fulfil the information requirements and addressing concerns under REACH. Report ECHA-UFZ contract ECHA/2014/341. 14 April 2016. 105p.
- Stengel, D. S. Wahby, and T. Braunbeck. 2018. In search of a comprehensible set of endpoints for the routine monitoring of neurotoxicity in vertebrates: sensory perception and nerve transmission in zebrafish (*Danio rerio*) embryos. Env Sci Poll Res 25:4066–4084.
- UBA (German Environment Agency, Umweltbundesamt) and ECHA (European Chemicals Agency). 2017. Joint Report ECHA and UBA, Expert Workshop on the potential regulatory application of the Fish Embryo Acute Toxicity (FET) Test under REACH, CLP and the BPR. 3-4 May 2017, Helsinki, Finland. 149p.
- USEPA (United States Environmental protection Agency). 2016. Weight of evidence in ecological assessment. Office of the Science Advisor, Risk Assessment Forum, Washington, DC. EPA/100/R-16/001. 69p. + Appendices.

DEADLINE FOR SUBMISSIONS: September 1st, 2019

Please see www.cefic-lri.org/funding-opportunities/apply-for-a-grant/ for general LRI objectives information, project proposal form and further guidance for grant applications.