



## CEPIC Long-range Research Initiative Request for Proposals (RfP)

### ***Code and Project Title:***

***LRI ECO40: Validation of an alternative, non-vertebrate, BCF test using the freshwater amphipod *Hyaella azteca****

### ***Background***

The ultimate decisive bioaccumulation criterion as part of the REACH regulation (Annex XIII) is the bioconcentration factor (BCF) reflecting the uptake of a test substance from the contaminated surrounding medium. Bioconcentration factors (BCF) for regulatory purposes are usually determined by fish flow-through tests according to TGD OECD 305 [1]. Alternatively, biomagnification factors (BMF) can be determined in dietary exposure tests for such groups of substances where this is considered more suitable than an aqueous exposure test. Fish bioaccumulation studies are time consuming, expensive, and use many laboratory animals. In particular, care is required to ensure test concentrations of poorly water soluble, highly hydrophobic test compounds are maintained throughout the duration of the study as well as having sufficiently sensitive analytical methods for analysis of water and tissue samples. ECHA promotes alternatives for testing on animals (vertebrates) that meet their regulatory needs so methods that replace the use of fish for BCF (BMF) testing are of particular value. An alternative approach by Fraunhofer IME which aims to replace fish as test organisms in bioaccumulation studies by a non-vertebrate species, the freshwater amphipod *Hyaella azteca* has shown promise. It has been shown that experimental BCF values from bioaccumulation studies with *H. azteca* are similar to those obtained from fish [2]. To date, 14 compounds of different lipophilicity (log Kow range 2.2-7.8) have been tested under flow-through conditions to determine steady-state and kinetic bioconcentration factors (BCF<sub>ss</sub> and BCF<sub>k</sub>). However, a more detailed understanding of comparative metabolic profiles, metabolic activity and transformation rates when employing *H. azteca*, compared to fish, is needed. Comparison of metabolism of fish and invertebrates has been reviewed, though not with involving *H. azteca* [3,4]. Preliminary studies carried out by Fraunhofer IME with *H. azteca* suggest CYP450 mediated bio-transformation comparable to fish. This is in accordance with the results obtained for other crustaceans e.g. *Daphnia magna* [5]. When performing BCF studies with *H. azteca*, although these organisms are much smaller than fish, collection of ten adult amphipods is sufficient to be able to quantify tissue concentrations and to determine uptake and elimination rates for *H. azteca*. In comparison to fish, shorter uptake and elimination periods are required using this species. It has recently been proposed that elimination rates constants generated during such studies could be also used as an alternative parameter for bioaccumulation in aquatic and terrestrial food chains [6]. Once sufficient comparative metabolic information has been generated, in addition to elimination rate constant data (phase 1), an inter-laboratory comparison of the BCF test protocol will be initiated (phase 2) which will provide the basis for a new OECD Test Guideline. The initiation of a new test guideline for BCF testing with *H. azteca* is supported by UBA, Germany.

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### **Scope**

#### Phase 1

- To investigate the biotransformation of xenobiotics (i.e. metabolite profiles, metabolic activity and transformation rates) in *Hyaella azteca* compared to fish
- To generate a database of elimination half-life data from in-house and company BCF studies in aquatic and terrestrial studies
- To compare experimental data from *H. azteca* and fish BCF studies and hence assess the suitability of the non-vertebrate test as alternative to OECD 305 fish tests.

#### Phase 2

- To organise an Inter-laboratory comparison ('ring-test') with known test compounds to validate the test protocol (at least six partners).

### **Objectives**

#### Phase 1

- To collate existing information as well as develop new data to provide convincing evidence to support comparable biotransformation of xenobiotics in *H.azteca* and fish
- To assess the use of elimination rate constants as an alternative parameter for bioaccumulation in aquatic and terrestrial food in a peer-reviewed article
- To prepare a detailed test protocol for use of *H.azteca* in aquatic BCF studies for the ring-test.
- To have regular dialogue with regulatory agencies, such as UBA and ECHA, over the acceptability of *H.azteca* as an alternative, non-vertebrate test species for bioaccumulation assessment

#### Phase 2

- To organise the ring-test comparison and report the findings with the intention of progressing the BCF test with the freshwater amphipod *H.azteca* as the basis for a new OECD Test Guideline.

### **Deliverables**

Validated test protocol for BCF tests with the freshwater amphipod *Hyaella azteca* providing the basis for a new OECD Test Guideline.

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 several peer reviewed publications, following poster(s) and presentation(s) at suitable scientific conference(s).

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### ***Cost and Timing***

Start in early 2017, duration 2 years.

Budget in the order of € 300,000. For phase 1, € 200,000 is allocated and a further € 100,000 would be required for the organisation of the ring test.

### ***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 how their proposal is aligned with LRI objectives. Furthermore, an indication on how the results could influence regulatory and policy areas should be provided.

Dissemination plans should also be laid down.

### ***Cited references***

- 1 OECD. 2012. OECD Guidelines for the Testing of Chemicals / Section 3: Degradation and Accumulation. Test No. 305: Draft TG 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure.
- 2 Schlechtriem, C., Kampe, S., Bruckert, H.J., Schaefers, C., L'Haridon, J., Bioconcentration tests with fish and the freshwater amphipod *Hyalella azteca*. Are the results comparable? SETAC Europe 25th Annual Meeting, 3-7 May 2015, Barcelona, Catalonia, Spain.
- 3 Seibel B.A. 2007. On the depth and scale of metabolic rate variation: scaling of oxygen consumption rates and enzymatic activity in the class Cephalopoda (Molluscs). J. Expt. Biology, 210: 1-11.
- 4 Seibel B.A., Drazen J.C. 2007. The rate of metabolism in marine animals - environmental constraints, ecological demands and energetic opportunities. Phil. Trans. R. Soc. B., 362: 2061-2078.
- 5 Ikenaka Y., Eun, H., Ishizaka, M., Miyabara Y. 2006. Metabolism of pyrene by aquatic crustacean, *Daphnia magna*. Aquatic Toxicology, 80: 158-165.
- 6 Goss K-U., Brown T.N., Endo S. 2013. Elimination half-life as a metric for the bioaccumulation potential of chemicals in aquatic and terrestrial food chains. Environ. Toxicol. Chem., 32:1663-1671.

**DEADLINE FOR SUBMISSIONS: 31 August 2016**

Please see [www.cefic-lri.org](http://www.cefic-lri.org) for general LRI objectives information, project proposal form and further guidance for grant applications.