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

Title and Code Number:
Development and validation of an abbreviated *in vivo* fish bioconcentration test – ECO14

Background
Bioaccumulation potential is a key parameter used in regulatory prioritization and chemicals management, e. g. in REACH. Currently, the OECD 305 fish bioconcentration test serves as the gold standard for substance-specific bioaccumulation assessment. However, this test is costly, involves maintaining relatively constant aqueous concentrations over long exposure durations and requires tissue measurements during both uptake and depuration phases which consume a large number of test organisms. For substances which have sufficient aqueous solubility, a simpler screening test would address a practical need for obtaining reliable information necessary to support decision-making in a less resource intensive manner.

References
Bopp, S.K., N. C. Bols, K. Schirmer (2006). DEVELOPMENT OF A SOLVENT-FREE, SOLID-PHASE IN VITRO BIOASSAY USING VERTEBRATE CELLS Environmental Toxicology and Chemistry 25:1390–1398
Gerofke,A. P. Kömp, M. S. McLachlan (2004). Stir bar contamination: a method to establish and maintain constant water concentrations of poorly water-soluble chemicals in bioconcentration experiments Water Research, 38: 3411-3419
Kiparissis, Y.; Akhtar, P.; Hodson, P. V, Brown, R. S. (2003). Partition-Controlled Delivery of Toxicants: A Novel In Vivo Approach for Embryo Toxicity Testing
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Mayer, P.; Wernsing, J.; Tolls, J.; de Maagd, P. G.-J.; Sijm, D. T. H. M. (1999). Establishing and Controlling Dissolved Concentrations of Hydrophobic Organics by Partitioning from a Solid Phase, Environ. Sci. Technol. 33:2284-2290
Parkerton, T., J. Arnot, K. Woodburn, A. Weisbrod, C. Russom, R. Hoke, T. Traas, M. Bonnell, L. Burkhard, M. Lampi (2008) Guidance for Evaluating in-vivo Fish Bioaccumulation Data; Integrated Environmental Assessment & Management, 4:139-155.

Objectives
The objective of this proposal is to develop a shorter, more cost-effective bioconcentration test that uses much fewer fish. The development of such a test was recently concluded as a promising area for research based on the findings of an ILSE/HESI bioaccumulation workshop (Parkerton et al. 2008).

Scope
In support of this RfP, a training set of potential test compounds has been selected that investigators should consider in responding to this request for proposal (Table 1). These compounds were chosen to span a wide range of structural classes and physical properties (e. g. octanol-water partition coefficients) and susceptibility to bio-transformation based on reliable aqueous BCF data that are available in the literature for either trout or carp (or both species). The bioaccumulative compounds chosen have been identified as bioaccumulative by the EU PBT Group. Most of the remaining substances in Table 1 are also being investigated in a separate research project that is

focused on in-vitro methods for estimating fish biotransformation. Therefore, data from this project will complement efforts to validate in-vitro techniques that are intended to further limit animal use.
The results of this research project should deliver BCF estimates using substance-specific analysis of parent compounds. It is recommended that either trout or carp be used as the fish test species.
Additional elements that should be considered in responding to this proposal are:

- Advances in passive dosing techniques that can be used to improve delivery and maintenance of aqueous exposure concentrations under static test conditions (e.g. Mayer et al. 1999; Kiparissis et al. 2003; Gerofke et al 2004; Bopp et al. 2006)
- Alternative experimental designs (e. g. short uptake phase and subsequent depuration phases in which fish tissue concentrations are measured at fewer time intervals, testing of multiple compounds simultaneously) that still allow steady-state BCFs to be reliably estimated.
- Experimental factors relevant to the test procedure (e.g. feeding) and endpoint interpretation (lipid content of fish, TOC concentration of exposure media) should be specified or determined

Short interim reports on progress are required at 3 to 6-monthly intervals. It is expected that the findings will be developed into a peer reviewed publication, following presentation at a suitable scientific conference.

Table 1 .Training Set of Test Compounds

Category	Chemical	CAS No.	Log Kow	Biotrans-formation	ECB PBT list	HESI testing list
Fragrance	2,4,6-trinitro-5-tert-butyl-1,3-xylene	81-15-2	4.5	Low	x	
Aromatic Ester	fluroxypyr 1-methylheptyl ester	81406373	4.5	High		x
Organophosphate	chlorpyrifos	2921882	5.0	Low		x
Aliphatic Alcohol	n-dodecanol	112538	5.1	High		x
Chlorinated benzene	hexachlorobenzene	118741	5.7	Low	x	
Alkyl phenol	4-nonylphenol	104405	5.8	High		x
Alkyl naphthalene	diisopropyl naphthalene	38640629	6.1	Moderate		x
Olefin	1-tridecene	2437-56-1	6.6	High		
Aliphatic Amine	n-hexadecylamine	143271	6.7	High		x
Organochlorine	p-DDT	50-29-3	6.9	Low	x	

Cost and Timing

Budget in the order of €150,000
Start end of 2008, duration up to 24 months