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

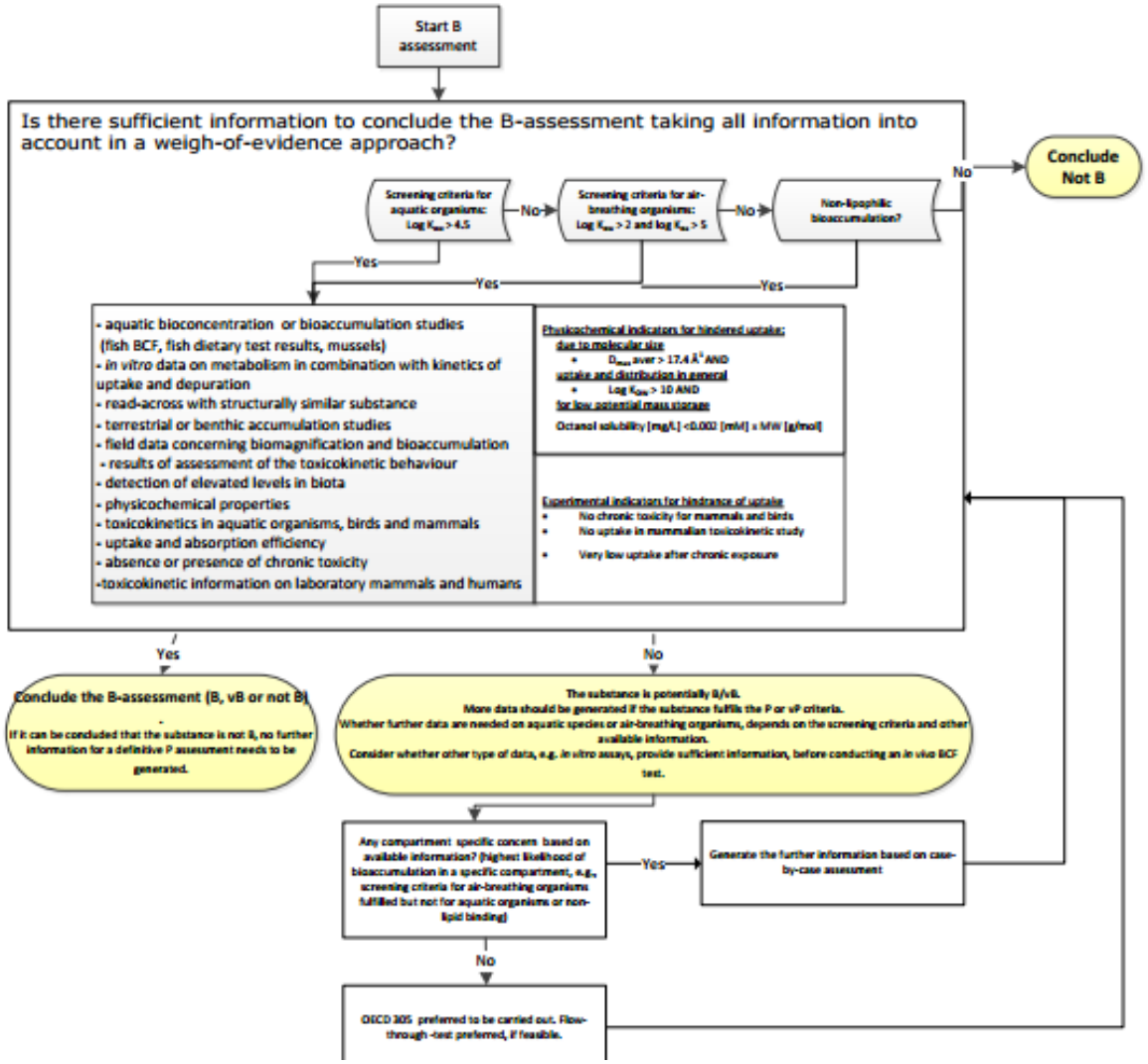
Title and Code Number:

Enhanced Screening Methods to Determine Bioaccumulation Potential of Chemicals in Air-Breathing Species – **LRI ECO41**

Background

The globally accepted model for bioaccumulation determination currently is fish (*c.f.* OECD 305). The basis of bioaccumulation potential (B) screening and assessment, and subsequent testing requests, is driven by the Octanol-Water Partition Coefficient (K_{OW}).

In the recent revamping of REACh Guidance Chapter R11: PBT / vPvB Assessment (latest version at time of writing RfP is Draft to CARACAL on 28/03/2017), ECHA has included B / vB screening threshold criteria for air-breathing organisms linked to logK_{OW} and logK_{OA} (K_{OW} >2 in conjunction with K_{OA} >5):



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

Chapter R.11 further states: An efficiently absorbed, non-biotransformed neutral organic substance with a log K_{OA} ≥ 5 in combination with a log K_{OW} ≥ 2 has the potential to biomagnify in terrestrial food chains and air-breathing marine wildlife as well as in humans, while the substances with log K_{OW} < 2 are being quickly eliminated by the urinary excretion, and therefore do not biomagnify even though their K_{OA} is high.

At the same time, it is acknowledged that considerations of absorption efficiency and biotransformation rates[1, 2] are also necessary for bioaccumulation assessment.

This additional evaluation approach to include K_{OA} as an evaluation screen, for potential bioaccumulation in air-breathing (terrestrial) organisms, may lead to unnecessary regulation and testing of substances without sufficient scientific justification. At present, there is no tiered testing approach to facilitate the testing and regulation of chemicals with regards to potential bioaccumulation in air-breathing organisms.

In addition, although standardized test guidelines exist to determine the Octanol-Water Partition Coefficient (K_{OW} – OECD 107, 117, 123), no such disposition exists for the experimental determination of the K_{OA}, the establishment of which is, currently, a function of the Octanol-Water Partition Coefficient and Henry's Law Constant. There is also a need to critically examine the applicability of the K_{OA}/K_{OW} screening criteria set out in the draft update of R.11.

To start to address the lack of a suitable K_{OA} test method and clear subsequent testing strategy for air-breathing organisms, there is a need to consider the following:

1. Review the accuracy of the theoretical calculation approach for K_{OA}, and develop a standard and practical Phys-Chem screening test to establish K_{OA}.
2. Develop a Tier 1.5* *in vitro* screening approach to determine metabolic turnover kinetics and adsorption efficiency for air-breathing organisms.

To date, the development and validation of *in vitro* testing and implementation of recognized test guidelines has been restricted to the field of mammalian toxicity. Whilst mammalian metabolism rate and toxicokinetic data is relevant in the bioaccumulation assessment of air-breathing organisms, it is necessary to also develop *in vitro* screening approaches adapted to the environmental / ecotoxicological-relevant domain.

Fish liver hepatocytes and S9 cells have been successfully employed to demonstrate metabolic turnover of chemicals under *in vitro* test conditions and have been used to construct IVIVE (*In vitro* to *In vivo* Extrapolation) models [3, 4, 5, 6, 7]. Draft OECD test guidelines are currently under development for this approach, which, in time, will serve as a Tier 1.5 evaluation step, in respect of the 3Rs (Reduction, Refinement, Replacement) criteria, before an eventual need to move to a full *in vivo* study, and remaining within the realms of additional 3Rs (reproducibility / reliability, ecological relevance and regulatory acceptance).

The Octanol-Water partition coefficient (K_{OW}) is not a robust indicator for the prediction of a chemical's potential to bioaccumulate, where metabolism and elimination are the main driving factors. In addition, current *in silico* models inadequately simulate metabolism, and, thus, rely heavily on K_{OW} to generate their output.

It is envisaged that a similar approach to the fish liver hepatocyte / S9 cells could also be considered to develop knowledge on the metabolic turnover and adsorption efficiency of ingested chemicals in air-breathing species.

Objectives and Scope

This CEFIC LRi project intends to address the lack of a tiered testing approach to facilitate the testing and regulation of chemicals with regards to potential bioaccumulation in air-breathing organisms. This will be done by proposing a standard and practical Phys-Chem screening test to establish K_{OA}, and the development and validation of a Tier 1.5* *in vitro* screening approach.

Cefic LRI project monitors and the research team for this project will discuss the range of chemistries to be covered at the project start, and appropriate substances will be defined based on appropriate property threshold criteria.

The objectives of the project are:

(i) Review the accuracy of establishing K_{OA} for diverse chemistries via the theoretical calculation approach [8], including consideration of applicability domain (this will be partly via literature review, but experimental data from (ii) will also be relevant);

(ii) Propose and evaluate a standard and practical Phys-Chem screening test to establish K_{OA} – via a) review of existing state-of-the-science with respect to K_{OA}; b) compare the validity and representativeness of the generator-column method [9, 10, 11, 12], the GC multi-column retention time approach[13,14], headspace sampling techniques[15], and, any other practical approaches for experimentally measuring K_{OA} which might be applicable and could be taken forward for proposal as an OECD Series 100 Draft test guideline;

(iii) Perform literature search to review and compile existing data on bioaccumulation and toxicokinetics in air-breathing species. This will advise on the relevance of the K_{OA} and *in vitro* metabolism data for a tiered testing approach for B assessment. It may also inform as to which substances to test in (iv);

(iv) Develop and validate a Tier 1.5* *in vitro* screening approach, inspired from fish *in vitro* liver hepatocyte and S9 metabolism / bioaccumulation, and corresponding IVIVE models [3, 4, 5, 6, 7], relevant to air-breathing species. These will consider exposure and metabolism, intrinsic clearance rates and elimination pathways in liver hepatocytes and/or S9 cells of air-breathing species [16].

(v) Identify and quantify the main factors influencing and affecting the proposed experimental set-up for the Tier 1.5* *in vitro* screening, and standardize procedures to permit high level of reproducibility (standardization of hepatocyte, S9 isolation, storage, activity; optimization of exposure concentration; effect of addition of co-factors; analytics; etc.).

Deliverables

1. Review of accuracy of establishing K_{OA} for diverse chemistries via the theoretical calculation approach, including consideration of applicability domain.
2. Proposal for a standard and practical Phys-Chem screening test to establish K_{OA}. [Ultimately, this would facilitate the set-up of an inter-laboratory study as the first step in the preparation of a draft OECD Series 100 test guideline to experimentally determine K_{OA}.]

3. A detailed and validated protocol describing the set-up and execution of a Tier 1.5 *in vitro* screening method to determine metabolic turnover kinetics and adsorption efficiency in a relevant model for an/various air-breathing species, which can be adopted for further development in an inter-laboratory study, and development of an applicable IVIVE model.

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).

Cost and Timing

Start in January 2018, duration 36 months
Budget in the order of €480K

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.

It is recognized that the scope of this RfP spans a range of expertises (physical chemistry, *in-vitro*/metabolic biology), and thus proposals are anticipated from teams comprising a collaboration from diverse disciplines.

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 should also be laid down.

References

[1] Armitage, J.M. and Gobas, F.A.P.C. A terrestrial food-chain bioaccumulation model for POPs. *Environmental Science & Technology*, 2007, 41, 4019-4025.
[2] Goss, K.-U., Brown, T.N. and Endo, S. Elimination half-life as a metric for the bioaccumulation potential of chemicals in aquatic and terrestrial food chains. *Environmental Toxicology and Chemistry*, 2013, 32, 1663-1671.
[3] Nichols, J.W., *et al*.. Towards improved models for predicting bioconcentration of well-metabolized compounds by rainbow trout using measured rates of *in vitro* intrinsic clearance*. Environ. Toxicol. Chem*., 2013, 32(7), pg. 1611-1622;
[4] Weisbrod, A.V., *et al*.. The state of *in vitro* science for use in bioaccumulation assessments for fish*. Environ. Toxicol. Chem*., 2009. 28(1): p. 86-96
[5] Cowan-Ellsberry, C.E., *et al*.. Approach for extrapolating *in vitro* metabolism data to refine bioconcentration factor estimates*. Chemosphere*, 2008, 70(10), pg. 1804-1817;

[6] Nichols, J.W., *et al*.. *In vitro-in vivo* extrapolation of quantitative hepatic biotransformation data for fish. I. A review of methods, and strategies for incorporating intrinsic clearance estimates into chemical kinetic models. *Aquat Toxicol*., 2006, 78(1), pg. 74-90;
[7] Laue, H., *et al*.. Predicting the Bioconcentration of Fragrance Ingredients by Rainbow Trout Using Measured Rates of *in Vitro* Intrinsic Clearance. *Environ. Sci. Technol*., 2014, 48(16), pg. 9486-9495;
[8] Meylan, W. M., Howard, P. M.. Estimating octanol-air partition coefficients with octanol-water coefficients and Henry's law constants. *Chemosphere*, 2005, 61, pg. 640-644;
[9] Harner, T., Mackay, D.. Measurement of octanol-air partition coefficients for chrlorobenzenes, PCBs, and DDT. *Environ. Sci. Technol*.,1995, 29, pg. 1599 –1606;
[10] Harner, T., Bidleman, T. F.. Measurement of octanol-air partition coefficients for polychlorinated biphenyls. *J. Chem. Eng. Data*, 1996, 41, pg. 895-899;
[11] Harner, T., Bidleman, T. F.. Measurement of octanol-air partition coefficients for polycyclic aromatic hydrocarbons and polychlorinated naphthalenes. *J. Chem. Eng. Data*, 1998, 43, pg. 40-46;
[12] Harner, T., *et al*.. Measurements of octanol-air partition coefficients for PCD/Fs : A tool in assessing air-soil equilibrium status. *Environ. Sci. Technol*., 2000, 34, Pg. 3109-3114;
[13] Zhang, X., *et al*.. A method to estimate the octanol-air partition coefficient of semivolatile organic compounds. *Anal. Chem*., 1999, 71, pg. 3834-3838;
[14] Su, Y., *et al*.. Determination of octanol-air partition coefficient (K_{OA}) values for chlorobenzenes and polychlorinated naphthalenes from gas chromatographic retention times. *J. Chem. Eng. Data*, 2002, 47, pg. 449-455;
[15] Abraham, M. H., *et al*.. The solubility of gases and vapours in dry octan-1-ol at 298 K. *Chemosphere*, 2001, 44, pg. 855-863.
[16] Watanabe, K. P. *et al*.. Cytochrome P450-mediated warfarin metabolic ability is not a critical determinant of warfarin sensitivity in avian species : *In vitro* assays in several birds and *In vivo* assays in chicken. *Environ. Toxicol. Chem*., 2015, 34(10), pg. 2328-2334.

DEADLINE FOR SUBMISSIONS: 31 August 2017
Please see www.cefic-lri.org for general LRI objectives information, project proposal form and further guidance for grant applications.