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

Title and Code Number:
Improving IVIVE extrapolation models to predict bioconcentration using *in vitro* biotransformation rates for bioaccumulation assessment in fish **– LRI-ECO47**

Background

Biotransformation represents the largest source of uncertainty in *in silico* bioaccumulation predictions as part of PBT assessment for chemicals in the absence of an *in vivo* bioaccumulation study (commonly OECD TG 305) in fish. To close this gap, *in vitro* systems measuring biotransformation rates of chemicals to refine BCF model estimates in fish have been established. The reliability and reproducibility of *in vitro* substrate depletion assays using rainbow trout hepatocytes or liver subcellular fractions have been demonstrated in a recently completed multi-laboratory ring trial. Two OECD draft test guidelines and a guidance document have recently been approved [1-3].
It has been shown that incorporation of biotransformation rates determined *in vitro* substantially improves model performance. However, there remains a general trend towards underprediction of apparent *in vivo* biotransformation rates resulting in overprediction of bioaccumulation potential. Overprediction is particularly observed when hepatic clearance is assumed to be controlled by the unbound (free) chemical concentration *in vitro* and *in vivo*.
In vitro biotransformation rates are used to calculate a whole-body metabolism rate (k_{MET}) which is an important parameter in the *in vitro-in vivo* extrapolation (IVIVE) model to predict BCFs. The model includes a term (fraction unbound, f_U) that corrects for potential binding effects on clearance. F_U is calculated (f_U calc) as the ratio of free chemical fraction in blood plasma and the *in vitro* S9 (or hepatocyte) system [4]. This correction assumes that biotransformation enzymes operate against the free or unbound chemical fraction *in vivo* (in plasma) and *in vitro*, with faster metabolic rates *in vitro* vs. *in vivo* due to lower protein concentration in the test system compared to *in vivo* plasma protein concentrations. IVIVE results are often improved for pharmaceutical compounds in mammalian *in vitro* systems when f_U is calculated. However, it has been shown by several authors that f_U calc results frequently in an overprediction of the BCF in fish. For some hydrophobic chemicals setting f_U equal to 1.0 resulted in much better predictions [5-8]. Setting f_U to 1.0 assumes that chemical availability to metabolic enzymes *in vitro* and *in vivo* is the same, either because binding does not limit kinetic turnover under the dynamic flow-through situation of the liver, or, because the fraction available in both systems is identical. Most mechanistic work in this field had been done on polyaromatic hydrocarbons (PAHs), and generalization to industrially relevant chemicals is lacking.
The difference in predicted BCFs using f_U calc and setting $f_U = 1.0$ is in particular important for B assessment for values close to the regulatory cut-off criterion for bioaccumulation (i.e. >2000 L/kg for EU REACH; >1000 L/kg for US EPA). For slowly to moderately biotransformed chemicals which also have a high log K_{ow}, predicted BCFs are frequently <2000 L/kg if f_U is set to 1.0 and substantially greater than this B cut-off value if f_U calculated is used, raising the critical question which values are relevant for final bioaccumulation assessment.

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Two draft OECD test guidelines on determination of fish *in vitro* hepatic clearance using hepatocytes and liver S9 sub-cellular fractions from rainbow trout and an accompanying guidance document were approved by the OECD WNT in April 2018. Regulatory acceptance will be needed to support a broad application of IVIVE models for bioaccumulation assessment. Thus, relevance of these models has to be established, too. Further understanding of the impact of the fraction unbound and particularly improvement of modelling for the extrapolation from the *in vitro* metabolic rate to the whole-body metabolism rate (k_{MET}) is therefore important to evaluate *in vitro* biotransformation measurements and BCF predictions based on IVIVE. This is considered as a key issue for regulatory acceptance of *in vitro* data. Only with broad regulatory acceptance of the IVIVE predictions, unnecessary higher-tier testing can be avoided to fulfil the 3 Rs approach to significantly reduce the use of vertebrate testing.

The goal of this CEFIC LRI project is to improve the IVIVE approach for fish by addressing the major uncertainties of the predictions. If improvements of the modelling of e.g. protein binding as one of the major uncertainties does not resolve the discrepancy between predicted and *in vivo* measured BCFs, a fundamentally different, refined IVIVE model is needed. This project will provide complementary results with the following CEFIC LRI projects: ECO 34, ECO 37, ECO 41, ECO 44 and ARC.3 which are briefly discussed below.

The objective of ECO 34 ("A tiered testing strategy for rapid estimation of bioaccumulation by a combined modelling - *in vitro* testing approach.") is to reduce the uncertainty related to the estimation of bioaccumulation of organic chemicals in fish. The project focuses on different *in vitro* approaches to estimate chemical uptake and biotransformation (liver, gill and intestine) with toxicokinetic and QSAR models. So, there are overlapping activities regarding the modelling part with this project. However, the ultimate goal of this current project is to improve IVIVE by addressing major uncertainties like protein binding and extrapolation from *in vitro* metabolic rates to predicted whole-body metabolism rates (k_{MET}) which will be complementary to ECO 34 and will result in a further improvement of the models for bioaccumulation assessment.

ECO 37 ("D-BASS: Developing a Bioaccumulation Assessment Strategy for Surfactants") aims to validate the combined use of partition coefficients, *in vitro* intrinsic hepatic clearance and IVIVE for ionogenic compounds (mainly surfactants), whereas this current project evaluates neutral hydrophobic chemicals from industrially relevant chemicals. Due to the chemical properties that distinguish ionogenic chemicals from neutral chemicals, specific considerations have to be applied for IVIVE extrapolation in ECO 37.

The project ECO 41 ("Enhanced screening methods to determine bioaccumulation potential of chemicals in air-breathing species.") aims to develop an approach for assessing bioaccumulation of neutral hydrophobic organic chemicals in air-breathing species. Whereas the methodology (i.e. *in vitro* biotransformation assays, IVIVE) is similar, ECO 41 targets metabolism of air-breathing vertebrates using rat as model species, which

requires IVIVE models which are adopted for air-breathing species with e.g. different routes of uptake and excretion compared to fish.

In contrast to this current project, ECO 44 ("Integrating Bioaccumulation Assessment Tools for Mammals (iBAT-Mam)") focus like ECO 41 on bioaccumulation assessment in mammals. ECO 44 aims to develop a toxicokinetic modelling framework to assess the bioaccumulation behaviour of chemicals in mammals.

The general objective of ARC.3 ("Development of the bioaccumulation assessment tool (BAT VER.1.0) to aid in the bioaccumulation assessment of organic chemicals") is to develop a tool for integrating various lines of evidence in a quantitative weight of evidence approach to aid regulatory decision-making for bioaccumulation assessment. Improvement of IVIVE for neutral hydrophobic organic chemicals as outlined in this current project may be possibly integrated in this approach to improve bioaccumulation assessment in fish for regulatory applications.

Objectives and Scope

This CEFIC LRI project intends to refine the current IVIVE models as an important step towards the regulatory acceptance of the IVIVE predictions using *in vitro* data for bioaccumulation assessment. This will be done by a thorough investigation of the major uncertainties of IVIVE models, like the fraction unbound and other uncertainties with respect to physiological parameters.

Importantly, the project aims to investigate chemicals of different industrially relevant classes. 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. High quality empirical BCF data should be available for the test chemicals for comparison.

The objectives of the project are:

1. Review the accuracy and applicability of existing methods to determine the fraction unbound for chemicals in order to choose a method which is suitable for a broad range of industrially relevant classes with different chemical properties, e.g. PDMS depletion method [6], thin-film solvent dosing [9], vial equilibration method [10-12].
2. Determine the fraction unbound for chemicals of different industrially relevant classes and a broad range of log K_{ow} values (i.e. >~4.5 and <8) including particularly higher log K_{ow} substances (i.e. >~6) in fish plasma vs. liver S9 fractions or hepatocytes to refine binding assumptions. As current method to determine the fraction unbound for chemicals with different properties, the vial equilibration method seems to be the most appropriate for chemicals with different properties [10-12]. However, this has to be verified according to objective 1.
3. Perform experimental studies to evaluate the accessibility of the bound chemical fraction for biotransformation enzymes using industrially relevant chemicals (e.g. in cellular systems [13] and ideally in isolated perfused liver [14,15]).

4. Understand to which extent kinetics of mass transfer limits substrate availability of the bound fraction under the dynamic flow-through conditions of the liver.
5. Improve current IVIVE models [4] with a focus on refined f_U calculations based on the experimentally determined f_U values (results from objective 2) to predict the bioaccumulation potential for industrially relevant, hydrophobic chemicals. Alternative IVIVE models may be explored (optional) which use other types of corrections for the impact of protein binding.
6. Address other uncertainties in current IVIVE models [4] with respect to physiological parameters like the apparent volume of distribution (VD) to further improve the prediction models.
7. An improved estimate of f_U may not resolve the discrepancy between BCF estimated by IVIVE and *in vivo* values as indicated in liver perfusion assays [15]. Thus, in this case, a fundamentally different, refined model, which takes e.g. into consideration the dynamic flow-through conditions of the liver, is needed to derive a whole-body metabolism rate (k_{MET}) from *in vitro* metabolism rates.
8. Apply the refined IVIVE model for comparison of predicted and measured BCFs for industrially relevant chemicals. This will involve a comparison of predicted BCFs based on *in vitro* biotransformation rates using the improved IVIVE model with empirical BCFs (OECD 305 studies, good quality data, same fish species, i.e. rainbow trout; in ideal case even matched species using the same strain).
9. Validate as alternative approach the refined IVIVE model by a comparison of modelled *in vivo* biotransformation rates based on *in vitro* data vs. *in vivo* biotransformation rates determined in fish. This approach allows a direct evaluation of the IVIVE without the potential influence of factors associated with the uptake and biotransformation independent depuration of the chemical, e.g. gill uptake rate constant, gill elimination rate constant.

Deliverables

1. Understand whether a mechanistically relevant and quantitatively correct f_U factor can improve current IVIVE models.
2. Test underlying assumptions of current IVIVE models for deriving whole body k_{MET} from *in vitro* metabolism data and apply findings to industrially relevant chemicals (beyond PAHs).
3. Test impact of IVIVE model refinement on predictivity for different chemicals and for regulatory purposes.

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 and platform presentations at suitable scientific conferences.
At least one article related to the research project shall be published in the open access literature.

Cost and Timing
Start in Q1 2019
Duration: 2 years
Budget in the order of €300.000

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.
It is recognised that the scope of this RfP spans a range of expertise (physical chemistry, biochemistry (*in vitro* assays), modeling) 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. OECD. Determination of in vitro intrinsic clearance using cryopreserved rainbow trout hepatocytes (RT-HEP). *OECD testing guidelines* 2017; **Draft TG**.
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3. OECD. Draft Guidance Document on the Determination of in vitro intrinsic clearance using RT-HEP or RT-S9 and extrapolation to in vivo intrinsic clearance. 2017.
4. Nichols JW, Huggett DB, Arnot JA, Fitzsimmons PN, Cowan-Ellsberry CE. Towards improved models for predicting bioconcentration of well-metabolized compounds by rainbow trout using measured rates of *in vitro* intrinsic clearance. *Environmental Toxicology and Chemistry* 2013; **32**(7): 1611-22.
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7. Laue H, Gfeller H, Jenner KJ, Nichols JW, Kern S, Natsch A. Predicting the bioconcentration of fragrance ingredients by rainbow trout using measured rates of in vitro intrinsic clearance. *Environ Sci Technol* 2014; **48**(16): 9486-95.
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9. Lo JC, Allard GN, Otton SV, Campbell DA, Gobas FA. Concentration dependence of biotransformation in fish liver S9: Optimizing substrate concentrations to estimate hepatic clearance for bioaccumulation assessment. *Environmental Toxicology and Chemistry* 2015; **34**(12): 2782-90.
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11. Gouliarmou V, Smith KE, de Jonge LW, Mayer P. Measuring binding and speciation of hydrophobic organic chemicals at controlled freely dissolved concentrations and without phase separation. *Anal Chem* 2012; **84**(3): 1601-8.
12. Nichols JW, Ladd MA, Fitzsimmons PN. Measurement of kinetic parameters for biotransformation of polycyclic aromatic hydrocarbons by trout liver S9 fractions: implications for bioaccumulation assessment. *Applied In Vitro Toxicology* 2017; **Online Ahead of Print: May 22, 2017**
13. Han X, Mingoia RT, Nabb DL, Yang CH, Snajdr SI, Hoke RA. Xenobiotic intrinsic clearance in freshly isolated hepatocytes from rainbow trout (*Oncorhynchus mykiss*): determination of trout hepatocellularity, optimization of cell concentrations and comparison of serum and serum-free incubations. *Aquat Toxicol* 2008; **89**(1): 11-7.
14. Nichols JW, Hoffman AD, Fitzsimmons PN. Optimization of an isolated perfused rainbow trout liver model: Clearance studies with 7-ethoxycoumarin. *Aquat Toxicol* 2009; **95**(3): 182-94.
15. Nichols JW, Hoffman AD, ter Laak TL, Fitzsimmons PN. Hepatic clearance of six polycyclic aromatic hydrocarbons by isolated perfused trout livers: Prediction from *in vitro* clearance by liver S9 fractions. *Toxicol Sci* 2013; **136**(2): 359-72.

DEADLINE FOR SUBMISSIONS: 2 September 2018

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