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

Code Number and Title:

LRI-ECO36: **Building improved in-vitro exposure assessment capability**

Background

Recent advances in high throughput in vitro assessments provide opportunities for improved mechanistic understanding of toxicity, and which will enable the efficient implementation of a framework for toxicity testing in the 21st century[1, 2]. Additionally, data obtained from a suite of in vitro bioassays, such as Toxcast, provides a relatively rapid viable animal alternative tool that enables hazard-based screening and prioritization assessments of thousands of chemicals[2]. In order to expand the utility of in vitro bioassay data as a risk assessment tool for human and environmental systems, however, requires quantification of exposure[3-6]. For instance, it is widely understood that in toxicological studies the freely dissolved concentration is considered a more relevant parameter, concerning toxic effects, than the nominal concentration that is used most frequently[7-10]. Depending on the physicochemical properties of a chemical, reliance on the nominal concentration used in producing dose-response relationships obtained from in vitro data may not always represent the freely dissolved concentration in the test system [3-6, 10]. Specifically, losses due to volatilization and/or sorption to the vessel walls and other media within the assay, as well as metabolic and abiotic transformation of the parent chemical can strongly influence the actual dissolved concentration used to relate observations of an effect. Consequently, in an effort to strengthen the interpretation of toxicity data obtained from in vitro data, quantitative tools aimed at measuring or estimating the freely available chemical in the test system assumed to be causing an effect are needed. It has been suggested that a key challenge in extrapolating between in vitro and in vivo activity may be a result of the narrow chemical space of the training sets, the limits of the assay biology, or other factors [1]. For instance, empirically-based models have been constructed to fit high-throughput in vitro data, but there is poor understanding with respect to how confidently the models can be used to prospectively extrapolate between in vitro and in vivo systems for chemicals outside the domain of applicability [11]. Thus, the primary emphasis of this RfP should be directed towards better characterization of exposure in vitro of both the parent and degradation products for hydrophobic and less stable chemicals (i.e. chemicals with properties outside the current applicability domain of QIVIVE models) in relation to a toxicological response.

It is anticipated that the quantification of exposure within in vitro systems will require the complementary use of modelling and analytical tools, aimed at quantifying more accurate estimates of the concentration of chemicals in the various compartments of an in vitro bioassay. Given the large number of in vitro assays available for testing, of varying composition and volumes, it is suggested that the development of tools be targeted towards considering the influence that changes in the volume fraction of components that comprise an assay have on influencing the distribution of a chemical within the assay. For instance, differences in the free concentration of a chemical between assays containing culture media with serum versus serum-free media; static versus dynamic cultures; suspensions versus adhesion cultures; 2D versus 3D cultures. Given that the emphasis is on characterizing the freely dissolved concentration in the test system, a challenge to consider is the application of tools that do not disturb the equilibrium within the test system, which is believed to become more problematic as the volume of the test system decreases and the hydrophobicity of a chemical increases.

Scope and Objectives

1. Analytical tools capable of accurately quantifying in vitro bioassay dose(exposure)-response relationships for chemicals with a broad range of physicochemical properties
2. Based on the strategic selection of a small number of case studies, provide guidance on how to best assess the behaviour and exposure of non-stable chemicals within in vitro systems and application towards extrapolating between in vitro to in vivo systems.

Deliverables

This project is intended to develop analytical methods capable of quantifying in vitro bioassay exposure concentrations, enabling more robust assessment of dose-response relationships. Additionally, a final report, shall contain an executive summary (2 pages max), a main part (max. 50 pages) and a detailed bibliography, aimed at providing guidance on best practice for extrapolating in vitro to in vivo exposure estimates. 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), and/or a workshop to discuss the findings.

Cost and Timing

Start in early 2017, duration 3 years. Budget in the order of €500.000

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 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.

References

1. Knudsen, T. B.; Keller, D. A.; Sander, M.; Carney, E. W.; Doerrer, N. G.; Eaton, D. L.; Fitzpatrick, S. C.; Hastings, K. L.; Mendrick, D. L.; Tice, R. R.; Watkins, P. B.; Whelan, M., FutureTox II: in vitro data and in silico models for predictive toxicology. *Toxicol Sci* **2015,** *143*, (2), 256-67.
2. Judson, R. S.; Houck, K. A.; Kavlock, R. J.; Knudsen, T. B.; Martin, M. T.; Mortensen, H. M.; Reif, D. M.; Rotroff, D. M.; Shah, I.; Richard, A. M.; Dix, D. J., In vitro screening of environmental chemicals for targeted testing prioritization: the ToxCast project. *Environ Health Perspect* **2010,** *118*, (4), 485-92.
3. Gulden, M.; Schreiner, J.; Seibert, H., In vitro toxicity testing with microplate cell cultures: Impact of cell binding. *Toxicology* **2015,** *332*, 41-51.
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7. Escher, B. I.; Hermens, J. L. M., Modes of Action in Ecotoxicology: Their Role in Body Burdens, Species Sensitivity, QSARs, and Mixture Effects. *Environmental Science & Technology* **2002,** *36*, (20), 4201-4217.
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10. Blaauboer, B., The use of biomarkers of toxicity for integrating in vitro hazard estimates into risk assessment for humans. *Altex* **2012,** *29*, (4), 411-425.
11. Wetmore, B. A., Quantitative in vitro-to-in vivo extrapolation in a high-throughput environment. *Toxicology* **2015,** *332*, 94-101.

DEADLINE FOR SUBMISSIONS: 31 August 2016

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For further assistance do not hesitate to contact the LRI Secretariat by e-mail at lri@cefic.be or by phone on 0032 (0)2 676 7368.