Dosing for Controlled Exposure (DoCE): Dosing strategies for characterising *in vitro* dose-responses with increased relevance for *in vivo* extrapolation

Background

Chemical safety assessments continue to transition from a reliance on observing apical toxicity endpoints (e.g. reproductive effects, cancer, allergy) in animal models towards a system that evaluates perturbations of the human-relevant biological pathways involved in these toxicity health effects (so called 'toxicity pathways' or 'Adverse Outcome Pathways'). These pathway test systems use suites of *in vitro* methods, coupled with computational models to describe the impact of changes in biological processes in response to chemical exposure (Blaauboer BJ, 2015). Despite the promising correlations between *in vitro* and *in vivo* 'dose-response' relationships, it is widely understood that better quantification and control of exposure of test compounds to cells / tissues in *in vitro* models would greatly improve the large inter-assay variation and the occasionally low absolute sensitivity of *in vitro* assays to predict *in vivo* toxicity.

Quantitative extrapolation from *in vitro* to *in vivo* (QIVIVE) is reliant on accurate *in vitro* 'dose-response' relationships, based on actual concentrations in the test system rather than intended (nominal) exposures. Only the freely dissolved, unbound concentration of a chemical (Cfree) is considered available for uptake into organisms, tissues or cells, to cause biological effects (Groothius FA *et al.,* 2015) and it is critical to understand the concentration-response relationships including its time dependency not simply the 'dose-response'. Typically, nominal concentrations are used to define *in vitro* dose-response relationships with concentrations of chemicals in tissues and cells rarely reported. This can lead to large errors with non-specific binding to extracellular matrices (*e.g.* serum proteins, plastic well plates), evaporation and degradation / metabolism of test chemicals significantly altering the actual concentrations in the test system. The physico-chemical properties of chemicals, such as volatility and hydrophobicity, need to be understood to allow appropriate *in vitro* test design and subsequent data interpretation. Mathematical models have been developed to estimate the distribution of neutral organic chemicals in *in vitro* systems and the corresponding chemical activity to facilitate the use of *in vitro* toxicity data for risk assessment (Armitage JM *et al*., 2014). However, these models have a limited applicability domain and specific challenges remain to model and measure hydrophobic, polar and charged chemicals in *in vitro* assays. For example, hydrophobic chemicals are difficult to dose and maintain at a constant exposure in *in vitro* assays due to sorption to vessel walls. The use of a co-solvent to dose hydrophobic chemicals can further lead to unrealistically high concentrations above the solubility limit and may alter the interaction with the biological target (Tanneberger K *et al*., 2010). These challenges are compounded further when trying to assess chemical mixtures (*e.g.* UVCBs - Unknown or Variable composition, Complex reaction products or Biological materials) which contain constituents with a range of physico-chemical properties.

The use of improved dosing systems to compensate for the loss of test chemicals in *in vitro* assay systems (*e.g.* partition-controlled dosing systems) offers a potential solution for better controlling and quantifying exposures *in vitro*. A number of studies have demonstrated that such approaches can maintain constant concentrations of test chemicals in *in vitro* systems and compensate for losses (*e.g.* due to evaporation, sorption) and eliminate the need for co-solvents (Kramer NI *et al*., 2010; Smith KE *et al*., 2010). Such developments could extend our ability to characterise concentration-response

relationships for a wider range of chemical types being developed across the bioscience sector (*e.g.* hydrophobic, volatile and unstable chemicals). A recent study compared the dose-dependent hormonal responses of H295R cells to 4-nonylphenol using two different dosing regimes (solvent versus partition-controlled). Whilst similar dose-dependent responses were observed, the partition-controlled system resulted in an up to 2-fold increase in progestogen and corticosteroid levels at the free concentration (Gilbert D *et al*., 2015). There has been good progress made recently with respect to the development of improved *in vitro* dosing systems, however, their routine use remains limited to low throughput assays due to technical challenges associated with their use in multi-well format (dosing and measurement) and also the wider acceptance of these approaches by the scientific and regulatory communities. This challenge aims to:

(i) Develop a framework to describe when and how to select appropriate dosing technologies to enable improved characterisation of bioavailable exposures *in vitro* for a diverse range of chemical types;

(ii) Develop increased throughput methods for controlled dosing and measurement of chemical concentrations in *in vitro* assays to enable their uptake, application and use in risk-based decision making; and a strategy to maximise the potential for regulatory acceptance of the approaches developed.

3Rs benefits

A number of animal-based studies have been, and continue to be used in certain industrial sectors to inform the safety and efficacy of chemicals in commerce (*e.g.* repeat dose and reproductive toxicity tests). Often such testing is driven by specific regulatory requirements before commercial use. The integration of information from different sources that combine: predictive chemistry assessments, high content human cell/tissue-based *in vitro* assays and mathematical modelling approaches (*e.g.* physiologically-based biokinetic modelling), offer the potential to eventually replace the need for *in vivo* animal studies. Implementation of such strategies for toxicological risk assessment purposes have been outlined in a recent series of National Research Council reports (National Research Council, 2007; National Research Council, 2012; National Academies of Sciences, Engineering and Medicine, 2017). A range of *in vitro* assays are proposed to provide the data to derive both the initial concentration-response relationship and the ADME (absorption, distribution, metabolism and excretion) parameters needed for *in silico* modelling to estimate toxic doses to humans and the environment (Blaauboer BJ *et al*., 2012).

The aim of this Challenge is to develop methods and tools that will better quantify and control the exposure of chemicals, with different physico-chemical properties, over a wide range of *in vitro* assay systems (*e.g.* those differing in terms of treatment duration, volume, serum content etc.). This will enable more robust characterisation of concentration-response relationships and improve the selection of the most appropriate and well designed *in vitro* assays for predicting *in vivo* toxicity. There has been good progress made recently with respect to the development of improved *in vitro* dosing systems, however their routine use remains limited to low throughput assays due to technical challenges associated with their use in multi-well format (dosing and measurement) and also the wider acceptance of these approaches by the scientific and regulatory communities. Developing more robust approaches that can be routinely and easily incorporated into assays would increase their uptake and the number of people / papers reporting relevant exposure data; ultimately leading to regulatory acceptance.

In industry sectors where animal safety studies are still required, the success of using *in vitro* approaches early in development to de-risk candidate molecules (and hence avoid unneccessary animal testing) will be greatly increased if there is better understanding of the *in vitro* concentrations used and how they may relate to *in vivo* exposure. Similarly, a better understanding of *in vitro* dosimetry will be beneficial in other areas when prioritising molecules for animal testing (*e.g.*

pharmacology screens). If successful long term, the Challenge will provide essential tools in the evaluation of safety in the pharmaceutical, chemical, petroleum and consumer product sectors without the use of animals.

Need for collaboration

Improved characterisation of concentration-response relationships from *in vitro* assays will only be achieved through combined expertise in the areas of *in vitro* biology, chemistry and mathematical modelling. The collaboration and sharing of expertise between contract research organisations (CROs), chemical industry, pharmaceutical, petroleum and consumer product sectors with cell culture technology and engineering / automation providers in *in vitro* assays will bring considerable added value to this Challenge.

Overall aim

To establish improved and increased throughput methods and approaches to better account for bioavailability through development of dosing and measurement strategies of test chemicals in *in vitro* assays. Successful completion of this Challenge will deliver new capability (dosing and measurement of chemicals *in vitro*) to ensure concentration-response relationships determined from a range of *in vitro* test systems, that are reflective of human and environmental species *in vivo* exposure conditions, to enable robust QIVIVE. Improving confidence in the relevance of *in vitro*-derived data, through better understanding and control of exposure parameters, will deliver the 3Rs benefits stated earlier.

Key deliverables

- A framework to describe when and how to select appropriate dosing technologies to enable improved characterisation of bioavailable exposures *in vitro* for a broad range of chemicals with diverse physico-chemical properties (*e.g.* including hydrophobic, volatile, and unstable chemicals) and chemical mixtures (*e.g.* UVCBs).

- Development of the necessary technology (both dosing and measurement of chemicals *in vitro*), with emphasis on increased throughput methods, to enable their uptake and use in risk-based decision making. Representative *in vitro* test systems / formats will need to be identified to address specific dosing requirements (*e.g.* taking account of duration of exposure, assay format and media composition).

- The technology described above will be used to provide measured Cfree and cellular concentration data for a range of case study chemicals/chemical mixtures with diverse physico-chemical properties. Consideration will be given to how this data informs the development of *in silico* approaches to aid the prediction of concentration-response relationships.

Phase 1 deliverables

- Framework development: Based on an understanding of physico-chemical properties of chemicals, which influences their bioavailability in *in vitro* test systems, describe a framework strategy to enable the controlled exposure of chemicals for relevant toxicity assay formats *in vitro*. Consideration should be given to the current landscape of on-going research in the area, identifying gaps and key technology development needs towards use in routine assessments.

- Technology development: Provide proof-of-concept data showing improved control of dosing and measurement of Cfree and / or cellular concentration *in vitro* towards the adoption of

increased throughput methods.

- Robust plans to deliver Phase 2 of the Challenge including commercialisation and dissemination.

Phase 2 deliverables

- Framework development: Finalisation of the chemical-specific framework and protocols for dosing and measurement of chemicals *in vitro* based on physico-chemical properties (ideally in collaboration with a CRO to enable commercialisation and uptake of the approach).

- Technology development: Development / application of a range of dosing technologies (*e.g.* direct spiking, repeat dosing, partition-controlled dosing) as required by the proposed framework (see Phase 1 above). Consideration should be given to study design to enable higher throughput control of exposure allowing better understanding of Cfree and cellular concentration over study duration to inform risk-based decision making.

- *In silico* prediction of exposure and concentration-response relationships: Use measured data in a reiterative process to improve current models for predicting *in vitro* bioavailable concentrations.

- A clear dissemination plan to not just inform, but actively engage with relevant industry, academic and regulatory stakeholders on key learnings from the project to maximise the potential for acceptance of the approaches developed.

It is important to note that the CRACK IT Challenges competition is designed to support the development of new 3Rs technologies and approaches, which will improve business processes and / or lead to new marketable products. The application must include a plan to commercialise the results into a product or service. This should be taken into consideration when completing your application.

Sponsor in-kind contributions

The Sponsors will provide:

Phase 1

- Identification of case study chemicals that have challenging physico-chemical property profiles for controlled dosing and testing.

- Scientific advice and modelling experience.

Phase 2

- In house assessment of the approaches developed through this Challenge, as appropriate, to facilitate industry uptake.

- Access to relevant findings from ongoing research programmes focussing on toxicity testing in the 21st century (TT21C) approaches to mechanistic-based risk assessment of human relevant toxicity (www.tt21c.org).

- Provision of risk assessment expertise for a range of relevant chemicals used in personal and home care, and petrochemical contexts, and understanding of their chemistries.

- Provision of expertise / knowledge gained from (i) in-house experimental approaches currently employed for improved characterisation of concentration-response relationships, and (ii) relevant external collaborative activities and initiatives.

Duration

Phase 1: six months, Phase 2: Up to 3 years

Budget

Phase 1: £100K, Phase 2: £1million

Sponsors

Unilever, Shell

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