Challenge 27: DoCE Surgery Q&As

Q. The primary focus of this Challenge is on improving dosing for controlled exposure of chemicals in *in vitro* test systems, but *in vitro* and *in silico*/mathematical modelling goes hand in hand. Can you explain what role you see *in silico*/mathematical modelling playing in this Challenge?

A. The primary focus of the Challenge is on improving dosing and exposure of chemicals in *in vitro* test systems. There are other ongoing initiatives where a greater focus is placed on *in silico*/mathematical modelling approaches e.g. Cefic LRI ECO36 (Paving the way for QIVIVE: from nominal to free to cellular concentrations in *in vitro* assays), and it is important not to duplicate efforts in this area. While *in silico* modelling approaches are a feature of the challenge, this is why they are not a primary focus.

In this Challenge, modelling approaches could be used to:

- Develop the dosing framework e.g. by predicting when a refined dosing method may be required to control the exposure of a given chemical/mixture in an *in vitro* test system.
- Predict what nominal dose will give x free concentration/exposure *in vitro*.
- Understand metabolism once the chemical has been dosed.

Q. Would the Sponsors consider using mathematical modelling to inform the development of the dosing system useful?

A. Yes, the Sponsors would consider using mathematical modelling to inform development of a dosing system useful.

Q. What is meant by 'dosing'?

A. Traditionally, the test substance is dissolved in a solvent (e.g. DMSO) and spiked into the test medium. This comes with a number of limitations such as the test chemical could come out of solution when added to the test medium, the solvent could cause toxic effects and chemicals could be lost through the pipetting process (e.g. by adhering to the plasticware). We would like to look at a range of methods to improve dosing for controlled exposure of chemicals in the *in vitro* test system. The focus is on dosing because if you do not get the dosing right then the results from the *in vitro* model are more difficult to interpret.

Q. Should dosing systems go beyond the state of the art e.g. flow-based systems?

A. All options are open. The focus was placed on well-based technologies as this is a challenge to address, but if there is a technological leap forward in other systems, such as flow-based systems, then this would be seen as useful for addressing the challenge.

Q. Higher throughput systems are mentioned in the Challenge brief. Does this mean being able to do it more quickly, more frequently or being able to integrate measurements?

A. Yes, all of these points are relevant. Currently, approaches to control the exposure of chemicals *in vitro* rely on chemical specific/bespoke method development. Improvements to enable increased throughput (e.g. through improved *in vitro* study design and automation), ultimately aiming to place technologies in the hands of service providers are in scope of this challenge.

Q. How much of an emphasis should be placed on development of a measurement technology?

A. The emphasis of the Challenge is on dosing, but being able to measure at the same time would be useful and should be included. There are automated systems available that can measure chemical concentrations in a 96 well format by taking small volumes of media without depleting the concentration in the system. However, this is not the only technology or approach that is available and all options should be explored.

Q. Is the aim to achieve constant exposure concentrations *in vitro*?

A. Yes, the concentration needs to be constant and known. A dosing technology that gives more constant concentrations throughout the assay duration is required.

Q. What level of throughput is required? Are 24 and 96 well formats the type of throughput being sought?

A. Yes. Current approaches to control the exposure of chemicals *in vitro* rely on chemical specific/bespoke method development and consequently throughput is low. Improvements to this are sought through this challenge e.g. 24 and 96 well formats. Flow-based technologies may also enable improvements in throughput over current methods.

Q. What about chemical metabolism? Is this considered to be a part of the Challenge?

A. The focus of the Challenge is on improving dosing and exposure of chemicals in *in vitro* test systems. Metabolism is not the primary focus of the Challenge, but it could be a component of it. *In silico* modelling approaches could be used to understand metabolism once the chemical has been dosed. There are other programmes looking at the influence of metabolism on the bioavailability of chemicals e.g. CRACK IT Challenge 20 (Metaboderm) has a focus on understanding the influence of metabolism in the skin.

Q. Can the Challenge include 3D tissue models?

A. Yes, 3D tissue models can feature in the solution to this Challenge, but are not the only option for creating relevant tissue models.

Q. Is it the intention that what is developed as part of the Challenge fits into packages such as Gastroplus and Simcyp or is something different being sought?

A. Gastroplus and Simcyp are *in silico* tools, the primary focus of this Challenge is on controlling exposure in *in vitro* test systems.

Q. Should solutions to this Challenge be focussed on particular chemical classes and effects/mechanisms of action?

A. No, the scope of the Challenge is not limited to one class of chemical or specific mechanisms of action/effects. The Challenge is about the dosing procedure, methodology and measurement technique(s) so that a chemical (or a mixture of chemicals) can be delivered at a known concentration. The framework developed through this challenge should inform the selection of appropriate dosing technologies to control exposure of a wide range of chemical classes/mixtures, and be applicable to a broad range of *in vitro* test systems.

Q. Is there a specific set of *in vitro* test systems that you are interested in?

A. There is scope for selection of *in vitro* test systems of interest to the applicant. What is developed in one system should be readily transferable to another system. We are using single cell, multicellular and tissue model-based systems to understand the potential toxicity of chemicals. The challenge regarding our ability to define/measure the bioavailable concentration of chemicals (single or within mixtures) introduced into these systems holds for all *in vitro* model systems.

Q. How will the framework be tested? Are there any benchmarks?

A. The framework would be tested with the proof of concept approaches, drawing on selected case study chemicals with a wide range of physical/chemical properties. The framework developed through this challenge should inform the selection of appropriate dosing technologies to control exposure of a wide range of chemical classes/mixtures, and be applicable to a broad range of *in vitro* test systems. In addition it should help with the adoption of refined dosing technologies by service based providers, and the acceptance of approaches taken by regulators.

Q. How does this Challenge also address the important consideration of relevant bioavailability of chemicals/mixtures after their introduction into an *in vitro* assay system e.g. linking exposure to toxicology, C_{free} and C_{cell} conc, what enters the target, what enters the cell?

A. The Challenge is about improving dosing to better understand the relevant metrics of bioavailability after the chemical/mixture has been dosed *in vitro*. It is about controlling the exposure and understanding the exposure over a relevant period of time in the *in vitro* test system.

Q. In Phase 1, should the focus be on developing a framework with different components or on developing the most promising dosing technology?

A. Development of a dosing framework and technology are key deliverables for Phase 1 and both should be included. The framework should describe chemicals that can be applied and tested using examples to show it will work. The framework will give some frame as to which the technological development will fit.

Q. Quantitative extrapolation of bioavailable exposure (*in vitro* to *in vivo*) is a challenge. For example, there are compensatory mechanisms *in vivo* that are not present i*n vitro* (*e.g.* in the brain there can be a 20 fold difference in concentration from one region to the next due to differences in transporters). Does this Challenge aim to address these factors (transporter function, persistence, bioaccumulation etc.)?

A. We are not specifically aiming to address the influence of transporters, persistence or tissue bioaccumulation through this Challenge. Instead the Challenge is focussed on dosing, and controlling and understanding bioavailable exposure *in vitro*. It is hoped that by better controlling exposure *in vitro* will enable extrapolation to relevant bioavailable exposure values *in vivo* e.g. comparing C_{free} *in vitro* (media) to C_{free} *in vivo* (plasma concentration). We recognise that this will not necessarily allow the effects of transporter function, persistence and bioaccumulation to be considered.