Challenge 5: Improved *in vitro* to *in vivo* extrapolation in chemical safety risk assessment of human systemic toxicity

Surgery Question and Answers

Q: What data will the companies be able to make available as part of their in-kind contributions?
A: Specific data to be made available has not yet been determined. This is likely to depend on individual project needs and applicants should specify what information they believe they would require in order to crack the Challenge.

Q: How does this challenge relate to other activities on Toxicity Testing in the 21st Century (TT21C), or programmes such as Toxcast (www.epa.gov/ncct/toxcast) in the USA?
A: Toxcast is primarily hazard based, focusing on using *in vitro* methods as high throughput screening tools that could be used to prioritise chemicals for *in vivo* safety testing. The aim of the present challenge is to translate the findings from *in vitro* findings to the *in vivo* human situation, in order to make safety assessment decisions without using animals. In particular, the challenge is seeking new, creative approaches with a greater emphasis on measures of exposure and relevant human exposure levels.

A number of research efforts are ongoing to realise the TT21C vision using a variety of approaches, but this is a long-term goal and needs extensive research and investment. This challenge is seeking new ideas on how TT21C can be put into practice and encourages a case study approach to demonstrate applicability.

Q: Would tools used to assess adverse effects caused by physical agents such as ultrasound be of interest?
A: The focus of the challenge is on assessing chemical toxicity, but if methods used to asses physical hazards could be translated to looking at effects of chemicals then this may be of value in coming up with new approaches.

Q: Is the challenge seeking a more critical approach to measuring in vitro exposure concentrations?
A: Yes. It will be important to consider how best to define and measure the concentration of test compound to which cells/tissues are exposed *in vitro* (e.g. free, bound, concentration within cells etc), and how these relate to human exposures. Applicants are also encouraged to consider the most relevant test systems and make use of recent technological developments in areas such as stem cells, tissue engineering and/or organotypic models.

Q: Are sponsors open to applications focusing on new in vitro models without seeking to characterise effects in a particular toxicity pathway(s)?
A: No. The preference would be for applications looking at new models to also look at application to toxicity pathways. If you only have expertise in one field we encourage you to find a partner in a another discipline to strengthen your proposal. Please post a comment on the CRACK IT LinkedIn group at http://linkd.in/p2tcID to try to find a suitable partner.

Q: Are sponsors looking for tests that could be used in a particular stage of chemical development (e.g. for initial screening)?
A: The ultimate goal is to develop strategies that could be used to completely replace the use of animal toxicity data in decision making about human safety, i.e. at any stage of development. It could be that a model could initially be used for screening before being a complete replacement, in which case it would be important for it to produce as few false positives as possible.

Q: Should the model be focused on acute or chronic exposure?
A: The focus should be on systemic toxicity following chronic exposure, i.e. weeks/months up to years of human exposure.

Q: What concentration ranges should be evaluated?
A: The focus should be on concentrations relevant to foreseeable human exposure scenarios, and on what would be of use in identifying safe human exposures. A range of concentrations that could be used to identify transitions between normal functioning, adaptive changes and toxicity is encouraged.

Q: The sponsors come from different sectors, do proposed solutions have to be applicable across all companies?
A: Solutions could cover all sectors or potentially be more specific to certain chemical classes or types of exposure. However, common to all sectors is the need for tools to assess effects of chemicals on biological pathways, and to develop new methods that enable risk in humans to be predicted from non-animal tools.

Q: Should any new approaches developed be validated in animal models?
A: The companies will have access to existing animal toxicity data if needed so it is not anticipated that there should be a need for new *in vivo* testing under this challenge. The aim is to develop new models that will be of most relevance to predicting effects in humans rather than experimental animals. Therefore, applicants are encouraged to consider new ways of validating their proposed approaches and challenging traditional approaches. However, formal validation is not anticipated to be part of this CRACK-IT research.

Q: If an application wanted to explore a recently identified mechanism of toxicity, should animal data be generated for validation?
A: See above. The sponsors encourage applicants to think creatively about how they could validate their proposed method(s), and not necessarily use animal models.

Q: Should test systems include metabolism?
A: Consideration of human metabolism would be critical in any proposed approach. The *in vitro* test system may not necessarily need to include metabolic capability itself, provided other means are used to predict metabolism and this information is taken into account.

Q: Is there a need for cross-validation of any new in vitro models with existing in vitro cell lines?
A: Not necessarily. Consideration should be given to the most appropriate means of demonstrating that the system is fit for purpose.

Q: How many test compounds should be included?
A: This is up to applicants to decide. Applicants may wish to start with a small number of test compounds in early stages and then move on to using larger numbers once an initial proof of principle has been established.

Q: The title of the challenge mentions in vitro to in vivo extrapolation, but could in silico or modelling approaches such as computational chemistry be included in an application?
A: Yes. Such approaches are likely to be valuable, for example in predicting metabolism, modelling toxicity pathways, characterising some of the initial molecular events that occur when a chemical encounters a biological system or in making extrapolations.

Q: Do Sponsors have expectations of the types of cells/tissues/organs that should be focused on? Should the focus be on multiple types?
A: There are no set criteria at this stage; this will be up to applicants to decide. Applications involving organotypic systems are encouraged, but it is recognised that the type of system(s) needed may vary depending on the chosen toxicity pathway.

Q: Will applications relating to immunological effects be accepted?
A: Yes.