CRACK IT

Challenge 36: Animal-free *in vitro**

Q. Is it an absolute requirement to not use human-serum?

A. It is not an absolute requirement (certainly not during Phase 1 of the Challenge), but we see distinct advantages of moving away from the use of human serum. The Challenges aims to address the disadvantages of human serum linked to: limited supply and ethical use (informed consent for commercial use and data protection); donor variability; health-related aspects (tissue screening); and acceptance of use by certain OECD member countries.

During Phase 1 of the Challenge, if no suitable alternative is available, then use of human-derived products will be accepted, but ideally the new protocol will progress towards the development of chemically-defined culture conditions and not contain human-derived products - including human serum. This is particularly relevant for OECD TG455, where treatment of serum with charcoal/dextran is currently necessary to remove endogenous hormones and hormone-binding proteins which, if present, can be a confounding factor in the interpretation of the results.

Q. Is there any flexibility on the cells / cell lines used out-with those described in the brief?

A. Yes, there is flexibility on the choice of cells/cell lines used, but we would ask that the chosen cells/cell lines are of human origin. For comparative purposes, it may be appropriate to select cells or cell lines typically used in TG487 and/or TG455 thereby allowing a performance assessment of the newly established assay with the current assay using the positive and negative controls and performance criteria outlined in the Guideline.

Q. The brief outlines an expectation to have early interactions with regulators/ the OECD. Are the Sponsors able to facilitate this?

A. OECD are very receptive to early discussion with test developers, and the Sponsors and the NC3Rs will be able to help facilitate early interactions with the OECD Test Guideline Programme and with National Coordinators. Scientists from both AstraZeneca and Unilever have supported the OECD in various capacities (e.g. contribution to expert working groups, working with National Coordinators or via BIAC (Business and Industry Advisory Committee to the OECD)) towards the development of new or revisions to existing Testing Guidelines.

Q. Are you expected to work on both TG in Phase 1?

A. You are expected to work on one or preferably both Test Guidelines in this Challenge (through Phase 1 and Phase 2).

Q. Is human S9 acceptable? Finding a defined metabolic system with optimal human relevance is challenging.

A. We would prefer that human S9 is avoided as its use alone is not recommended in *in vitro* genotoxicity screening assays. It is more often recommended for use in follow-up tests for example, where a question on human-relevant or rodent-specific metabolism arises.

Also, human S9 when compared to rodent S9 is more metabolically variable between donors (even when pooled), and often has a lower metabolic capacity as it is not induced like rodent S9, which can lead to false negative results. In addition, human S9 is more cytotoxic in *in vitro* micronucleus assays which may mask genotoxic effects. Human S9 also has similar issues to those seen with human serum including limited supply, biocontainment, and ethical considerations.

The ideal outcome of this Challenge would be a synthetic S9, with defined (and/ or tailored) metabolic enzymatic capability and which enables the detection of different types of metabolically activated genotoxins.

Q. I have expertise in certain areas, but not in all areas that are required to solve the Challenge. How can I find other expertise?

A. Speak to the NC3Rs office (crackitenquiries@nc3rs.org.uk) and we will do our best to help connect you with the expertise you are seeking. You can also make use of the Challenge-specific LinkedIn pages that have been established.

Q. Who should we email with questions?

A. General questions can be sent to the NC3Rs. Questions regarding a specific Challenge can be sent to the Sponsors, but enquiries should be sent to ALL Sponsor parties for a Challenge. If preferred, please email the NC3Rs to introduce you to the Sponsors at crackitenquiries@nc3rs.org.uk

* The Sponsors representatives are: Andrew Scott, Safety & Environmental Assurance Centre, Unilever; and Jo Elloway, Functional and Mechanistic Safety, Clinical Pharmacology & Safety Sciences, BioPharmaceuticals R&D, AstraZeneca, Cambridge, UK