COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Preface

Forward by David Lovell – Chair

I am pleased to present this report on the work of the Committee on Mutagenicity (COM) during 20198. As always, the COM would be happy to receive any feedback from readers of this report.

To be completed

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ONGOING WORK

COM Guidance Series Update

In 2018, a review of the COM guidance on a strategy for genotoxicity testing of chemical substances was initiated. This document was last updated in 2011. As there had been no significant changes to strategy developments or assay methodologies that merited a total re-write of the COM guidance the focus was to review content for accuracy and update references where necessary. MUT/2019/01 and MUT/2019/12 document the amendments and comments from members. Four new stand-alone sections have been drafted which will be published once complete. Methods for the assessment of the genotoxicity of nanomaterial were reviewed including OECD and EU projects. The guidance statement will include an opinion about the use of the Ames test in the testing of manufactured nanoparticles, and the use of cytochalasin B in the micronucleus assay (MUT/2019/02). Members previously considered a scoping paper (MUT/2018/2) on the use of QSARs to predict genotoxicity in February 2018, which formed the basis of the draft Guidance Statement (MUT/2019/03). The members concluded that that QSAR models should not be used to overrule test results but can be used to aid interpretation of test data. A paper on 3D models provided a summary of models currently used for genotoxicity testing and those under development and/or validation(MUT/2019/04). This is an area which is developing rapidly and members were aware of imminent publications thus this statement would be reviewed in the near future. The original guidance document contained discussed germ cell and somatic cell mutagens, a separate guidance statement has been drafted (MUT/2019/05). The aim of producing these separate short guidance statements is to be able to update or edit sections independently as new methods or evidence is published.

ToxTracker

The ToxTracker assay is a stem cell-based genotoxicity screening platform which utilises six unique reporter cell lines[1] to detect potential carcinogenicity and provide information relating to the mode of genotoxic action, if present. The COM first evaluated the technology in 2014. Since that time, ToxTracker has undergone further validation and development and Giel Hendriks from 'toxys', the Dutch Boiotech company that developed the assay, presented an update of recent developments, to the COM in October 2019.

The unique reporter cell lines can detect changes that may indicate potential carcinogenicity, including two types of DNA damage, activation of p53,

[1] Bscl2-GFP (mutagenic DNA lesions); Rtkn-GFP (DNA double strand breaks); Btg2-GFP (activation of p53); Srxn1-GFP (oxidative stress); Blvrb-GFP reactive oxygen species production); Ddit3-GLP (protein damage).

oxidative stress and/or reactive oxygen species (ROS) production, and protein damage. ToxTrackerACE (Aneugen and Clastogen Evaluation) includes the addition of DNA staining in wild type (wt) stem cells to detect aneugenicity leading to cell cycle block and polyploidy. To date, a large number (>1000) and range of substances have been tested using ToxTracker including: single molecules; polymers; complex mixtures; nanomaterials; and intermediates. As such, there is a growing trend to include the assay for early screening and hazard identification purposes, in addition to its use in follow up testing, identifying mode of action (MoA), for quantitative dose response modelling, threshold of toxicological concern (TTC) and for weight of evidence (WoE) considerations.

Technical in-house validation of ToxTracker indicated sensitivity and specificity to both be around 90% and this was supported by the findings of a small inter-laboratory validation exercise where two laboratories screened 28 blinded compounds. A much larger international inter-laboratory validation exercise is currently in progress, coordinated by a Validation Management Team, with the aim of evaluating and the adoption of the assay by The European Centre for the Validation of Alternative Methods (ECVAM) and The Organisation for Economic Co-operation and Development (OECD). This includes eight independent laboratories in the US, EU and Japan analysing 24 blinded compounds, with findings expected to be reported in early 2020.

Following the presentation, clarification was sought by COM around the influence of the dose range chosen for use in ToxTracker and the 'yes'/'no' categorisation of the assay. In this respect, procedures are inherent in the assay through the choice of a maximum dose, and by having defined increases that can signify a' true positive' and 'true negative' result. Members considered how chemicals producing border-line results are interpreted as it was recognised that these would not be straightforward to classify in the regulatory context. Dose-response analysis was explained to be crucial to help categorise such results which is currently dependant on expert judgement. However, a possible future development is a more sophisticated software including principal component analysis which will provide a learning ability to assist the classification of such chemicals. In addition, it was recognised that, as a greater number of compounds are run through the ToxTracker platform, unexpected results will provide learning opportunities regarding the limitations of the platform.

The sensitivity of ToxTracker in terms of being able to detect individual chromosome deletions was also considered. In this regard, if the deletion triggers an effect on cell cycle progression then it will be picked up in the assay. Members discussed the added value of using ToxTracker, particularly when equivocal data has been found using 'standard' *in vitro* testing methods. It was considered that information on the MoA provided by ToxTracker could help explain equivocal findings from other standard assays; including *in vivo* studies. In addition, ToxTracker could be used where *in vivo* follow up studies

are not permitted following a positive Ames test (for example when testing cosmetics).

Increased or more widespread use of ToxTracker was seen as necessary to trigger its inclusion in the standard battery of genotoxicity assays and to gain regulatory acceptance. The ongoing discussions of the development of ToxTacker within the OECD process has been positive to date, and the eventual outcome for these newer developments will decide if an OECD technical guideline is needed for the screening assay. However, although there has been much interest from Industry in using ToxTracker, the longer-term issue remains as to whether compounds can be accepted within a regulatory process if there is no approved OECD technical guideline.

In conclusion, it was agreed that the COM would keep an active watching brief on developments with the ToxTracker platform, particularly with regards to regulatory acceptance of its use for genotoxicity testing.

COM EVALUATIONS

These items will be completed after the minutes are agrred.
Risks to Human Health from the use of Azodicarbonamide as a Food Additive
Review of Genotoxicity of Cannabidiol (CBD)
Review of Genotoxicity of Patulin (PAT)

HORIZON SCANNING

At the February meeting the committee discussed potential items for further discussion under horizon scan.

It was suggested that *in vitro* multi-endpoint test systems were likely to become more important, including high-throughput test systems, imaging systems and 3D cell cultures. These could be used to evaluate a number of endpoints in addition to mutation that are relevant to cancer e.g. cell division rates and suppression of apoptosis. It was suggested that the COM could consider other such endpoints rather than focusing solely on mutation to give a clearer overall picture in terms of genotoxicity and cancer.

Another suggestion was for the COM to consider a weight of evidence approach to evaluating genotoxicity data and mutation potential. This could involve bringing various aspects together (e.g. mode of action, non-linear dose response relationships, quantitative genotoxicity analysis etc.) to aid consistency in the

interpretation of data. The multi-endpoint test systems (e.g. MultiFlow and Toxtracker) could also help with this.

The Pig-a *in vivo* assay was highlighted as a test that had the potential to be used to a greater extent in the future. Currently it is only used in blood cells. However, it was suggested that it could be conducted in other tissues and that this would provide a further option in addition to the *in vivo* transgenic rodent (TGR) gene mutation test, which was currently the only option for an *in vivo* gene mutation test.

A further suggestion was that the COM should consider a more holistic approach when considering potential harms to the public (e.g. disinfection by-product mixtures in swimming pools) rather than focussing on just the mutation aspect (i.e. consider the overall public health concern).

In June 2019 ~~A~~a two-day workshop "on the interpretation of genetic toxicology data in a regulatory environment". was held in June 2019 that brought together key people with an interest in developing views on the interpretation of genotoxicity data and discussed new methods and challenges for future testing strategies. From this workshop two papers were produced. The first paper (MUT/2019/08) provided notes of the presentations given and discussion sessions. The second paper (MUT/2019/09) provided an assimilated summary of the workshop. There was support for the publication of the workshop summary, once finalised. In addition, some members confirmed interest in helping to develop guidance to evaluate genetic toxicology data, one of the recommendations from the workshop. In addition, a further workshop, possibly in conjunction with UKEMs, was supported, with COM as the lead.

OECD

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