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

Forward by David Lovell – Chair

I am pleased to present this report on the work of the Committee on Mutagenicity (COM) during 2020.

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ONGOING WORK

COM GUIDANCE SERIES UPDATE

The updating of the overarching COM Guidance document continued through 2020 (papers in February (MUT/2020/03), June 2020 (MUT/2020/09), and November (MUT/2020/16)). The intention was to finalise this overarching document, with the publication of the updated COM Guidance in 2021, which would then be updated as part of a rolling revision. The topic of genomics would not be included in the overarching document because it was a rapidly developing field and likely to become out of date very quickly. A separate guidance document on genomics may be developed in the future.

Other separate COM Guidance documents developed through 2020 included: Germ cell mutagens (MUT/2020/12 and MUT/2020/17); 3D models (MUT/2020/11 and MUT/2020/18); Guidance on the genotoxicity testing of nanomaterials (papers MUT/2020/10 and MUT/2020/19); and Guidance on the genotoxicity testing of impurities (MUT/2020/21). These documents would be considered further in 2021.

GUIDANCE STATEMENT ON QSAR MODELS TO PREDICT GENOTOXICITY

At the February meeting a draft statement on QSAR models was presented (MUT/2020/02). There was also a presentation to the committee by Dr Robert Foster on the Lhasa Ltd *in silico* prediction models for genotoxicity. The talk introduced (Q)SAR systems, using Derek an expert rule-based model and Sarah Nexus, a statistical system, as examples, and discussed the performance of (Q)SAR systems and model development with respect to genotoxicity. For mutagenicity it was accepted that these models perform very well and are accepted for regulatory purposes. The ICH M7 guidelines state that one expert rule-based and a statistical-based model can be reviewed with expert knowledge to support the final conclusions for the mutagenic potential of impurities. Dr Foster noted that there is far greater Ames data available for model building compared to other tests for genotoxicity, such as chromosome aberration and micronucleus tests. A validation of Derek against chromosome aberration data showed that it performed well on chemicals which are expected to be DNA reactive. But Derek had low sensitivity for prediction of a set of compounds known to interact with either topoisomerase or tubulin. In Derek, chromosomal damage (CD) alerts primarily cover DNA/protein reactive compounds. This is an issue with rule-based systems where creating a valid SAR is incredibly difficult for complex, poly(hetero)aromatic ring systems. Dr Foster also demonstrated how a statistical system may be able to complement the rule-based system by creating a Sarah model for the prediction of CD. Data were taken predominantly from Vitic Nexus. Each time a compound is positive in both *in vitro* CA or *in vitro* MN data sets it is counted as positive in CD. This model is significantly more sensitive for prediction of chromosome

damage compared to Derek. However, it is important to note that Sarah was designed for the prediction of mutagenicity *in vitro* and, and in line with an European Food Safety Authority (EFSA) report (2019:EN-1598 Evaluation of the applicability of existing (Q)SAR models for predicting the genotoxicity of pesticides), additional refinement would be required to the model before it could be considered for use for prediction of chromosome damage *in vitro*. Following the presentation by DR Foster and COM discussion at the February 2020 meeting, a draft statement (MUT/2020/20) had been prepared for the November meeting. However, there was insufficient time for members to discuss the draft document at the November meeting due to a shorted meeting duration. Members were asked to send comments by email. The comments would then be considered, and a revised document prepared for discussion at a later meeting.

QUANTITAIVE ASSESSMENT OF GENOTOXICITY DATA

The COM first considered quantitative approaches for assessing genotoxicity data, and how they may be used in chemical risk assessment, at its Horizon Scanning exercise in June 2013 and a guidance statement was published in 2015. EFSA released a draft guidance on the assessment aneugenicity in 2020, which made proposals regarding the quantitative assessment of genotoxicity data. The draft EFSA document was reviewed by COM members and a Committee response to the public consultation was submitted. It was suggested that the COM guidance statement on quantitative assessment of genotoxicity data should be reviewed in light of the changes proposed in the EFSA 2020 document, and paper (MUT/2020/22) highlighted where updates could be made. The suggested updates were discussed by COM members and it was agreed that due to a number of concerns regarding the EFSA document, the COM guidance should not be updated at this time to reflect these. An alternative approach was agreed whereby the COM would prepare a directed statement in response to the EFSA document once it had been adopted and published.

TWO-DAY WORKSHOP ON THE INTERPRETATION OF GENOTOXICITY DATA HELD IN BIRMINGHAM IN 2019

A draft report (MUT/2020/14) and draft paper (MUT/2020/13) relating to the 2019 Two-day workshop on the interpretation of genotoxicity data were considered by the COM. The draft paper drew together the main outcomes and consensus points from the separate breakout discussion groups at the meeting under various topic headings. Members agreed that the draft paper was a good summary and representative of the workshop. It was suggested that a paper could be submitted for publication in a journal. Members also agreed that it would be useful to explore the possibility of holding similar future meetings.

PRESENTATION BY PROFESSOR DAVID PHILLIPS ON MUTATIONAL SPECTRA AND SIGNATURES OF ENVIRONMENTAL MUTAGENS

The COM keeps a 'watching brief' on the development of new methodologies for determining potential mutagenicity resulting from environmental exposures to chemicals. As part of this awareness programme, Professor David Phillips from King's College, London, provided an overview to COM of the current status of the use of mutational spectra and signatures to identify environmental mutagens.

For clarity, the key differences between 'spectrum' and 'signature' were outlined. Spectrum was defined as a mutation in a single gene in a test system, determined over many repeats in different cells and tumours, to build up a library of mutations. A 'signature' was taken to refer to mutations in the exome or across the whole genome of the test system, which is determined over a smaller number of repeats. An example of TP53 mutations in human cancer was discussed which has data available from a large number of studies (>1000). Professor Phillips described an experimental system in mice fibroblasts that his research team had developed for human TP53 genes, which showed concordance with human data in reproducing the spectrum in human tumours following environmental chemical mutagen exposure (e.g. aristolochic acid). Other mutations were also identified in the system using whole genome sequencing, with between 15,000 and 25,000 mutations identified, depending on the chemical exposure. Untreated cells have a background mutation rate of around 5000 which is thought to be due to reactive oxygen species (ROS) generation.

There are six possible base substitution point mutations, although insertions/deletions do also occur. Taking neighbouring bases into consideration, each signature has 96 possible substitution mutations in total. A study was described in which human induced pluripotent stem cells were exposed to 79 environmental agents and the base substitution signatures determined. There was no selection bias for type of mutation. Around half (n=41) of the agents produced a significant increase in mutations, once the 'cell-culture' signature, or background signature, had been subtracted. Similarity of signatures to those determined in the Sanger Institute Catalogue of Somatic Mutations In Cancer was demonstrated for aristolochic acid, benzo[a]pyrene (in presence of S9) and benzo[a]pyrene diol epoxide (with mutations similar to those seen in tumours from smokers). Other examples discussed included dibenzopyrans, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), platinum drugs, alkylating agents and ROS inducers. Dinucleotide substitutions are also possible, and solar radiation was associated with CC>TT and cisplatin with AG>TT and GA>TT. Insertion / deletion signatures were also seen with a limited number of agents (n=8), and stable signatures (i.e. reproducible) seen for 7 of these.

Professor Philips concluded that the study showed similar signatures for similar agents (e.g. cisplatin and carboplatin), however this did not apply in all cases and, in addition, some dissimilar agents also showed similar signatures (e.g. PhIP and BaP/BPDE). It has not been possible to date to compare tissue specific signatures.

The focus of research by Professor Phillips and his research team was on 3D systems, which were considered more relevant to the *in vivo* situation. Clonal organoid lines had been developed from human tissue and the assay time had been reduced by using Duplex Sequencing. Early results with a limited number of agents demonstrated proof of principle.

Following the presentation, clarification was sought around whether the methodology detected mutations in actively transcribed or silent regions and whether differences could be expected due to DNA repair. Members were informed that this was dependant on the agent. Further interesting results had been seen when early and late replicating regions had been compared as these did not mimic what was seen in tumours. As this is an evolving methodology however, it was considered possible that the mutation load may have been too small, or that the duration of exposure is important at low doses. The origin of the organoids used in the studies presented was also discussed as these can be derived from normal tissues, tumour biopsies and pluripotent stem cells; the ones described had been derived from normal tissue.

COM noted that a project being undertaken at HESI/GTTC was assessing the use of Duplex Sequencing for genotoxicity testing. The ultimate aim of this was to replace the transgenic rodent assay as the new methodology could be applied to any repeated dose study and potentially be used for detecting mutagenicity within *in vitro* assays. Further refinement of signature detail was also discussed which could be achieved using different bioanalytical software. However, Professor Phillips cautioned that there was still much work to do to verify that signatures are caused by specific agents.

It was agreed that the COM would keep an active watching brief on further developments with the methodology, particularly with regards to its use as part of a genotoxicity testing strategy.

COM EVALUATIONS

EVALUATION OF THE GENOTOXICITY OF CANNABIDIOL UPDATE

The Food Standards Agency (FSA) previously requested an opinion from the COM on the genotoxicity of cannabidiol (CBD). This was to assist the FSA in developing its advice relating to the increasing number of requests for a health risk assessment on CBD in consumer products. The COM had considered the genotoxicity data relating to CBD in 2019 and concluded that the *in vitro* and *in vivo* data were inadequate. In January 2020, the Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) received an update on available data, which included additional genotoxicity data. Therefore, the COT referred the consideration of the 'new' genotoxicity data to the COM. Paper MUT/2020/01 provided details of additional genotoxicity studies submitted to the European Medicines Agency (EMA) (available online) in relation to a medicinal form of CBD known as Epidiolex (used to treat seizures

in certain medical conditions e.g. Lennox-Gastaut syndrome and Dravet syndrome).

The *in vitro* data consisted of pure CBD tested in the Ames test conducted to GLP (in *Salmonella typhimurium* strains TA98, TA 100, TA 102, TA 1535, and TA 1537). Members had no concerns over the reported data and agreed with the conclusion of a negative result.

Two *in vivo* studies were reported, a bone marrow micronucleus test and a comet assay for chromosome damage. Pure CBD was evaluated for its potential to increase the incidence of micronucleated polychromatic erythrocytes (MNPCEs) in rat bone marrow cells. Male rats received two oral gavage doses of 0 (sesame oil), 125, 250 and 500 milligrams per kilogram of body weight per day (mg/kg bw/day. The positive control group was dosed once with cyclophosamide (CPA 20 mg/kg) on the second day of dosing. In addition to animals tested for micronucleus formation, two groups of satellite animals were dosed with vehicle and pure CBD (500 mg/kg/day) for confirmation of exposure (this did not include toxicokinetic data). Clinical signs of exposure (e.g. lethargy, ataxia, piloerection, anogenital soiling and unkempt appearance) were observed on day 3. CBD treated rats showed mean MNPCE frequencies similar to those of the vehicle control group and fell within the laboratory's historical vehicle control range. Members noted that they could not see any information provided on whether the target tissue had been exposed (e.g. toxicokinetic or plasma levels) but assumed that because this study related to a medicinal product that appropriate toxicokinetic data would be available, which would be informative regarding bone marrow exposure. The COM agreed that from the information provided that the study appeared to be robustly conducted and gave a negative result.

In a rat alkaline comet assay, rats were given single oral gavage doses of 0 (sesame oil), 125, 250 or 500 mg/kg/day CBD oral solution. Liver samples were taken 24 hours after the initial dose. No clinical signs of toxicity were observed at any dose. Members agreed that from the information provided the study appeared to be robustly conducted and gave a negative result.

Overall, the COM concluded that from the information provided, the studies appeared to be well conducted and gave negative results. However, the COM asked whether it could see all the relevant data for the *in vivo* studies to confirm that there was sufficient target tissue exposure and to evaluate whether there was any important species difference in metabolism (i.e. between humans and rats) because the potential for this this was mentioned in the summary information provided.

WHO JECFA RESPONSE TO CONSULTATION

The Committee was provided with comments from COM members that had already been sent to the Joint FAO/WHO Expert Committee on Food Additives (JECFA) secretariat on its draft revision of EHC 240 chapter on genotoxicity (MUT/2020/07). Members were asked whether they wished to submit any additional comments. JECFA were expected produce a final version and provide responses to any not taken into consideration. The COM had no further comments.

HORIZON SCANNING

It was noted that the item on the two-day workshop on the interpretation of genotoxicity data contributed to horizon scanning. For example, there was a proposal to form a working group to develop a framework or guidance (perhaps, similar to that of the Bradford-Hill criteria) on how to evaluate genotoxicity data from different sources (e.g. unpublished GLP studies conducted to OECD test guidelines and non-GLP studies published in the scientific literature). A few members expressed an interest in contributing to this. It was also noted that an additional COM led workshop could be organised in the future to further discuss unresolved questions that came out of the Birmingham meeting.

~~The committee was informed of an email from the DHSC assessor that said the UK would start formal negotiations with the EU in March 2020. It was anticipated that the UK would soon publish its mandate for negotiations with the EU. This would likely include UK objectives for the chemical sector and rules/regulations relating to future trade.~~ [SR1]It was also anticipated that Defra would be developing a new chemical strategy. Additionally, it was expected that there would be a call for evidence in Spring relating to human health and chemicals in the environment. The COM assessors considered at that time that is was difficult to predict how the various government departments/agencies may require COM input in the future.

Members noted a few topics that the COM may need to consider in the future and these included the baseline for spontaneous inherited mutations; environmental DNA (eDNA) collected from environmental samples (e.g. soil, water or air), which could be informative for monitoring various aspects, such as biodiversity (via DNA sequencing without having to collect individual living organisms); and new techniques for evaluating DNA damage. Additionally, it was noted that horizon scanning needed to be targeted with a need to avoid duplication or unnecessary work (e.g. in terms of regulatory response to technological changes). The COM was also informed that the COT was holding a workshop on exploring dose-response analysis at Manchester on the 11th March 2020.

OECD

PIG-a UPDATE

The COM was provided with paper MUT/2020/06 relating to the PIG-a gene mutation assay, mainly for information. This included UK comments that had been submitted to the OECD on the development of its test guideline. Member were asked if they had any additional comments.

The COM agreed this did not contain anything controversial and was generally content. It was noted that although there was nothing wrong with the assay, it did not appear to fill any useful gaps i.e. it did not enable anything to be investigated that couldn't already be done with existing methods. It would be useful if it could be developed further to examine other tissues in addition to peripheral blood.

Additionally, an update on the development of OECD Test Guideline 488 on transgenic rodent somatic and germ cell mutation assays was circulated to the COM (just a day before the meeting). Members were aware that there had been some disagreement between some countries over the text for sampling time in relation to rat germ cells. Members were also aware of reported evidence and modelling of rat spermatogenesis that suggested that a 28 day + 28-day (i.e. sampling 28 days later, after 28 days of dosing) designs was a better germ cell design than 28-day + 3-day (i.e. sampling 3 days later, after 28 days of dosing) for both the mouse and rat. The UK had previously commented that the data on appropriate sample times were not as good for the rat as the mouse. The relevant paragraph had been reworded to create a 'quick fix' for TG 488.The COM was content with the new wording that had been circulated (e.g. regarding sample times).