Committee on MUTAGENICITY

MUT/MIN/2020/2

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

Minutes of the meeting held at 10.30 on 9th June 2020 via teleconference.

Present:

Chairman:	Dr D Lovell
Members:	Dr C Beevers Professor D Harrison (Ex officio) Professor S Doak Dr S Dean Professor P Fowler Dr R Morse Dr A Povey
Secretariat:	Dr O Sepai (PHE Scientific Secretary) Mr S Robjohns (PHE Secretariat) Dr C Mulholland (FSA Secretariat) Dr B Maycock (FSA Secretariat)
Secretariat Support:	Dr R Bevan (WRc/IEH Consulting) Dr P Rumsby (WRc/IEH Consulting)
Assessors:	Ms E Blenkinsop (DHSC) Dr L Koshy (HSE) Dr H Stempleski (MHRA) Mrs R Pearson (VMD)
Observers	Professor J O'Brien (FSA Scientific Council) Professor D Phillips (Kings College) Dr G Johnson (Swansea University)

Dr J Kenny (GlaxoSmithKline)
Mrs M Wang

ITEM 1: ANNOUNCEMENTS/APOLOGIES FOR ABSENCE

1. The Chair welcomed the COM members, assessors and secretariat. Professor J O'Brien (FSA Scientific Council) attended as an observer. The Chair also welcomed Dr G Johnson (Swansea University), and Dr J Kenny (GlaxoSmithKline) and Mrs M Wang who would soon be joining the COM as new members and were attending this meeting as observers.

2. Apologies for absence were received from the member Dr Mike O'Donovan.

3. Members were requested to declare any interests before the discussion of any items.

ITEM 2: MINUTES OF MEETING ON 20th February 2020 (MUT/MIN/2020/1)

4. Members agreed the minutes of the COM meeting held on the 20th February 2020, subject to minor typographical changes. Item 5 on a presentation on QSARs and update of the COM guidance was not complete. This would be sent out for agreement at a later date.

ITEM 3: MATTERS ARISING

5. There were no matters arising not on the agenda.

ITEM 4: PRESENTATION BY PROFESSOR DAVID PHILLIPS ON MUTATIONAL SPECTRA AND SIGNATURES OF ENVIRONMENTAL MUTAGENS

6. 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.

7. 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.

8. 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.

9. In conclusion, 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 current focus of research by Professor Phillips and his research team is on 3D systems which are considered more relevant to the *in vivo* situation. Clonal organoid lines have been developed from human tissue and the assay time has been reduced by using Duplex Sequencing. Early results with a limited number of agents have shown proof of principle.

10. 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.

11. 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 can currently 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.

12. 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.

ITEM 5. FINAL UPDATES OF COM GUIDANCE (MUT/2020/09)

ITEM 5a. COM Guidance Series update (MUT/2020/09)

13. Amendments to the overarching COM Guidance document as a whole have been ongoing and previously considered at Committee meetings in July 2018 (paper MUT/2018/09), October 2018 (paper MUT/2018/13), February 2019 (MUT/2019/01), October 2019 (MUT/2019/12) and February 2020 (MUT/2020/03). At the last consideration, the Committee suggested amendments to the main updated text to remove 'historical data', with reference given to accessing this text within older versions of the Guidance documents. This also applied to Annex 3.

14. The paper presented (MUT/2020/09) contained amendments made to date to all sections. Members discussed each query that was outstanding to allow finalisation of the document and a number of further suggestions for amendment were agreed. With regards to Table 1 'Supplementary *in vivo* genotoxicity tests' it was agreed that some of the information was outdated and members were asked to provide updates for Table 1 to rectify this prior to publication. Members were also requested to check that references quoted in the Guidance document were as current as possible.

15 All changes agreed by members will be incorporated into a new version of the overarching Guidance Document to be reviewed by the Committee prior to the next meeting in November 2020.

ITEM 5b. Guidance – Strategies for nanomaterials (MUT/2020/10)

16. The COM Guidance on genotoxicity testing strategies for manufactured nanomaterials is a new document as this area has not been considered previously within the overarching COM Guidance. A draft paper was considered at the Committee meeting in February 2019 (MUT/2019/02) and in October 2019 (MUT/2019/12).

17. The paper presented (MUT/2020/10) provided an update of recent activities in the area, together with revisions requested when the paper had been previously considered. Members highlighted further areas for inclusion in the document and suggested a revised format for the Guidance. The anticipated update from OECD regarding testing of nanomaterials had not been released and was not thought to be due for publication in the near future.

18. The changes suggested by members will be undertaken and a revised document sent to the Committee for review prior to discussion at the next meeting in November 2020.

ITEM 5c. Guidance – Use of 3D models for genotoxicity testing (MUT/2020/11)

19. The COM Guidance on the use of 3D models for genotoxicity testing is a new document as this area has not been included previously within the overarching COM Guidance. A draft paper was considered at the Committee meeting in February 2019 (MUT/2019/04) and in October 2019 (MUT/2019/12).

20. This paper (MUT/2020/11) presented updates of recent activities in the area (particularly from the 7th IWGT meeting), together with revisions requested when the paper had been previously considered. Members discussed how the phrases 'false positive' and 'misleading positive' should be used within the paper to better reflect the utility of such assays. Some further specific changes were suggested and, in addition, some discussion concerning the context of these assays from a regulatory perspective.

21. The changes suggested by members will be undertaken and a revised document sent to the Committee for review prior to discussion at the next meeting in November 2020.

ITEM 5d. Guidance – Genotoxicity testing strategies for germ cell mutagens (MUT/2020/12)

22. The overarching COM Guidance has previously included discussion of the genotoxicity testing strategies for germ cell mutagens. However, a stand-alone Guidance document was considered necessary to allow for more frequent updates to this fast-moving area. As a consequence, a draft paper was considered at the Committee meeting in February 2019 (MUT/2019/05) and in October 2019 (MUT/2019/12).

23. The paper presented (MUT/2020/12) provided updates on any recent activity in the area and revisions previously requested by members. Outstanding queries within the paper were discussed and changes to the organisation of the OECD study information, prior to finalisation, also suggested.

24. These revisions will be undertaken, and a revised document sent to the Committee for review prior to discussion at the next meeting in November 2020.

ITEM 6. TWO DAY WORKSHOP IN BIRMINGHAM ON THE INTREPRETATION OF GENOTOXICITY DATA - REPORT AND DRAFT PAPER (MUT/2020/13 and MUT/2020/14)

25. At the previous COM meeting in February 2020, it was agreed that some outstanding matters and questions relating to the draft notes (MUT/2020/04) and summary document (MUT/2020/05)) could better be addressed by the relevant questions being sent to the members by email after the meeting. Four specific questions were circulated via email and were subsequently resolved and agreed.

26. Since then, the notes of the meeting were amended accordingly, and the various presentations were simplified and summarised into a bullet point format

(in MUT/2020/14). Additionally, at the February 2020 meeting it had been agreed that a paper should be produced from the meeting and submitted for publication in a journal. In light of this, a draft paper was produced (MUT/2020/13). This attempted to draw together the main outcomes and consensus points from the separate breakout groups at the meeting under various topic headings. Members were asked for their views on the approach adopted in terms of the structure and format and whether they agreed with the overall conclusions.

27. Members agreed that the draft paper was a good summary and representative of the workshop. It was suggested that it would be useful to include an executive summary that highlighted the main conclusions already covered at the end of the current document. It was also suggested that the introduction could set the scene and context of the meeting and explain the reasons behind it (e.g. different views from different organisations and countries) and to include the questions that the delegates were asked to consider. Members also agreed that it would be useful to explore the possibility of holding similar future meetings.

ITEM 7: EFSA PUBLIC CONSULTATION ON THE DRAFT GUIDANCE ON ANEUGENICITY ASSESSMENT (MUT/2020/15)

28. The COM was reminded that it had previously provided comments to the European Food Safety Authority (EFSA) in May 2020 on its public consultation on draft guidance on the assessment of aneugenicity. Members were asked whether there were any aspects relating to this that they wished to discuss further.

29. Regarding the quantitative assessment of genotoxicity, it was agreed that this would be better considered as part of the COM's update of its statement on the quantitative assessment of genotoxicity data. They were no further comments.

ITEM 8: ANY OTHER BUSINESS

30. A draft glossary of scientific terms used by the COM, COC and COT was circulated. It was noted that it would be useful if the set of terms used by the different sister committees were consistent. Members were asked to send any comments (e.g. for any inconsistencies or definitions that needed to be updated) to the secretariat via email.

ITEM 13: DATE OF NEXT MEETING

31. 25 November 2020 – venue and date to be confirmed.