MUT/MIN/2021/02

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

Minutes of the meeting held at 10.30 on 10th June 2021 via ~~teleconference.~~MS Teams

Present:

Chairman: Professor G Jenkins

Members:
Mr Bhagwat
Beevers
Dr G Johnson
Professor D Harrison (Ex officio)
Professor S Doak
Dr S Dillan
Professor P Fowler
Ms J Kenny
Dr R Morse
Dr A Povey
Mrs M Wang

Secretariat:
Dr O Sepai (PHE Scientific Secretary)
Mr S Robjohns (PHE Secretariat)
Ms C Mulholland (FSA Secretariat)
Dr D Gott (FSA Secretariat)
Ms C Tsoulli (FSA)

Secretariat Support:
Dr R Bevan (IEH Consulting)
Dr K Vassaux (IEH Consulting)
Dr S Bull (IEH Consulting)

Assessors:
Henry Penrose (DHSC)
Dr F Fernandez (VMD)
Dr H Stempleski (MHRA)
Dr L Johnstone (BEIS)

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	Dr L Koshy (HSE)
	Dr F Hill (BEIS)
In attendance	Dr M Jacobs (PHE)
	Jocelyn Frimpong-Manso (FSA)
	Dr Robert Smith (Covance)
	Dr S Auerbach (Pennsylvania State University)
	Dr D Lovell (St George's Medical School)

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ITEM 1: ANNOUNCEMENTS/APOLOGIES FOR ABSENCE

1. This was the first meeting for Professor Gareth Jenkins as the new chair of the COM. The Chair welcomed the COM members, assessors and secretariat. The Chair also welcomed Dr Ruth Bevan, Dr Kate Vassaux and Dr Sarah Bull from IEH Consulting providing support to the COM secretariat.

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

ITEM 2: MINUTES OF MEETING ON 11th FEBRUARY 2021 (MUT/MIN/2021/01)

3. Members agreed the minutes of the COM meeting held on the 11th February 2021 (MUT/MIN/2021/01), subject to minor typographical amendments.

ITEM 3: MATTERS ARISING

4. The COM member Dr Mike O'Donovan had unfortunately resigned due to ill health. This meant there was currently a vacancy for a member of the COM. An advert to fill this vacancy would be published later this year.

5. A number of COM guidance documents had been completed and would be published on the COM website. These included the overarching COM guidance Document, the document on 3D models and the document on germ cell mutagens.

ITEM 4: REVIEW OF THE OPINION ON TITANIUM DIOXIDE (E171) PRESENTED BY THE FOOD STANDARDS AGENCY (MUT/2021/03)

6. Professor S Doak had written to the secretariat prior to the meeting and declared that her laboratory carried out research on the uptake and toxicity of titanium dioxide nanoparticles in human cultured cells. No evaluation of the genotoxicity of titanium dioxide was carried out and Professor Doak had not been involved in the European Food Safety Authority (EFSA) evaluation. The declaration was regarded as a non-personal specific interest and did not preclude Professor Doak from contributing to the discussions. Julia Kenny informed the Chair that authorisation of the use of titanium dioxide in pharmaceuticals was contingent on the EU legislation/classification and therefore of interest to the pharmaceutical industry. As Julia Kenny had not been involved in data generation for titanium dioxide, was not a co-author of the EFSA opinion and had not received any funding for research in relation to titanium dioxide that there was no conflict of interest and therefore could take part in the COM discussion of this item.

7. The Food Standards Agency has asked for advice on the genotoxicity of Titanium Dioxide, following a recent re-evaluation from the European Food Safety Authority (EFSA).

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8. Titanium dioxide is an authorised Food Additive in the EU and under GB Food Law (retained EU law Regulation No 1333/2008 on food additives). It is used in food as a colour to make food more visually appealing, to give colour to food that would otherwise be colourless, or to restore the original appearance of food.

9. Titanium dioxide has been the subject of multiple safety evaluations. Following an evaluation in 2016 by EFSA, it was determined that E 171 mainly consisted of micro-sized Titanium dioxide particles, with a nano-sized (< 100 nm) fraction less than 3.2% by mass. Uncertainties around the identity and characterisation of E 171 were however highlighted, noting that no limits for the particle size of E 171 were set in the EU specifications. With regards to genotoxicity, based on the available genotoxicity data and considering other absorption, distribution, metabolism and excretion parameters (ADME) the EFSA Panel on food additives and flavourings (FAF) concluded that orally ingested Titanium dioxide particles (micro- and nanosized) were unlikely to represent a genotoxic hazard *in vivo*. With regards to other endpoints additional testing was required to establish an ADI, such a multigeneration or extended-one generation reproduction toxicity study according to the current OECD guidelines.

10. However, following the review of Titanium dioxide specifications in 2019, and based on the fraction of nanoparticles present in E171, the food additive fell under the scope of the EFSA guidance on nanotechnology for "a material that is not engineered as nanomaterial but contains a fraction of particles, less than 50% in the number–size distribution, with one or more external dimensions in the size range 1–100 nm" and a recommendation for re-assessment of the safety of Titanium dioxide was proposed.

11. In the most recent evaluation published in 2021, data evaluated was for the food additive Titanium dioxide E171 as well as titanium dioxide other than E171 containing a fraction of nanoparticles <100nm or nano titanium dioxide. Concerning the genotoxicity studies, combining the available lines of evidence, the EFSA FAF Panel concluded that Titanium dioxide particles have the potential to induce DNA strand breaks and chromosomal damage, but not gene mutations. No clear correlation was observed between the physico-chemical properties of Titanium dioxide particles – such as crystalline form, size of constituent particles, shape and agglomeration state – and the outcome of *in vitro* or *in vivo* genotoxicity assays(*i.e.* a cut-off value for Titanium dioxide particle size with respect to genotoxicity could not be identified). The EFSA FAF Panel concluded that several modes of action (MOA) may operate in parallel and the relative contributions of the different molecular mechanisms resulting in the genotoxicity of Titanium dioxide particles are unknown. Based on the available data, no conclusion could be drawn as to whether the genotoxicity of Titanium dioxide particles is mediated by a mode (s) of action with a threshold(s). Therefore, the EFSA FAF Panel concluded that a concern for genotoxicity of Titanium dioxide particles cannot be ruled out.

12. Paper MUT/2021/03 summarised the EFSA 2021 evaluation and included a number of questions that the COM were requested to consider.

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13. The COM had concerns over the quality and robustness of some of the studies considered by EFSA to draw its conclusions and noted that the overall data considered by EFSA was heterogenous (e.g. the range of particles evaluated was diverse; different types of approach and assays; different doses; different cell models; some studies were published in obscure or non-genotoxicity journals and the inclusion of non-GLP studies, which all contributed to the difficulty in making comparisons and an overall evaluation). Members were also concerned over the potential for publication bias in the studies evaluated by EFSA (i.e. where negative studies were less likely to be published). It was also noted that until relatively recently, the specification of E171 was poorly defined, which contributed to uncertainty and difficulty in evaluation.

14. Regarding mode of genotoxic action, the COM agreed that the evidence indicated an indirect interaction with DNA with a threshold for genotoxicity. Although some *in vitro* tests reported a positive result these appeared to mainly relate to nanoparticles with the micro-sized particles mainly giving negative results[SR1][SR2]. The *in vivo* studies tended to be of better quality and negative. The relatively low nano-fraction in E171 (i.e. often less than 3.2%) and its low bioavailability, could be important factors when considering risk assessment.

15. Members considered that the lack of quality in the evidence (e.g. mixed particle sizes (micro and nanoparticles) and a wide variety of testing approaches) did not allow definitive conclusions to be drawn and therefore did not agree with the EFSA overall conclusions on the genotoxicity of E171 Titanium dioxide. A review of more reliable and robust dataset may be required before conclusion could be drawn on the mutagenicity of titanium dioxide particles. Members noted that EFSA made no clear distinction between the genotoxicity of nano-sized and micro-sized titanium dioxide particles. EFSA seemed to have put a lot of emphasis on the evidence from nano-sized particle studies when nanoparticles made up only a small fraction of E171. The COM suggested that that if practicable, restricting the amount of nanoparticles in the specification for E171 may reduce any potential genotoxicity risk. Additionally, the COM considered that the wording of EFSA's conclusion was not helpful from a risk communication perspective. Due to the heterogenous data and equivocality of the evidence further refinement of the data evaluated may be needed before definitive conclusions on the genotoxicity and safety of titanium oxide could be made. Currently, the EFSA conclusions were not justifiable based on the available evidence and this may create unnecessary concern for the public.

ITEM 5: HORIZON SCANNING

5a Forward look from the Chair

16. The Chair suggested two main areas of potential interest to the COM, which were genomics and next generation sequencing, and the use of genotoxicity markers in human biomonitoring. It was anticipated that in the next few years genomics and sequencing would be seen more in genotoxicity, including Duplex sequencing. There was a potential for this to support or even replace genotoxicity testing, particularly testing for gene mutation or point mutation. Developments in these areas may also provide an opportunity to

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gain more information from biomonitoring, occupational exposure or environmental exposure.

5b Presentation by HSE

17. Dr Lata Koshy gave a presentation on the work of the HSE post the UK exit from the EU. HSE are involved in a number of activities within UK REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals), which includes identifying hazards, such as mutagenicity, and identifying substances of Very High Concern (SVHC). Most of the HSE work on Classification, Labelling and Packaging regulation relates to hazard identification for industrial chemicals. The HSE is also involved in the regulation of biocides and pesticides. Additionally, the HSE produces summaries for ministers and HSE opinions on the mandatory classification of substances and whether to align with EU opinion. The future work programme of the HSE is still being worked out post EU Exit and will be limited by resource and recruitment. HSE anticipated that it would complete the evaluation of two to three active substances per year. Evaluation of mutagenicity is a key part in determining whether an active substance will be given approval. Mutagenicity is also a key factor in the UK review of new and existing substances and import tolerance for pesticides. Due to the short timeline, it may be difficult consulting with COM, which has three meetings per year.

18. Some key differences for HSE since the UK exit from the EU is that the HSE has to act in isolation from ECHA and ECHA and from that peer review process. Its independence meant that it had to improve its own individual peer review process and has set up various expert groups and developed links with various other expert advisory groups. HSE may consult the COM in the future in relation to complex genotoxicity data sets and for advice in reviewing GHS for germ cell mutation category 1 and 2. The COM guidance documents and expert advice will be useful to the HSE and its advice on specific areas, for example, on mode of action/threshold mode of genotoxic action and the use of QSARs.

Government assessors

19. Assessors from other Government Departments and agencies were asked for any horizon scanning topics they wished to highlight. VMD had an interest in biopharmaceutical molecules and their potential for mutagenicity. VMD were not aware of any guidance on how to assess the mutagenic potential, for example, of modified stem cells or monoclonal antibodies, particularly those sourced from different species (e.g. xenogeneic stem cells). VMD may seek the view of the COM of this area in the future. BEIS noted that it had set up its own expert scientific advisory groups following UK exit from the EU and that it would be seeking to develop links with secretariats for other expert advisory groups, such as the COM.

20. Members of the COM were asked to send in any thoughts on horizon scanning topics to the COM secretariat.

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ITEM 6 - Toxicogenomics and Risk Assessment: Application of Transcriptomics and Next-Generation Sequencing to Genotoxicity and Carcinogenicity Assessment (MUT/2021/04)[SR3]

21. At the COM meeting in February 2021, during discussions of some preliminary literature on 'toxicogenomics and risk assessment' (MUT/2021/06), Members noted that this field could at present be considered to comprise two different major elements; the more highly established field of transcriptomics, and the newer area of next-generation sequencing technologies. It was felt that it would be useful for a document to be prepared providing a preliminary overview of these two areas and their potential applications to risk assessment in the fields of mutagenicity and carcinogenicity. Discussion paper MUT/2021/04 [KV4][SR5]provided an overview of these two areas, summarising narrative from three recently published review articles.

22. Members noted that overall this is a very fast-developing area. For this reason, it may be difficult for the COM to establish a specific guidance document, as this would rapidly become out of date. However, Members also considered that this is a very important area in the development in genotoxicity assessment and should be kept under evaluation by the Committee.

23. Some major areas of work in this field were highlighted by individual COM Members for consideration. These included: Current efforts to obtain mutational signatures and match these to environmental exposures, which was noted as an area that the COM would probably wish to focus on further; Progression of work on TGx-DI, noting that data is being passed to regulators with the aim to be able to provide guidance; Development of duplex sequencing at Health Canada, which is starting to be useful for investigations of germ-cell mutagenesis and for dose-response analysis; Use of cancer-driver mutations via the 'CarcSeq' method at FDA.

24. In terms of document progression, a more detailed paper could be envisaged, noting techniques and methodologies that are becoming available, and describing some examples of how these techniques may be becoming applicable to investigation of genotoxicity. It was agreed that further development of any paper from COM concerning the use of toxicogenomics for risk assessment purposes would be discussed by a small sub-group of interested members prior to the next meeting.

ITEM 7 - Guidance on Genotoxicity Testing Strategies for Manufactured Nanomaterials (MUT/2021/05)

25. As part of an update of the overarching COM guidance document, several additional topics have been included for consideration. One such area addresses genotoxic testing strategies for manufactured nanomaterials (NMs). It is recognised by the Committee that this is a rapidly developing area and updates will be carried out as new information becomes available.

26. This paper (MUT/2021/05) presents a first draft of the suggested guidance, prepared to a format previously agreed by COM at the meeting in

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November 2020 (MUT/2020/19). Members considered that it was important to add a note to clarify that 'Stage 0' of the COM recommended approach for genotoxicity testing would not apply to NMs. Other small changes to the document were also highlighted.

27. A question was raised regarding whether COM should recommend a positive control for NM testing. This was not considered feasible at present as this would probably need to be both assay and cell line specific, due to differing sensitivities. Members requested that this information be added to the document. It was also agreed that a note should be added to consider the most appropriate dispersion technique for a specific NM.

28. It was agreed that, following the amendments agreed above, the final version of the document could be signed off by Chair's Action.

RESERVED ITEM

ITEM 8 - Presentation on OECD development of the Mini-Ames
Dr Robert Smith, Covance

29. Dr Robert Smith is the UK representative on the OECD expert group developing the mini-Ames test. The presentation was a personal summary of the activities of the OECD expert group on miniaturised bacterial mutation assays and did not necessarily reflect the views of the expert group as a whole.

30. The bacterial reverse gene mutation assay, or Ames test (OECD TG471), is one of the most widely used tests for detection of mutagenicity. The OECD TG describes the Agar plate incorporation method and/or pre-incubation method and recommends conducting the test using a panel of 5 strains to screen for a broad selection of potential mutagens. Several miniaturised versions have been developed which differ from the standard Ames assay through the use of multi-well plates, use of liquid media rather than agar plates, fewer bacterial strains and reduced numbers of bacterial cells (and volumes etc). These are already extensively used as they offer higher throughput with a significant reduction in the amount of test material required (around ~5-fold and ~20-fold reduction for the 6- and 24-well formats respectively).

31. A Detailed Review Paper (DRP) is being prepared by OECD to evaluate the various mini-Ames assays and to compare these to the standard Ames test. Data indicate excellent agreement between the miniaturised and standard assays, with liquid suspension assays showing lower sensitivity (higher frequency of false negatives) relative to the 6- and 24-well plate assays. There was an improvement in correspondence between mini- and standard Ames based on 5-strain compared with 2-strain which was more marked for the liquid suspension assays.

32. Following the presentation members considered the possibility of whether data obtained from Ames II™ assays, which can be bought 'off the shelf', run by inexperienced laboratories may have influenced the findings of the DRP. However, there had been a requirement for laboratories to show proficiency prior to submitting data for inclusion. Although there was good

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concordance between the 4 assays evaluated, there was some remaining discussion around comparison of top doses as the fluctuation assay expressed dose as µg/ml and the Ames as µg/plate. It was also considered that exposure might be enhanced for the fluctuation assay, as less cells are present. The effect of pre-incubation with the fluctuation assay was queried and had been associated with a small increase in sensitivity and specificity. The maximum limit on concentration per well/plate was considered by members to be a critical factor for take up of the assays once finalised.

33. It was confirmed that the Secretariat would circulate the DRP once issued and that members would provide feedback on the more detailed document. Although there will be two commenting rounds in total, members were encouraged to provide detailed comments in the early stages of review. It was noted that the inclusion of nanomaterials would need to be carefully considered, particularly in light of previous considerations where the full Ames test was deemed unsuitable for nanomaterials.

OPEN SESSION

ITEM 9 – Presentation on Toxicogenomics in toxicology testing
Dr Scott Auerbach, Division of the National Toxicology Program, National Institute of Environmental Health Sciences, USA.

34. Functional omics technologies are a powerful tool for the characterisation of chemical effects on biological systems. Historically the primary use of omics technologies, transcriptomics in particular, has been to characterise chemical mode action to understand toxicological mechanisms and human relevance. More recently effort has been put into use of transcriptomics as a means to identify a biological effect point of departure that roughly approximates a point of departure derived from much more resource intensive studies such as the two-year cancer bioassay.

35. The presentation discussed how transcriptomics has been used for qualitative characterisation of chemical effects and how it is being modelled to derive a genomic-based point of departure. In addition, some of the current scientific challenges that need to be addressed to facilitate more widespread use of genomic point of departure values for health-based guidance value determination were also discussed.

36. Following the presentation, the sensitivity of the methodology was queried as some genotoxic compounds may not have a strong genotoxicity signal over the shorter exposure time. This is addressed by the inclusion of doses of test substance up to the maximum tolerated dose during screening which should produce a signal if it is genotoxic. The limitation of precision of toxicogenomics in its ability to determine what proportion of cells are affected to produce the measured 'fold' change was highlighted. This was anticipated to be a chemical specific issue as those only affecting a small number of focal points (e.g. nitrosamines) would take longer to produce a signal than chemicals affecting multiple sites (e.g. TCAB) and should be taken into account to avoid inaccuracies. The use of gene-set dose response data (as a point of departure) with BMD modelling was also discussed. There is no standard model to use with such data as the adverse effect size (BMR) for a particular gene is not

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known for many chemicals. It is also not possible at this time to take into account the effect of co-variables, which is an important consideration for human data, however this is being actively addressed by a number of groups.

ITEM 10: ANY OTHER BUSINESS

37. The COM had recently been sent a consultation on a new draft Test Guideline on the mammalian erythrocyte Pig-a gene mutation assay. Members were requested to send any comments to the secretariat by the 14th July, so that these could be collated and sent to the OECD by the deadline on the 16th July.

ITEM 11: DATE OF NEXT MEETING

38. 12 October 2021

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