Committee on MUTAGENICITY

MUT/MIN/2018/3

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

Minutes of the meeting held at 10.30 am on 18th October 2018 at Public Health England, Wellington House, 133 – 155 Waterloo Road, London, SE1 8UG.

Present:

Chairman:	Dr D Lovell
Members:	Mr A Bhagwat Dr C Beevers Dr G Clare Professor S Doak (via teleconference) Dr M O'Donovan Dr S Dean Professor D Harrison (Ex Officio) Professor D Kirkland Dr R Morse Dr A Povey
Secretariat:	Dr O Sepai (PHE Scientific Secretary) Dr D Gott (FSA Secretariat) Mr S Robjohns (PHE Secretariat)
Secretariat Support:	Dr R Bevan (WRc/IEH Consulting) Dr S Bull (WRc/IEH Consulting)
Assessors:	Mrs R Pearson (VMD) Dr L Pippin (HSE) (via teleconference) Dr H Stemplewski (MHRA)
In Attendance:	Dr M Fellows (Astra Zeneca – for item 4)

Observer: Dr P Fowler (FStox Consulting Ltd)

ITEM 1: ANNOUNCEMENTS/APOLOGIES FOR ABSENCE

1. The Chair welcomed new members Dr Ruth Morse (University of the West of England) and Mr Amit Bhagwat (Lay member), the secretariat and assessors. Dr D Gott attended from the Food Standards Agency (FSA). Professor S Doak (member) and Dr L Pippin (HSE - assessor) attended via online teleconference. Dr Paul Fowler (FStox consulting Ltd) attended as an observer and Dr Michael Fellows (Astra Zeneca) attended for Item 4.

2. Apologies for absence were received from Professor G Jenkins (member), Ms P Hardwick (member), Dr C Ramsay (Health Protection Scotland), Dr I Martin (EA assessor), Dr Lata Koshy (HSE Assessor) and Dr E Lawton (Defra Assessor).

ITEM 2: MINUTES OF MEETING ON 26th JUNE 2018 (MUT/MIN/2018/2)

3. Members agreed the minutes subject to minor typographical changes.

RESERVED BUSINESS

ITEM 2: MINUTES OF MEETING ON 26th JUNE 2018 - para-CHLOROANNILINE PRESENTATION

4. The minutes for this item were considered as reserved business as it relates to commercially sensitive information.

OPEN SESSION

ITEM 3: MATTERS ARISING

5. The Committee were informed that developments on the OECD Test Guidelines on the screening Ames test (e.g. Ames II and Ames MPF) were ongoing. It was noted that there had been a recommendation from the International Workshop on Genotoxicity Testing (IWGT) that the use of TA1535 was no longer required for the Ames test. Also, from the choice of the strains TA1537, TA97 and TA97A, only the use of TA97 should be recommended.

6. Members were informed that OECD Test Guideline 488 on the Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay (TGR) would be updated to recommend sampling times that would cover all stages of spermatogenesis. Regarding the development of an OECD Test Guideline for the *Pig-a* assay, a proposal would be submitted next month. It was noted that there was some work to be done on this. Questions had been raised, such as, whether the observed phenotypic changes were due to mutation. It was understood that comparisons could be made between positive and negative responses in the *Pig-a* and the TGR, using the TGR as the standard.

7. The Chair also informed the COM that he was now a member of the OECD *Pig-a* working group and had taken part in the last teleconference. There had been some discussion of a detailed review paper and a retrospective performance assessment. Furthermore, the Committee heard that there was going to be a meeting between the OECD and regulatory authorities from China, relating to the harmonisation of regulatory processes in November 2018.

8. The FSA noted that the 2017 annual report of the Committees on Toxicity, Carcinogenicity and Mutagenicity of Chemicals in Food, Consumer Products and the Environment was due to be published within the next few weeks.

ITEM 4: PRESENTATION ON CRISPR TECHNOLOGY

9. A brief scoping paper concerning CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology, its application as a genome editing tool in human medicine and the potential for viral vector mediated genotoxicity was presented to the COM Committee at the July 2018 meeting (MUT/2018/10). Members considered that CRISPR was an interesting technique. However, as mutations leading to cancer had been reported with a commercial product, members had requested an expert overview presentation before giving further consideration to this technique.

10. Dr Mick Fellows from the Innovative Medicines & Early Development Biotech Unit at Astra Zeneca, UK, was invited to present his research in this area to the Committee, in a presentation entitled 'Nucleotide therapeutics: preclinical safety case studies'. Dr Fellows outlined the development of precise genome editing techniques in general (including CRISPR) as research tools and the process by which these were being translated into clinical practice.

11. This was followed by a more detailed explanation of the mechanism behind the CRISPR/Cas9 methodology which has applications to multiple types of genetic modifications. Dr Fellows also outlined the pre-clinical safety considerations surrounding therapeutic genome editing, as the occurrence and consequences of off-target (as seen with gene therapy trials) and on-target effects would be crucial to the acceptance of the use of CRISPR.

12. Currently used standard *in vitro* and *in vivo* genotoxicity assays were unsuitable for pre-clinical assessment of CRISPR technologies as they often used single gene targets and had low sensitivity for double strand breaks. Bioinformatics analysis, next generation sequencing, detection of off target translocations and assessment of carcinogenicity using a humanised mouse model may offer suitable approaches and were under development.

13. Dr Fellows emphasised that therapeutic CRISPR is still at an early stage and 'safer' reagents are being developed. Key aspects for its successful use would also include identification of immunogenicity and carcinogenicity, along

with off-target and on-target adverse effects. The safety paradigms adopted would depend on whether delivery of therapy is *ex vivo* or systemic, but in either case, regulatory interaction would become increasingly important.

14. Members asked what the COM role would be in relation to the use of CRISPR technologies. The committee was informed that the remit of the COM would relate to potential environmental exposure rather than medical or therapeutic use.

15. The regulation of CRISPR technologies and associated guidelines was highlighted as a requirement by the Chair as there was a need to evaluate the hazards to people using the technique. It was not certain who this would be done by. The technology is also being used in the agrochemical arena to develop pest resistant plants and these were being classified as GMOs. The speaker suggested that the *ex vivo* entity produced using CRISPR was considered to be a Genetically Modified Organism (GMO), until it is re-injected into the patient. But, there is a current debate about whether the regulation should be changed.

16. Members also discussed activity at ICH (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) in this area. The speaker commented that there are currently working groups discussing the use of CRISPR technologies, in an attempt to influence ICH guidance. One member suggested that the COM should wait until guidance is released, for example from ICH, which could then be considered by the COM.

17. There was some discussion of the potential risk for the use of CRISPR as a clinical therapy. The speaker noted that hazard identification in terms of potential clinical use is difficult, but the key to how quickly the technology is accepted would depend on the particular disease target. Issues that had arisen during gene therapy clinical trials carried out in the past were key considerations for reducing risk, including monitoring for compensatory effects, ensuring specificity and the design of delivery vectors.

18. The presentation highlighted that one current issue in assessing the pre-clinical safety of the CRISPR technology is that many of the existing standard techniques cannot be used as testing methods. It was suggested that new assays that are being developed may not only be useful for looking at CRISPR effects, but could also feed into the wider field as a very sensitive method for detecting DNA lesions. The measurement of translocations would be a valuable tool to assess the actual consequences of a strand break. The importance of including tissue-specific studies was also highlighted and the speaker stated that this was being addressed using a humanised mouse model.

19. The Chair concluded that as the technique is fairly widely used in academic research, and the COM should keep a watching brief on mutagenicity aspects. A summary document will be prepared for inclusion in

the annual COM report for 2018 and a short paragraph added to the COM guideline document.

ITEM 5: PRESENTATION ON SELECTION OF TISSUES FOR IN VIVO GENOTOXICITY TESTING BY PROFESSOR DAVID KIRKLAND

20. The COM member Professor David Kirkland provided a presentation on evaluations conducted by a working group of the IWGT (International Workshops on Genotoxicity Testing) on *in vivo* genotoxicity testing strategies. The presentation focussed on three main areas including: comparison of the transgenic rodent mutation (TGR) assay with the *in vivo* comet assay; recommended tissues to be sampled in the comet assay; and the status of the validation of the micronucleus (MN) test in non-haematopoietic tissues.

21. In light of some decisions and recommendations made by certain regulatory organisations relating to strategies for *in vivo* genotoxicity testing, a working group of the IWGT investigated whether a substance that induces gene mutation in an *in vitro* test should be followed up by an *in vivo* TGR study, or whether a follow up in an *in vivo* comet assay would also be acceptable.

22. A database of 91 chemicals, which had been tested both in the TGR and comet assay, was compiled from various suitable sources,. The data were imbalanced because the vast majority of the results were positive for both endpoints. This required a specially adjusted statistical analysis (e.g. PABAK and AC1, which are preferable to Cohen's Kappa). Only three tissues were evaluated, namely, the liver, bone marrow and the gastrointestinal tract (GI). Only positive and negative results were included in the analysis (inconclusive and equivocal results were excluded). Data from rats and mice were combined.

23. Two different evaluation approaches (i.e. weight of evidence and curation) were adopted and led to the same conclusion that if a chemical induces TGR mutations in liver or the GI tract, then it is also highly likely to induce DNA strand breaks (comets) in those same tissues. The same was not true for the bone marrow, where sensitivity and agreement between endpoints was poor. Thus, for evaluation of *in vivo* genotoxicity in the most commonly studied tissues (i.e. liver and GI tract) the comet assay appeared to be as effective as the TGR in detecting a positive response. However, if the objective of *in vivo* testing was to study gene mutation, then the TGR assay appeared to be more appropriate.

24. Additionally, comparisons were made between TGR and comet responses in any tissue of rats or mice with Ames test results. Further comparisons were made between TGR and comet responses in any tissue of rats or mice with rodent carcinogens that were positive in the Ames test and rodent non-carcinogens that were negative in the Ames test. This also required the same adjusted statistical approach as referred to above. For bacterial mutagens and Ames positive carcinogens, the results did not support a preference for the use of one *in vivo* assay over the other (i.e. TGR or comet) for detecting *in vivo* genotoxicity. The identified TGR/comet studies reported

effects in a wide variety of tissues, but often only one or a few tissues in each study, unlike cancer bioassays, which made it difficult to incorporate results from less frequently sampled tissues into a robust analysis. For investigation of a gene mutation mode of action, the TGR was considered to be the most appropriate *in vivo* genotoxicity assay.

25. An evaluation was also conducted to determine whether an *in vivo* site of contact tissue needed to be sampled in addition to the liver (in a comet assay) and the bone marrow (in a micronucleus test) i.e. in relation to an *in vivo* follow up of a positive *in vitro* MN test. It was concluded that for routine assessment of *in vivo* genotoxicity involving an *in vivo* bone marrow MN test and an *in vivo* liver comet where there was adequate systemic exposure, an additional site of contact comet assay was not necessary, unless there was reason to investigate a specific tissue other than the liver. This was a majority view of the working group. A minority view was that a comet in the liver and two sites of initial contact (stomach and duodenum in the GI tract) may be needed due to physiological differences.

26. Some circumstances may require testing at a site of contact tissue (e.g. low systemic exposure, chemical instability, or bacterial metabolism). The IWGT working group investigated whether there was a need to include the glandular stomach in addition to the duodenum for comet site of contact tissue analysis. Data were evaluated for 70+ chemicals that had comet data in more than one GI tract tissue. Substances that were positive in the stomach were also positive in the duodenum and substances negative in the stomach were also negative in the duodenum. It was concluded that a default tissue of liver (initial site of xenobiotic metabolism) in combination with one GI tract site of contact tissue (either the stomach or duodenum/jejunum) would detect such positive genotoxic substances administered orally.

27. The intraperitoneal (i.p.) route of administration had been requested by some regulatory groups. The working group considered the choice of an appropriate route of administration and agreed that a physiologically relevant route should be used (e.g. oral). It was considered that there was likely to be sufficient exposure to the liver whether the oral or intraperitoneal route of administration was used. When high quality data were available from both i.p. administration and a physiologically relevant route of exposure (e.g. oral), then more weight should be given to data from the physiologically relevant study i.e. for risk evaluation.

28. Regarding evidence of bone marrow tissue exposure, the working group agreed various lines of evidence could be considered to indicate bone marrow exposure e.g. decrease in PCE ratio, measurement of substance in blood, ADME data from a relevant study or appropriate clinical signs of exposure (e.g. representing effects on the CNS). Evidence from blood plasma analysis could also indicate exposure of other systemic tissues e.g. the liver. It was noted that this was qualitative evidence and it was difficult to define when systemic exposure was “sufficiently high”.

29. Additionally, the working group considered that the repeat dose liver MN test was sufficiently validated for an OECD guideline to be developed in terms of number and types of chemicals evaluated. But, further work was required on the potential impact of dosing animals at different ages (i.e. Japan currently dosed from 6 weeks of age while other countries dosed from 8 weeks of age). A high correlation between a positive result in the repeat dose liver MN test and chemical induced liver carcinogenicity had been noted by the working group.

30. Overall, important IWGT conclusions were that if there was systemic exposure to any substance that is mutagenic *in vivo*, then, a positive genotoxic response would be detected by the use of a combination of the bone marrow MN test with a liver comet test. If systemic exposure to a substance does not occur, then a sample from a single tissue in the GI tract (e.g. the duodenum) would be sufficient to detect a substance that is a GI tract site of contact in vivo mutagen.

31. There followed some discussion on how to address differences of opinion and inconsistencies in strategies for *in vivo* genotoxicity testing between various regulatory bodies. Related to this, the Chair informed the committee that he had previously written to EFSA to ask whether representatives from EFSA could attend a COM meeting to enable some discussion of various aspects of strategies for in vivo genotoxicity testing. EFSA had replied that if the COM provided specific questions in advance, then EFSA could decide whether to attend such a meeting. Overall, members suggested that it was important to explore ways of harmonising the approach to strategies for *in vivo* genotoxicity testing, interpretation of results and application of weight of evidence that may, for example, involve organising a suitable workshop and inviting relevant National and International regulatory organisations.

ITEM 6: EFSA CONSULTATION ON GENOTOXICITY OF MIXTURES (MUT/2018/12)

32. The European Food Safety Authority (EFSA) launched a consultation on its draft Guidance statement on ‘Genotoxicity assessment of chemical mixtures’ in July 2018. A few COM members provided comments submitted on behalf of the whole of the COM on the 7th September 2018. These comments were provided to the committee for information as paper MUT/2018/12. The comments submitted by the COM to EFSA related to concerns over the following *in vivo* test strategies in light of recommendations from the International Workshops on Genotoxicity Testing (IWGT) e.g. tissue selection; a recommendation that mixtures containing a large number of substances with positive *in vitro* results should be considered to be genotoxic without *in vivo* follow up testing; lack of consideration of concentrations of genotoxic substances (which is inconsistent with the EU Classification, Labelling and Packaging (CLP) regulations and guidance); use of dose addition when applying Margin of Exposure (MoE) or Threshold of Toxicological Concern (TTC) approaches i.e. different genotoxicants in a mixture may have different

modes of genotoxic action; no discussion of dose response modelling to determine a point of departure for *in vivo* genotoxicity data; a heavy reliance on hazard rather than risk; and lack of consideration of low levels of exposure to genotoxic substances as part of a mixture. The timing of the consultation and the deadline for comment had not allowed the committee as a whole to consider the EFSA consultation or to have a full discussion of the response before it was sent out. However, Members did not have any additional or substantial comments on the response that had already been submitted on behalf of the COM.

33. The COM was informed that a COM member had attended a workshop organised by EFSA to consider the consultation on the genotoxicity of mixtures in terms of comments received and suggestions for amendments to the draft EFSA Guidance statement. The COM member informed the COM that EFSA had said that it would only discuss the consultation comments that it considered to be relevant. There were about 67 comments from about 16 different organisations. There was some discussion over what was meant by a fully characterised mixture and a mixture of unidentified components. A simple mixture was where the components could be identified and a complex mixture where individual components could not be robustly identified.

34. A potential difficulty was that the EFSA document covered a wide range of different regulatory areas, such as food flavourings, animal feed, and residues in the environment. Therefore, EFSA intended the document to be generic, overarching and not too specific. For example, EFSA did not want to provide a fixed percentage or cut off value to distinguish between a fully characterised mixture and an uncharacterised mixture.

35. Attendees at the workshop had expressed some concern that the draft EFSA document may be attempting to overrule CLP classification. EFSA said that it did not intend to overrule or redefine CLP Guidance, but aimed to identify a mutagenic hazard. EFSA acknowledged that wording needed to be amended to clarify the intention to produce Guidance and not a statement on regulation. There was no intention to overrule data requirements for existing legislation.

36. For most of the comments and areas of concern raised by the COM, EFSA did not want to provide specific guidance or a specific response and indicated that a case by case approach should be adopted. Concern was expressed by some attendees that some of the EFSA comments on hazard were so bold that this could be interpreted as risk by some regulators.

37. Other examples of areas of discussion included: clarification of food constituents and botanicals; definition of a mixture; contaminants in pesticides; mode of genotoxic action and dose addition; and suitable *in vivo* testing to follow an *in vitro* positive. The question of the use of benchmark dose modelling was raised. EFSA did not wish to consider this as it believed that currently there was no consensus on the use of this for genotoxicity data or how to compare with human exposure.

ITEM 7: COM GUIDANCE STRATEGY UPDATE (MUT/2018/13)

38. Updates to the QSAR and *in vivo* genotoxicity assay sections of the COM Guidance on a strategy for genotoxicity testing of chemical substances were presented to the Committee in February 2018 (MUT/2018/02 and MUT/2018/03 respectively). Although it was agreed by members at that meeting that there had been no significant changes to the overall strategy for genotoxicity testing that merited a re-write of the COM Guidance, it was agreed that other aspects of the document required updating.

39. At the COM Committee meeting in June 2018, paper MUT/2018/09 was presented, which provided an initial draft update of the full COM Guidance document, incorporating amendments agreed in February 2018. During the meeting members suggested some changes and updates, up to Annex 1 and following the meeting, one member provided an annotated copy of the document containing more detailed comments to the Secretariat.

40. All suggested amendments were subsequently collated by the Secretariat and presented to the COM Committee as paper MUT/2018/13. The Chair requested that 3 additional members go through the amended document, adding their updates in turn. Other members were also asked to forward any comments to the Secretariat. Once complete, all changes would be incorporated as an updated version of the guidance document to be reviewed at the next COM Committee meeting in February 2019.

41. Members also considered that due to the frequent updates anticipated for some of the methodologies within the guidance document, those relating to QSAR, germ cells and 3D models should be taken out as a stand-alone guidance documents that could be updated more regularly. In addition, a separate stand-alone guidance document concerning the testing methodologies used for nanomaterials was agreed as this was currently absent from the guidance document.

ITEM 8: ANY OTHER BUSINESS

RESERVED BUSINESS

EXIT OF THE EU

42. A discussion of planning for potential implications of Brexit was considered under reserved business.

OPEN SESSION

OECD TEST GUIDELINES

43. One member informed the committee that the OECD was ready to re-initiate Project 4.95 – A Guidance document on the adaptation of *in vitro* mammalian cell-based genotoxicity Test Guidelines for testing manufactured nanomaterials. The OECD is intending to hold a meeting in January or February in 2019 to discuss the appropriate follow up actions required with input from relevant experts. For example, there was a need for an inter-laboratory test exercise, Standard Operating Procedures, and adjustments to the methods, selecting of testing materials and selection of cell lines. The OECD has requested that the national coordinators nominate experts to contribute to the meeting and the inter-laboratory testing. Professor Shareen Doak had been nominated by Defra and it was important that other UK genotoxicity experts attend the meeting.

ITEM 9: DATE OF NEXT MEETING

44. Date of next meeting 28th February 2019.