Committee on MUTAGENICITY

MUT/MIN/2017/2

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

Minutes of the meeting held at 10.30 am on Thursday 22nd June 2017 at Public Health England, Wellington House, 133 – 155 Waterloo Road, Lambeth London, SE1 8UG.

Present:

Chairman:	Dr D Lovell
Members:	Dr C Beevers
	Dr M O'Donovan
	Dr G Clare
	Professor S Doak
	Dr S Dean
	Professor H Drummond
	Professor D Harrison
	Ms P Hardwick
	Professor D Kirkland
	Professor F Martin
	Dr A Povey
Secretariat:	Dr O Sepai (PHE Secretary)
	Mr B Maycock (FSA Secretariat)
	Dr K Burnett (Imperial College)
	Mr K Okona-Mensah (Imperial College)
	Mr S Robjohns (PHE Secretariat)
	Miss H Smith (PHE Secretariat)
Assessors:	Dr L Dearsly (HSE)
	Dr R Pearson (VMD)
In attendance:	Miss B Gadeberg (PHE COC & COT Secretariat)

ITEM 1: ANNOUNCEMENTS/APOLOGIES FOR ABSENCE

1. The Chair welcomed members, the secretariat and assessors. Mr B Maycock attended for the secretariat from the Food Standards Agency (FSA) and Miss B Gadeberg (PHE) attended for the COC and COT Secretariat.

2. Apologies for absence were received from Professor G Jenkins (member), Dr H Stemplewski (MHRA), Dr C Ramsay (Health Protection Scotland), Dr I Martin (EA), and Ms T Netherwood (DH).

3. The committee was informed that the triennial review of the COM had been published on the DH website, but the secretariat had not yet received any confirmation that it been formally signed off.

4. The COM heard that the current contract for scientific writing for the COM had come to an end and the contract had gone out to tender via open competition. The process of agreeing a new contract had not yet been finalised. The committee thanked Dr K Burnett and Mr K Okona-Mensah for their hard work in providing the scientific writing services for the committee and wished them the best for the future.

5. The members were asked to review and provide any declarations of interest to the secretariat. Members were also reminded to declare any interests before discussion of items.

ITEM 2: MINUTES OF MEETING ON 23 FEBRUARY 2017 (MUT/MIN/2017/1)

6. Members agreed the minutes subject to minor changes.

ITEM 3: MATTERS ARISING

7. The assessor from the Health and Safety Executive informed the COM that the European Food Safety Authority (EFSA) had recently concluded that glyphosate was not classified as an endocrine disruptor. Members were also informed that a full opinion from the European Chemical Agency's (ECHA) Risk Assessment Committee (RAC) on the harmonised classification and labelling of glyphosate had recently been published on the ECHA's website.

ITEM 4: FIRST DRAFT OF A STATEMENT ON QUANTITATIVE RISK ASSESSMENT OF GENOTOXICITY

8. The Chair declared that he sat on a number of the International Life Sciences Institute and the Health and Environmental Sciences Institute (ILSI/HESI) committees that have discussed this topic.

9. At the COM meetings in October 2016 and March 2017, members considered papers on recent developments in Quantitative approaches to the risk assessment of genotoxicity data. This included overviews of reports from the International Workshops on Genotoxicity Testing (IWGT) working group on quantitative approaches to genetic toxicology risk assessment (the QWG); publications arising from a workshop organised by HESI; and publications in a recent edition of Mutagenesis on this topic. Aspects, such as, the development of different benchmark dose (BMD) software (PROAST[1] and US EPA BMDS), point of departure metrics, and application in carcinogenicity risk assessment were considered.

10. The COM had agreed that it would be useful for it to present its views and opinions in a statement. A first draft had been produced (MUT/2017/03) for consideration and comment by members.

11. Members had a general discussion of the draft statement before going through each paragraph with specific comments and suggested amendments.

12. The COM considered that it was important to emphasise in the statement that the area of quantitative analysis of genotoxicity data by the various experts in this field was a work in progress and was developing with new ideas. The changing nature of the topic made it difficult for the COM to come to overall conclusions or make recommendations. For example, the BMD software tools appeared to be frequently updated, which made it difficult to make comparisons between the US EPA BMDS and PROAST.

13. Members agreed that it is difficult to understand the sophisticated algorithms and the detailed mathematical based work that had been conducted. It would be helpful if the experts in the field could provide relatively simple explanations of their work and explain the various strengths and weaknesses of the two main types of BMD software used. For example, to explain the arguments relating to critical effect size (CES) and whether a percentage change or a one standard deviation was the preferred option. The COM considered that it was very important to determine the most appropriate approach before the use of quantitative risk assessment of genotoxicity data could be developed further.

14. Members had some reservations over how informative complex analysis applied to relatively limited data could be (e.g. when there were just three dose levels, with just one giving a positive response). The COM also had concerns over the quality of the data analysed and reiterated its request for guidance on a cut-off point where the ratio of the upper confidence interval to the lower was too large i.e. when the quality of the data were too poor to analyse.

15. The COM believed that insufficient consideration had been given to the biological relevance of the genotoxic endpoints analysed and to the biological meaning or significance of the size of the effect seen. Members questioned

[1] This includes the EFSA-PROAST platform

how the the biological relevance of the formation of micronuclei, the comet assay, and transgenic mutation assays could be compared quantitatively. The COM noted that it had been suggested that comparisons could be made between maximum effects of various endpoints; however, chromosome aberrations measured on a percentage basis would have a maximum of 100%, whereas mutation frequency had no realistic limit. Further, the size of any response would depend on sampling time. It would not be known whether the maximum response occurred before or after the selected sampling time. The COM also noted that there was a difference between quantal and continuous data.

16. Currently, members understood that there would likely be two main uses for analysis of the dose-response relationship; one would be to help determine whether there was likely to be a threshold for genotoxicity; and the other as a predictor for potential carcinogenicity (i.e. where the intention was to avoid undertaking a carcinogenicity study). The COM was aware that analysis had suggested that a point of departure (POD) derived from genotoxicity dose-response data would give a more conservative and health protective exposure value than a POD derived from carcinogenicity dose-response data. However, such analysis had only been conducted with a relatively small number of chemicals with the same mode of action. The COM considered that further work needed to be done with a larger number of chemicals and with different genotoxic modes of action, before any conclusions could be drawn on a potential correlation between dose response analysis for genotoxicity and carcinogenicity data. Also, further work was required on different genotoxic endpoints and tissues before the COM could draw any conclusions. Currently, the COM did not consider that carcinogenic potency could be estimated from genotoxicity data.

17. Overall, the COM considered that quantitative dose-response analysis of genotoxicity data was work in progress and that further work was required. It was important to address a number of the points referred to above such as, the most suitable BMD software; documentation and explanation of the various versions of the BMD software; clearer explanation of the analytical quantitative approaches; difference between quantal and continuous data; suitable sampling time; a cut-off point for poor quality data; suitable genotoxic endpoint and tissues; biological relevance of CES or BMR; and analysis of a larger number of chemicals and classes with different modes of genotoxic action.

18. The revised statement would be circulated to the committee via email for comment initially.

ITEM 5: CONSOLIDATED SUMMARY OF GERM CELL MUTATION DISCUSSIONS

19. The COM had previously considered germ cell mutation at a meeting in June 2013, October 2015 and more recently in February 2016. A number of aspects had been considered, such as germ cell mutation assays; the effect of paternal age (e.g. increase in the number of mutations in sperm with paternal

age); the sperm chromatin structure assay (SCSA) and the TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labelling) assays and their potential for investigating germ cell mutagenesis in humans; and the suggestion that air pollution is a germ cell mutagen. As a number of different aspects relating to germ cell mutation had been considered by the COM it was agreed that a consolidated summary document could be produced to communicate the Committee's view. A draft COM summary document (MUT/2017/04) had been prepared and members were asked for comments.

20. There was some discussion of the appropriate sampling time to detect mutations in sperm and the potential implications for current guidance on germ cell gene mutation assays (e.g. OECD Test Guideline 488). Members were aware of suggestions that a sampling time of 28 days post dosing in *in vivo* studies may be more appropriate than the current recommendation of a 3 day post dosing sampling time to detect DNA effects in sperm. It was agreed that this should be addressed in the draft COM summary document.

21. The COM noted that there was evidence that the number of mutations in sperm increased as paternal age increased. It was not clear whether this increase in mutations was due to an individual being older per se (i.e. due to the aging process) or whether it was a consequence of a longer duration of exposure to environmental mutagens.

22. Regarding the suggestion that air pollution was a germ cell mutagen, the COM considered that the sperm assays used in providing evidence for this assertion had not been sufficiently validated for detecting germ cell mutations. Members had previously agreed that the SCSA and the TUNEL assays were difficult to interpret in terms of germ cell mutagenicity and had not been sufficiently validated for detecting mutation.

23. Members went through the draft COM summary document on germ cell mutagenicity paragraph by paragraph with various suggested amendments and comments, which would be addressed in the next revised version. The document would be amended accordingly and circulated to the committee for comments.

RESERVED BUSINESS

ITEM 6: TOXICOLOGICAL EVALUATION OF NOVEL HEAT – NOT BURN TOBACCO PRODUCTS: FOLLOW UP INFORMATION FROM JOINT COMMITTEE DISCUSSION (MUT/2017/01)

24. In line with the previous meeting, three members declared an interest in the item. Dr G Clare declared a personal specific interest as she has analysed anonymised slides possibly relating to studies included in the scoping paper. Dr C Beevers declared a non-personal specific interest as the company she

works for has conducted toxicity testing on heat-not-burn (HNB) tobacco products. Professor D Kirkland declared a personal specific interest as he had undertaken consulting work for one of the manufacturers to optimise test methods used for tobacco products, including HNB. None of the declarations were considered a conflict of interest and all members were able to fully participate in the discussion.

25. This item was discussed as reserved business as it relates to commercially sensitive information.

OPEN SESSION

ITEM 7: UPDATE ON HORIZON SCANNING

i) Statements from EU Regulatory Agencies

26. One member provided an update on ongoing work to address concerns expressed at previous meetings (June 2016, October 2016, and February 2017) on four statements from regulatory reviews by ECHA/EFSA.

27. The first statement was that for *in vivo* genotoxicity assays the intraperitoneal (IP) route of administration should be preferred over oral and inhalation as it leads to a by-pass of some first pass metabolism in the liver, and therefore, produces a more sensitive test. However, at the meetings in October 2016 and February 2017 it was noted that for the majority of compounds the IP route of administration does not represent a realistic route of exposure. At the last meeting the committee were informed that the ILSI/HESI Genetic Toxicology (GTTC) Committee were gathering information to address this issue.

28. However, the COM was informed that only limited information could be identified by the GTTC to address this issue. A study conducted in Japan in the late 90s compared the difference in genotoxicity of approximately 24 compounds when tested via the IP and oral route. The study reported that overall there was no reason to prefer the IP over the oral route. It was noted that a representative from ECHA had recently joined the GTTC and would feed back the findings of the GTTC to the chair of the ECHA Member State Committee (MSC).

29. The second statement was that for the *in vivo* mouse micronucleus test, even if a test compound is detected in the plasma, it does not necessarily indicate that the target tissue in the bone marrow had been sufficiently exposed to the test compound.

30. The committee were informed this was being considered by the EFSA Scientific Committee and Emerging Risks Working Group on Genotoxicity who had been requested to address this and other questions in a mandate from the

European Commission. A COM member attended a number of the meetings as a hearing expert and informed the committee that the working group recommendations will be published for a public consultation period during which members may wish to provide comments.

31. The third statement was that even if it can be demonstrated that a test chemical has reached the bone marrow at a concentration that exceeds anticipated human exposure, it may not be considered adequate, as higher exposure could have been achieved in an *in vivo* site-of-contact comet assay.

32. The committee were informed that the mandate issued by the European Commission to EFSA also requested the consideration of 'the use of data in a weight of evidence approach to conclude on the genotoxic potential of substances and the consequent setting of health-based reference values for the use in human health risk assessment' and therefore, this issue may be covered in the recommendations of the Scientific Committee and Emerging Risks Working Group on Genotoxicity.

33. The fourth statement was that the glandular stomach (in addition to the liver and duodenum) should be sampled for site of contact assays to help account for tissue variables; such as tissue structure/function, pH conditions, absorption rates and differences in breakdown products.

34. One COM member had drafted a discussion paper on this statement, which was shared with other COM members in advance of the last meeting and contained information available in the public domain on studies that had used both the duodenum and glandular stomach. Additional supportive data had subsequently been provided by other members of the COM for incorporation into the paper. A database of 90 chemicals collated by the ILSI/HESI GTTC was also being reviewed. Based on this preliminary analysis, almost all of the chemicals that produced positive results in an *in vivo* carcinogenicity study were identified as genotoxic chemicals using a combination of a bone marrow micronucleus assay and a liver comet assay (often conducted as a combined assay); suggesting that a site of contact comet assay may not be required. It was agreed that the members involved in the analyses would draft a discussion paper for submission to EFSA and ECHA. A COM paper would be produced, if required.

ii) General horizon scanning

35. The committee were reminded that at the last meeting one member was invited to give a presentation on the 'development of chronic and passive in vitro dosing systems for genotoxicity assessment', which had recently been covered at the joint National Centre for the Replacement Refinement & Reduction of Animals in Research (NC3Rs) and Unilever Workshop on 'applying exposure science to increase the utility of non-animal data in efficacy and safety testing'. It was also suggested that a presentation could be given on the US Environmental Protection Agencies (EPA) Benchmark Dose Software (BMDS).

36. The committee agreed to discuss the key themes and outcomes addressed at the International Workshop on Genotoxicity Testing (IWGT) (8-10th November in Tokyo, Japan) and the Industrial Genotoxicology Group (IGG) meeting (December) at the COM meeting in February 2018.

ITEM 8: UPDATES ON OECD

37. The committee were informed that the work programme for OECD includes a detailed review paper on the miniaturised version of the Ames test. A nominated expert attended an expert group at the OECD on behalf of UK where this was discussed. A survey has been circulated by the OECD to experts (including many members of COM) asking for information on what is already known and if more validation is needed. It was noted that COM members may wish to comment.

ITEM 9: ANNUAL REPORT

38. The draft annual report had been distributed to the Committee. Members were asked to email any comments to the secretariat.

ITEM 10: ANY OTHER BUSINESS

39. One member informed the committee that they had received final and draft decisions from the European Chemicals Agency (ECHA) requesting the conduct of a transgenic rodent (TGR) somatic and germ cell gene mutation assay (OECD TG 488) with analysis of mature sperm 3 days after the 28 day dosing period. As discussed under agenda item 5, the COM considered that sampling 3 days after dosing produces unreliable data in mature sperm and it was acknowledged that the current OECD guideline does not clearly state the sampling time that should be followed, depending on whether you are interested in somatic or germ cells. This was also acknowledged by ECHA who subsequently requested that mature sperm was analysed 7 weeks post-dosing, which would increase the number of animals required. The COM member noted the importance of raising awareness that the current OECD TG 488 is considered inappropriate for germ cell testing and that the guideline is under review. The committee were informed that Health Canada were conducting modelling of spermatogenesis in mice to establish whether a single sampling time could be used to investigate effects in both somatic and germ cells.

40. Members discussed the relationship between ECHA, EFSA, the European Medicines Agency (EMA) and the Home Office regarding setting requirements for animal testing. It was noted that this relationship would change as a result of Brexit. The committee agreed to discuss this further as part of the horizon scanning exercise at the joint committee meeting in October 2017.

ITEM 11: DATE OF NEXT MEETING

41. 9th October 2017, Joint Committee meeting, Public Health England, CRCE, Chilton, Oxfordshire