Committee on MUTAGENICITY

MUT/MIN/2016/3

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

Minutes of the meeting held at 10.30 am on Thursday 20th October 2016 at the Department of Health in Room 140B Skipton House, Elephant and Castle, London, SE1 6LH.

Present:

Chair:	Dr D Lovell
Members:	Dr C Beevers Dr G Clare Professor S Doak Dr S Dean Professor D Harrison Professor G Jenkins Professor D Kirkland Dr M O'Donovan Ms P Hardwick
Secretariat:	Dr O Sepai (PHE Secretary) Mr B Maycock (FSA Secretariat) Dr K Burnett* (PHE Tox Unit) Mr K Okona-Mensah (PHE Tox Unit) Mr S Robjohns (PHE Secretariat) Miss H Smith (PHE Secretariat)
Assessors:	Dr L Koshy (HSE)
In attendance: *participated by phone	Miss B Gadeberg (PHE COC Secretariat) Dr G Johnson (Swansea University item 6)

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ITEM 1: ANNOUNCEMENTS/APOLOGIES FOR ABSENCE

1. The Chair welcomed members, the secretariat and assessors. Mr B Maycock was substituting for Dr D Benford as secretariat for the Food Standards Agency (FSA) and Miss B Gadeberg (PHE) was attending for the COC Secretariat. Professor D Harrison, the chair of the COC, was attending as an ex-officio member. The Chair also welcomed the assessor Dr L Koshy (HSE).

2. Apologies for absence were received from Professor F Martin (member), Dr D Benford (Secretariat FSA), Dr H Stemplewski (MHRA) and Dr C Ramsay (Health Protection Scotland).

3. The committee was informed that two new members had been appointed; Dr Andrew Povey (University of Manchester) was appointed as an expert member and Dr Helga Drummond (University of Liverpool) as a lay member. Four members had not received their reappointment letters due to delays in ministerial sign off, but are able to continue to attend committee meetings based on informal correspondence.

4. The Chair noted that recent correspondence had referred to members as non-executive directors instead of members. It was clarified that the COM is an advisory non-departmental public body which therefore has members.

5. No members declared a conflict of interest for the items on the meeting agenda.

ITEM 2: MINUTES OF MEETING ON 16 JUNE 2016 (MUT/MIN/2016/2)

6. Members agreed the minutes subject to minor changes.

ITEM 3: MATTERS ARISING

7. One member asked for an update on glyphosate. The secretariat informed the committee that the public consultation on the proposal for harmonised classification and labelling of glyphosate had closed; the proposal and comments would be considered by the European Chemicals Agency's (ECHA) Committee for Risk Assessment (RAC). The European Food Safety Authority (EFSA) was due to publish the raw data used in its recent evaluation of glyphosate as part of a commitment to increase transparency. One member had contributed to a special issue of 'Critical Reviews in Toxicology' which presents an independent critical review of the four main aspects of the International Agency for Research on Cancer (IARC) review: i) epidemiology, ii) exposure, iii) carcinogenicity and iv) genotoxicity. The special issue was in press at the time of this COM meeting, but could be viewed online: http://www.tandfonline.com/doi/full/10.1080/10408444.2016.1214677.

RESERVED BUSINESS

ITEM 4: DISCUSSION OF GENOTOXICITY STUDIES INVESTIGATING EMISSIONS FROM INCINERATORS

8. This item was discussed as reserved business as it relates to pre-publication research. Once the research has been published, the minutes will be made available.

OPEN SESSION

ITEM 5: QUANTITATIVE APPROCHES TO THE ASSESSMENT OF GENOTOXICITY DATA (MUT/2016/07)

18. The COM first considered quantitative approaches to assessing genotoxicity data and how they could be used in chemical risk assessment at its horizon scanning exercise in June 2013. This topic was also addressed in a special issue of Mutagenesis published in June 2016 following an ILSI/HESI Genetic Toxicology Technical committee (GTTC) and European Environmental Mutagen Society/UKEMS workshop held in Lancaster in July 2014. The International Workshop on Genotoxicity Testing (IWGT) working group on Quantitative Genetic Toxicology Risk Assessment (the QWG) also recently published the outcome of its discussions and consensus views in two publications.

19. MUT/2016/07 was produced as an introductory scoping paper to outline the current approaches to the quantitative risk assessment of mutagenic substances. It also summarised recent developments in the use of genotoxicity data in health risk assessment and included a discussion of thresholds and genotoxicity endpoints. The scoping paper listed a number of questions that was intended to aid a COM discussion of this topic.

20. Members noted that amendments to paper MUT/2016/07 were needed in paragraphs 7 and 9 to clarify the level of risk in relation to the Margin of Exposure (MOE) and also to refer to the assumption of a linear non-threshold dose response for mutagenic substances.

ITEM 6: PRESENTATION – DR GEORGE JOHNSON – QUANTITIVE ASSESSMENT OF GENETIC TOXICOLOGY DATA: A GLOBAL PERSPECTIVE

21. To help facilitate discussions and for information on this subject, Dr George Johnson from Swansea University presented some of the work that had been undertaken by ILSI/HESI GTTC and IWGT groups on quantitative approaches to the evaluation of genotoxicity data. Health Canada had also contributed to this work.

22. Professor Johnson suggested that a paradigm shift was taking place in genetic toxicology with a move away from a dichotomous (yes or no) hazard evaluation of genotoxicity test results towards a quantitative dose-response

analysis e.g. involving the estimation of a point of departure from the dose-response data to assess human health risk. It was suggested that to enable such a broader approach to examining genotoxicity data, a next generation testing strategy may be required to allow a more flexible approach to testing and subsequent modelling of the test data.

23. A large number of studies and genotoxicity endpoints had been evaluated for a few known genotoxic substances (e.g. EMS, ENU, MMS and MNU). Various Points of Departure (POD) metrics were investigated such as the No Observed Genotoxic Effect Level (NOGEL), the Breakpoint dose (BPD), the Slope transition Dose (STD) and Benchmark Dose (BMD). The Bi-linear models (i.e. the NOGEL and the BPD) were considered to have some advantages and disadvantages. For example, the NOGEL is easy to determine, but it is dependent on study design and sparse data tends to give higher PODs. Similarly for the BPD, advantages are that it is a simple bi-linear form and appropriate for some Modes of Action (MOA), but it is also dependent on study design. Overall, a consensus was reached by the study group that use of the BMD was the preferred option.

24. Advantages included that it is a flexible methodology, which uses all the available data points, covariate analyses can be performed, confidence limits can be derived and that it is less affected by experimental design (e.g. dose selection and dose spacing). A disadvantage is that it requires consensus on the Benchmark response (BMR) size for each genotoxicity endpoint evaluated. There were currently two main approaches used for the BMD. The US Environmental Protection Agency (EPA) BMD uses the best transformation of the response data for analyses, whereas the Netherlands National Institute for Public Health and the Environment (RIVM) PROAST model uses the default assumption of a log-normal distribution. Furthermore, the Benchmark Dose Response (BMR) uses an increase relative to a negative control either by one standard deviation (US EPA) or a percentage (e.g. 5 or 10%) increased response (RIVM PROAST).

25. Professor Johnson discussed how the working group considered how PODs could be used to determine human exposure levels expected to present a low or negligible risk to health. This involved consideration of a number of case studies and *in vivo* genotoxicity data sets.

26. For example, a case study using the Muta^{TM} Mouse and 28 day repeat oral dosing with benzo(a)pyrene was illustrated. The most sensitive BMDL (the lower confidence limit of the BMD) for micronuclei formation in the small intestine was used to estimate a human Tolerable Daily Intake (TDI) following allometric scaling, calculation of a human-equivalent dose and the application of uncertainty factors. A margin of exposure approach could also be used by comparing the estimated human exposure with the lowest *in vivo* BMDL.

27. In another case study involving MeIQX there appeared to be a trend of increasing values of the BMDLs for different endpoints progressing towards tumour development (i.e. from DNA adducts, mutations, pre-neoplastic lesions to tumours). However, further consideration demonstrated that endpoints were

not directly comparable because the increase in tumour incidence is quantal and so a 10% increase in DNA adducts or mutation frequency is not comparable to a 10% increase in tumour incidence. Analyses of B(a)P and BMD_{10}s across different genotoxicity endpoints (e.g. DNA adducts, lacZ mutations, Pig-a mutations and chromosome aberrations) showed that the fold increase in response above background for each endpoint varied considerably (e.g. 5 fold for chromosome aberrations and 250 fold for DNA adducts). It was relatively easy to get a 10% response increase for adducts; moderately easy for Pig-a mutations; and harder for chromosome aberrations. The trend of the BMDL values across the different genotoxicity endpoints was said to be not necessarily meaningful. The impact of identical treatments across different genotoxicity endpoints may differ depending on the ranges in responses available. A possible solution to this was suggested to be the use of endpoint specific BMR values accounting for the relative differences in response maxima across endpoints. It was noted that a statistical framework demonstrating how to define suitable BMR across endpoints would be published soon.

28. Professor Johnson also suggested that BMDLs should not be used in themselves to assess the reproducibility of studies. This was demonstrated by a case study that looked at the reproducibility of EMS $BMDL_{10}$s across Muta Mouse and Gpt delta Mouse. The $BMDL_{10}$s were much lower for mutations in Gpt-Delta mouse than in Muta Mouse. This was considered to be due to the Gpt-Delta data being more uncertain. It was stated that it was important to note that where the confidence intervals overlapped across the test systems (as in this case), it could not be concluded the two test systems reported differently. The confidence intervals for mutations in the bone marrow, small intestine and the liver overlapped for these two *in vivo* gene mutation test systems.

29. Analysis of a further case study consisting of the benzimidazole compounds that act as aneugens, illustrated that BMD derived potency rankings could be a useful starting point to define equipotent chemical grouping for data gap/read across purposes i.e. when supported by relevant structural and mechanistic information. Individual compounds can only be rank-ordered by potency where the confidence intervals show no overlap.

30. A further case study provided evidence that lowest BMD_{05} for the *in vivo* micronucleus study correlated with lowest BMD_{10} for carcinogenicity for a number of investigated compounds.

31. In summary, Professor Johnson concluded that the use of the BMD was the best approach for deriving a POD from genotoxicity dose-response data; that it is critically important to consider confidence intervals when comparing across covariates (e.g. compound, tissue etc.); confidence interval plots provide a visual tool for assessing effects of covariates in genotoxicity studies; BMD derived potency estimates may provide a basis for categorization of equipotent compounds for read across; BMD derived health based exposure values from B(a)P exposed transgenic rodent studies give health based values that are in line with those derived from the $BMDL_{10}$ from carcinogenicity

studies; and that there is a correlation between the lowest *in vivo* BMD_{05}s for the micronucleus test and the lowest $BMDL_{10}$s from carcinogenicity data.

32. Following the presentation there was a discussion by the COM. Members noted that there was now better quality *in vivo* genotoxicity data available than there had been in the past, which was more amendable to dose-response analysis. For example, there were more genotoxicity endpoints and a greater number of tissues that could be evaluated. Also, more dose groups tended to be used in *in vivo* genotoxicity studies than previously and there was better exposure data available (e.g. plasma levels), which was more conducive for dose-response analysis. However, the COM agreed that it was important to have good quality *in vivo* data for the dose-response analysis to be meaningful. It was noted that good quality data was generally considered to produce confidence intervals with a ratio below 10 fold and data producing confidence intervals greater than 100 was suggested to be unacceptable. It was also considered desirable to analyse more than one data set. Analysis of a combination of data sets would help avoid misleading results arising from a single 'rogue' or poor quality data set. It was noted that more case studies were needed to test the theory of using endpoint specific Benchmark response analysis. Members suggested it would be useful to investigate whether there were an optimum number of dose groups for *in vivo* genotoxicity testing. The committee was aware that it was generally considered preferable from a statistical point of view to have a larger number of dose groups with fewer animals per dose group i.e. as opposed to a lower number of dose groups with more animals per dose.

33. The COM noted that there were currently the two different approaches to Benchmark dose analysis used (e.g. the US EPA one standard deviation approach and the RIVM-PROAST percentage response approach) and suggested that it would be helpful if agreement could be reached on the use of one approach. The committee also agreed that if quantitative dose-response analysis of *in vivo* genotoxicity is developed and becomes accepted as an approach to estimate human cancer health risks, then there must be confidence that it is sufficiently precautionary and health protective. To aid the development of quantitative dose-response analysis and the evaluation of its potential use, it would be helpful to obtain better quality *in vivo* genotoxicity and carcinogenicity data, such as unpublished well conducted modern studies conforming to GLP held by industry.

34. It was noted that it was not possible to prove a threshold for *in vivo* mutagenicity statistically, but mechanistic evidence could demonstrate the likelihood of its occurrence. Determining whether a threshold for mutagenicity was likely is important, as currently two different risk assessment approaches are adopted depending on whether there is a threshold or not e.g. a Tolerable Daily Intake can be derived for threshold chemicals and a margin of exposure approach is applied to chemicals assumed to have no threshold for adverse effects.

35. Regarding the suggested questions for consideration, the COM agreed that there has been a change in the quality of available *in vivo* genotoxicity

data (e.g. more endpoints, tissues and dose groups) and developments in dose response modelling that allow *in vivo* genotoxicity data to be analysed quantitatively rather than only qualitatively, but that the analysis needed be conducted on good quality and consistent data to be informative. Aspects that needed to be considered in terms of risk assessment included what test systems and endpoints were the most suitable (e.g. gene mutations or micronuclei), what tissues should be analysed, what critical effect size should be used (e.g. $BMDL_{05}$ or $BMDL_{10}$), and what BMR values were needed for each genotoxicity endpoint. It was anticipated that quantitative approaches to genotoxicity data would be considered further by the COM at future meetings.

ITEM 7: ANY OTHER BUSINESS

i) Statements from EU Regulatory Agencies

36. One member provided further details on concerns expressed at a previous COM meeting regarding four statements from regulatory reviews by EFSA/ECHA. The first was a statement that, for *in vivo* genotoxicity, the intraperitoneal route of administration should be preferred to oral and inhalation because it appears to produce a more sensitive test. It was noted that one agency had requested another *in vivo* study by the intraperitoneal route for some substances with a positive *in vitro* genotoxicity assay, which had been followed up with a negative *in vivo* assay by the oral route. The committee agreed that there are a number of examples where intraperitoneal administration is not a reliable route of exposure, as the compound precipitates out and collects in the peritoneal cavity. For the majority of compounds it was agreed that the intraperitoneal route of administration does not represent a realistic route of exposure.

37. The second statement was that for mouse micronucleus tests, 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. It was noted that the ILSI Health and Environmental Sciences Institute (HESI) Genetic Toxicology (GTTC) Committee are likely to have access to relevant data (including tissue distribution data) that could be utilised to address this statement.

38. 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. This is because a higher exposure could be achieved in an *in vivo* site-of-contact comet assay. This could lead to the requirement for further comet/site of contact tests to be conducted at a higher exposure and therefore use of more animals.

39. Fourthly, the ECHAs Member State Committee (MSC) recently requested that, for site of contact assays, in addition to the liver and duodenum, the glandular stomach should also be sampled following oral administration. The justifications proposed by the MSC for such requests were that an additional tissue would help to account for variables such as different

tissue structure/function, different pH conditions, variable physicochemical properties/fate, different local absorption rates and differences in breakdown product(s). However, the committee noted that these principles would apply to every tissue within the body and that requests for such studies would lead to additional animal testing. It was proposed that a request for data that has been conducted in both the duodenum and glandular stomach could be sent to UKEMS members to evaluate this fourth statement.

40. It was agreed that the COM would consider these regulatory genotoxicity testing requests at an upcoming meeting and that details of the specific examples discussed would be shared with the secretariat to aid in drafting a paper(s). It was proposed that the second and third statements could be addressed by data collection. However, the first and fourth statements related to general principles in genotoxicity testing and it was therefore agreed that the committee would consider producing a statement or addendum to the testing guidance to address these principles. A working group at GTTC is addressing the first statement, so COM can review their findings in the future. One member had started drafting a paper regarding the fourth statement, which would be shared with the committee for further discussion. It was also proposed that the committee may wish to co-opt a member from the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) as the statements from EFSA/ECHA involved requests for the conduct of further animal tests and to consult with a metabolism expert.

ii) Horizon Scanning

41. The chair invited the committee to contribute to an informal horizon scanning exercise. One member proposed that the committee could consider reviewing ecological screening methods for the conduct of genotoxicity tests on environmental pollutants. It was noted that there are a number of research groups working on developing high dimension/high output studies that measure multiple endpoints (including P53, polyploidy, gamma-H2AX and phosphor-H3) within a single 96-well plate. It was noted that these techniques could provide useful mode of action information in addition to standard genotoxicity tests; however, the committee may want to consider how these tests could fit into the overall testing strategy for genotoxicity. It was noted that two modified versions of the Ames test had been developed. The Ames Multi Plate format (MPF) uses the same bacterial strains as the standard Ames test, but is conducted in a 384-well plate. Whereas, Ames II differs from Ames MPF in that it uses TA98 and TAMix, consisting of a series of TA7000 strains. It was suggested that the COM should monitor the progress of these assays and noted that an OECD Test Guideline was under development for Ames MPF. It was also suggested that the COM monitor developments in Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology, gene editing tools and off-target effects in relation to genotoxicity. The committee were informed that the COT were reviewing e-cigarettes and novel tobacco products and would consult the COM for an opinion on the available genotoxicity data.

iii) BREXIT

42. The committee discussed the possible impacts of Brexit on genotoxicity research, regulatory submissions and testing. It was noted that there were uncertainties regarding whether UK universities could continue to lead Horizon 2020 EU funded projects. The Government stated that they would continue to fund universities to participate in EU projects; however, the detail of this proposal was still unclear. It was noted that if UK universities are not able to directly contribute to EU projects in the future it could have a negative impact on the training of UK scientists in the affected disciplines. These projects can also feed into developments in regulatory practice; therefore, there is potential the UK could lose some scientific influence in policy making at the EU level. For pharmaceuticals, it was noted that the testing requirements are driven by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and are therefore unlikely to be affected; however, regulatory submissions may not continue to be submitted to the European Medicines Agency. It was noted that, as an expert committee, the COM could continue to engage with European agencies (e.g. ECHA/EFSA) and provide influential advice.

ITEM 8: DATE OF NEXT MEETING

43. 23rd February 2017.