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MUT/MIN/2015/1

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

Minutes of the meeting held at 10.30 am on Thursday 5th March 2015 at the Department of Health in Room LG20 Wellington House, Waterloo, London, SE1 8UG.

Present:

Chairman:	Dr D Lovell
Members:	Dr G Clare Dr S Dean Professor M O'Donovan Dr B Elliot Professor G Jenkins Professor D Kirkland Professor A Lynch Professor F Martin Professor D Phillips
Secretariat:	Dr O Sepai (PHE Secretary) Dr B Maycock (FSA Secretariat) Dr K Burnett (PHE Tox Unit) Mr S Robjohns (PHE Secretariat)
Assessors:	Dr Lata Koshy (HSE)
In attendance:	Mr K Okona-Mensah (PHE Tox Unit) Miss B Gadeberg (PHE)

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ITEM 1: ANNOUNCEMENTS/APOLOGIES FOR ABSENCE

1. The Chair welcomed Members, the secretariat and assessors. Dr B Maycock was attending in place of Dr D Benford from the FSA. The Chair also welcomed Ken Okona-Mensah (PHE Toxicology unit) and Britta Gadeberg (PHE).

2. Apologies for absence were received from the members Ms P Hardwick and Professor M Rennie. Apologies were also received from the assessors Dr S Fletcher (VMD), Dr C Ramsay (Health Services Scotland) and Dr H Stemplewski (MHRA).

3. The Chair informed the Committee that Mary Lyon a renowned geneticist had recently died (25 December 2014) at the age of 89. Mary Lyon graduated from the University of Cambridge in 1946 when women did not officially receive degrees from the University. She went on to pursue research at Cambridge and Edinburgh University before moving to her own research facility at the MRC in Harwell in 1955. She was head of the genetics section from 1962 to 1987. Mary Lyon developed the idea of X-chromosome inactivation, the random switching off one of the two X chromosomes in each cell of female animals. This process was named after her and is known as lyonization.

4. The Chair also informed the COM that it was the last meeting of Dr B Elliot who had come to the end of his term as a member of the committee. The chair thanked Dr Elliot for his hard work over the years and the immense help he had provided to the COM.

5. Members were reminded of the need to declare any interests before discussion of items.

ITEM 2: MINUTES OF MEETING ON 16th October 2015 (MUT/MIN/2014/2)

6. Members agreed the minutes subject to minor editorial changes.

ITEM 3: MATTERS ARISING

7. The committee was updated on vacancies in the COM secretariat. Following a re-structuring process within PHE, there had been a significant reduction in support. This meant that PHE staff were under pressure to balance committee work with their other non-committee workload.

8. The Chair had so far been unable to have a meeting with the interim Director of CRCE Chilton to discuss matters relating to the COM and the support that the Committee required. However, it was hoped that the Chair would be able to have a meeting with the interim or new Director once the re-structuring process had been completed.

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9. The Committee was informed that there were on-going difficulties with the new website, such as incorrect information on the role of the Committee on the front page. It was hoped that this would be resolved and that new COM documents would be able to be placed on the website in the future.

10. Members were asked to send in declarations of interest to the secretariat so that these could be included in the COM annual report for 2014.

ITEM 4: Statement on the mutagenicity of alcohol (ethanol) and its metabolite acetaldehyde: Update review of the mutagenicity of alcohol (MUT/2015/01)

11. Dr G Clare declared a personal, non-specific interest. It was agreed that Dr Clare would not participate in the discussion or conclusions for this topic.

12. A review of the mutagenicity of alcohol (and its primary metabolite acetaldehyde) had been conducted and considered at the previous meeting. This followed a request from COC to support its on-going review of alcohol induced carcinogenicity. This would help the COC regarding possible modes of carcinogenic action causally associated with the consumption alcoholic drinks.

13. Following the previous COM discussions, a statement had been produced, which provided details of the conclusions reached by the Committee (i.e. regarding the published information on ethanol, acetaldehyde and alcoholic beverages from January 2000 to May 2014). At the October 2014 meeting, members had agreed that there was a need to modify the conclusions drawn by the Committee in the 2000 statement.

14. An updated version of MUT/2014/05 was included for discussion with the current draft statement. The revised paper MUT/2014/05 included a study by Yukawa et al (2012) (tabled at the last meeting) and further information on studies presented in the tables to reflect members' comments on study quality and methods. Efforts had also been made to standardise the units of concentration used throughout the review paper, wherever possible.

15. Members were asked to review the statement and to provide any comments to ensure that the draft statement (MUT/2015/01) reflected the COM views and the discussions from the previous meeting.

16. Members were also asked to address some specific comments in the paper and to comment on the general content and conclusions.

17. Members suggested that there was a need to clarify the terms 'mutagenicity' and 'genotoxicity' in accordance with the COM guidance and to ensure there was consistent use throughout the statement. For example, micronuclei represented genotoxicity and not mutagenicity (i.e. the formation

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of MN is associated with lethality and would not result in heritable changes in DNA).

18. Regarding the specific question on why the use of antibodies may result in an artefact, it was explained that acetaldehyde can form cross links with protein. This indicated that if acetaldehyde were to cross link with kintechore, then antibodies may not be able to bind to kinetochore, which could lead to a false negative result. This also meant that paragraph 16, which referred to acetaldehyde interaction with kinetochore, would need to be restructured. A revised paragraph would be circulated to members for agreement.

19. The COM agreed that acetaldehyde was the metabolite of most concern with regard to the genotoxic effects of alcohol. However, Members considered that it was difficult to come to overall conclusion over the genotoxicity of ethanol in humans as there were inconsistencies in the data.

20. The Committee agreed that the ALDH2-deficient genotype was likely to play a key role in the overall genotoxicity of ethanol. Available data for polymorphisms in other enzymes were considered to be inconsistent. However, the COM considered that genotype may play a role in the degree of individual risk.

21. Members agreed that there could be multiple and complex modes of action that could be responsible for the formation of MN. There was limited, but emerging evidence to support the hypothesis that acetaldehyde induces MN via formation of replication-associated double strand breaks (DSBs) in dividing cells.

22. Members agreed that a single table outlining the papers reviewed was sufficient and that comments on the quality of the individual papers should be removed. The conclusions relating to oxidative damage were considered in the next item.

ITEM 5: THE POTENTIAL ROLE OF OXIDATIVE DAMAGE IN ALCOHOL'S MUTAGENIC AND CARCINOGENIC MODE OF ACTION (MUT/2015/02)

23. Dr G Clare declared a personal, non-specific interest. It was agreed that Dr Clare would not participate in the discussion or conclusions of this topic.

24. This paper was prepared following discussions at the last COM meeting when Members reviewed brief summaries of papers retrieved during the alcohol and mutagenicity review. This described alcohol-induced mechanisms that could lead to oxidative damage to DNA, including the generation of reactive oxygen species and induction of CYP2E1. Members had expressed an interest in considering these aspects as part of the on-going alcohol MOA discussions. A systematic review of the literature was not practical as the literature was too large. The Committee had considered that the previous review paper (MUT/2014/05) provided a good representation of

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available literature. However, it was noted that many of the review articles considered were from the same 'camp' (Seitz and/or Stickel), which could have led to some bias in generating an oxidative damage hypothesis. A recent review was attached for Members consideration (Linhardt et al., 2014). It was also noted that there were no studies or significant attempts to discredit this hypothesis, although this may have been a consequence of publication bias.

25. The Committee agreed that the hypothesis that alcohol-induced oxidative stress could be important in alcohol induced liver disease and carcinogenesis was plausible. Reactive oxygen species generated from oxidative metabolism or inflammatory processes could give rise to lipid peroxidation products, which may lead to subsequent mutagenic adducts.

26. The COM also recognised that ethanol consumption results in the induction of CYP2E1 primarily in the liver, but also in other tissues, such as the oesophagus and intestine. The COM agreed that it was plausible that the induction of CYP2E1 enhanced the metabolism of alcohol to acetaldehyde; the generation of reactive oxygen species; and adduct formation. A correlation between CYP2E1 levels and DNA etheno adducts had been demonstrated in animal models and humans.

27. However, an association between CYP2E1 polymorphisms and alcoholic liver disease/alcohol induced carcinogenesis was not well defined and appeared to be weak. Overall, Members agreed that oxidative damage to DNA was a plausible hypothesis and mode of action for genotoxicity and carcinogenicity for alcohol and its metabolite acetaladehyde, but that further evidence was required. The COM requested clarification on which adducts were consistent with oxidative damage resulting from acetaldehyde and alcohol exposure and those that were not.

28. The paper on oxidative damage to DNA would be amended in light of members' comments. The Committee's views on alcohol induced oxidative damage to DNA would be inserted into the conclusions in the revised statement on alcohol and genotoxicity.

ITEM 6: STATEMENT ON THE USE OF MUTATION SPECTRA IN GENETIC TOXICOLOGY – SECOND DRAFT (MUT/2015/03)

29. The topic of mutation spectra had been initially considered by the COM at its meeting in March 2014, when a paper summarising a selection of relevant studies was presented (MUT/2014/02). A statement had been drafted and amended according to discussion and suggestions at the last meeting. There had also been consultation with one member on the inclusion of appropriate new information on next generation sequencing. A cover paper listed the main changes that had been made. Members were requested for any further comments and it was intended that the revised statement would be finalised by Chair's action.

30. The Committee corrected a number of minor typographical errors and agreed on revised wording in the section on next generation sequencing and in the conclusions. Members confirmed that there were only a few examples

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of chemical mutation spectra. Members agreed that mutation spectra could be used in the evaluation of mutagenicity, for example it could be used in a weight of evidence approach to hazard assessment.

31. The draft statement would be revised in line with members' comments and cleared by Chair's action.

ITEM 7: CHROMIUM VI – IS THERE A THRESHOLD FOR MUTAGENICITY AND CARCINOGENCITY?

32. The Environment Agency had asked Public Health England whether a paper published by Thompson et al., 2013a (A chronic oral reference dose for hexavalent chromium-induced intestinal cancer. Journal of Applied Toxicology. 34(5): 525-36) and associated work by that group had demonstrated a threshold for the genotoxicity of Chromium (VI) following oral exposure. However, this paper was a description of the derivation of an oral Reference dose (RfD) using the assumption that there was a threshold for tumours seen following oral exposure. Another paper by Thompson et al., 2013b (Critical Reviews in Toxicology. 43(3): 244-274) applied the US Environmental Protection Agency (EPA) Mode of action framework to contend that there is a threshold for genotoxicity and carcinogenicity. This was based on evidence that mutagenicity may not be an early key event in the carcinogenic process and that villous cytotoxicity and crypt cell proliferation were more likely key events that lead to tumour formation (i.e. that tumours arose subsequent to cytotoxicity and regenerative cell proliferation rather than due to an early initial mutation).

33. The study by O'Brien et al., 2013 reported no increase in the mutation frequency of K-Ras in the proliferating tissue of mouse duodenal epithelium at carcinogenic doses. The authors contended that this suggested that mutation is not an early key event of Cr (VI) induced small intestine carcinogenesis. The study investigated site specific genotoxicity; cytotoxicity; and cytogenetic damage in duodenal crypt and villous enterocytes in mice. K-Ras mutations and MN frequency were examined in scraped duodenal epithelium (including both villi and crypts). The duodenal crypt area was examined histologically and the number of MN, karyorrhectic nuclei (KN), mitotic figures, apoptotic nuclei and cytotoxicity were recorded. The authors claimed that this technique/endpoint (i.e. measuring mutation in the K-*Ras* gene) was suitable for detecting early dose related increases in mutations relevant to the carcinogenic process and was particularly relevant to the development of intestinal cancer

34. MUT/201504 also provided an overview of available data relating to the genotoxicity of Cr (VI). This included a summary of the views on the genotoxicity of Cr (VI) in recent evaluations of Cr (VI) by the European Food Safety Authority (EFSA 2014) and the US Agency for Toxic Substances and Disease Registry (2012). The studies by Thompson et al., 2013 and O'Brien et al., 2013 were appended.

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35. It was recognised that a detailed evaluation of the pathological aspects of the MOA were not within the remit of the COM. However, members were asked for their opinion on the evidence for a threshold for the mutagenicity of Cr (VI) following oral exposure presented in the O'Brien study and the paper by Thompson et al., 2013b.

36. Members noted that there were two separate questions that could be considered. One was whether data provided by Thompson et al., and O'Brien et al., demonstrated a threshold for the mutagenicity of Cr(VI) via oral exposure and the other was whether there was a potential threshold due to the conversion of Cr (VI) to the non-mutagenic Cr (III) in the gastrointestinal tract. It was decided that the discussion would focus on the former.

37. The COM queried the selection of K-*Ras* as the most sensitive marker for mutagenicity and suggested that it may have been better to look for other key mutated genes. The Committee was also not convinced that it was sufficient to look solely at K-*Ras* in just one tissue (i.e. the small intestine) to demonstrate a lack of mutagenicity below a threshold dose.

38. A key criticism of the evidence was the lack of the use of a relevant positive control. The COM agreed that it would have been advisable to have used a suitable positive control that acted via the same expected mechanism, such as a direct acting alkylating agent. Furthermore, the reported background frequency of K-*Ras* mutation was very high, which would have made it very difficult to detect an induced mutagenic effect.

39. Members were not convinced that the dose related increase in MN in the duodenal villi described in the O'Brien paper could be regarded as solely due to cytotoxicity.

40. Members commented on the validity of the methods used. It was felt that the use of described paraffin sections were unlikely to have included a sufficient number of cells to detect a significant increase in MN. Furthermore, the nature of the sections may have meant that the nuclei were truncated and therefore were not accurately analysed. The type of cells examined, primarily short lived villi cells, may slough off in to the lumen before going through cell division. This may explain the lack of detection of mutation.

41. The COM noted elevations of Cr-DNA binding with increased Cr (VI) dose had been reported in other tissues. The paper had argued that because there was no correlation with K-Ras mutations that the Cr-DNA binding was not representative of pre-mutagenic DNA damage. This was also said to be complicated by the potential for Cr-DNA binding to occur during overnight digestion for DNA isolation. Members were not convinced by the arguments presented in the paper and did not consider that they sufficiently discounted the concern for potential mutation following Cr-DNA binding. A blood sample taken to examine DNA damage would also have been useful.

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42. One member pointed out that the cells examined in the mouse gastrointestinal tract (i.e. the area of the small intestine) may not be relevant to the main target tissue in humans. This was because colon cancer was a greater risk in humans rather than cancer of the small intestine. What goes on in the small intestine was regarded as complex and not yet characterised. It would have been better to look for MN in the more relevant Progenitor cells.

43. Overall the committee agreed that whilst the hypothesis was plausible, there were limited data to demonstrate a threshold for genotoxicity for Cr (VI) and it was not convinced that there was a clear negative result for genotoxicity at low doses. This was for a number of reasons, which included a lack of a suitable positive control; a high background level of MN formation; a question over whether K-*Ras* mutations were a sensitive marker for genotoxicity; the need for the investigation of another tissue in addition to the small intestine; and that evidence of Cr VI binding in a number of tissues had not been sufficiently discounted. Although the work so far conducted by O'Brien et al., 2013 and Thompson et al., 2013 was fairly thorough and the contention for a threshold was plausible, further work was required to demonstrate a threshold for genotoxicity.

44. There was some discussion over whether there was an apparent threshold for an effect due to lack of exposure at the target tissue following the conversion of Cr VI to Cr III in the gastrointestinal tract. The secretariat noted that this aspect would be considered in any subsequent risk assessment.

ITEM 8: OECD UPDATES

45. Test Guidelines (TG) four draft TGs (listed below) were sent to the members for comment. These TGs will be tabled for approval at the next OECD Working Group of National Coordinators of the Test Guidelines Programme (WNT) meeting in April 2015.

- Updated TG 478: Rodent Dominant Lethal Test
- Updated TG 483: Mammalian Spermatogonial Chromosomal Aberration Test
- Updated TG 476: In Vitro Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes
- New TG TK: In vitro mammalian cell gene mutation assays using the Thymidine kinase gene

ITEM 9: HORIZON SCANNING 2015 (MUT/2015/05)

46. The COM undertakes an annual horizon scanning exercise, which provides an opportunity for members and assessors from Government Departments/Regulatory Agencies to discuss and suggest topics for further work.

47. A formal horizon scanning exercise was not carried out in 2014.

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48. Paper MUT/2015/05 provided a brief outline of topics recently reviewed (cell transformation assays; mutation spectra); topics still under consideration and topics proposed for consideration (e.g. gene expression profiling; integration of *in vivo* genotoxicity assays in repeat dose toxicity testing; quantification of genotoxic response; epigenetics and mutations; and 3D tissue models).

49. Members noted that a review of combining genotoxicity testing with other *in vivo* toxicity studies may be useful, but acknowledged that this was increasingly becoming standard practice (e.g. including MN and comet evaluations in toxicity tests) in order to comply with the 3Rs principles.

50. One member pointed out that a paper would soon be published on a quantitative approaches to genotoxicity which would be useful the Committee to be aware of or consider.

51. One other topic of potential interest was the importance of mutation of mitochondrial DNA. Regarding 3D tissues models for the assessment of genotoxicity, the COM noted that there had been some dermal validation studies in the USA and that the methods used could be transferable to other uses, such as the assessment of cosmetic products. It was recognised that this was a growth area of research. Another area of increasing interest was modelling of toxicokinetics. It was important, particularly for the pharmaceuticals industry to model plasma concentrations.

52. Another suggested potential topic of interest was the screening for age related mutations and consideration of mutation rates in in certain health conditions e.g. individuals with schizophrenia.

53. Members were requested to provide their views or suggestions for future COM areas of work to the secretariat.

ITEM 10: ANY OTHER BUSINESS

54. It was the last meeting of Dr Barry Elliot who had come to the end of his term of membership to the COM. Dr Elliot said how much he had enjoyed working on the Committee.

ITEM 11: DATE OF NEXT MEETING

55. 18th June 2015.

Item	Actions	Responsibility
Item 4: Update review of the mutagenicity of alcohol (ethanol) and its metabolites	Draft updated COM statement?	Secretariat
Item 5: Alcohol and oxidative damage to	Revise draft	Secretariat

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DNA – a preliminary review		
Item 6: Draft statement on the use of mutation spectra in Genetic Toxicology	Revise draft statement	Secretariat

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