MUT/MIN/2014/2

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

Minutes of the meeting held at 10.30 am on Thursday 16th October 2014 in Room 125A Skipton House, Elephant and Castle, London, SE1.

Present:

Chairman:	Dr D Lovell
Members:	Dr G Clare Dr S Dean Professor M O'Donovan Ms P Hardwick Professor G Jenkins Professor D Kirkland Professor A Lynch Professor D Phillips
Secretariat:	Dr O Sepai (PHE Secretary) Dr D Gott (FSA Secretariat) Dr K Burnett (PHE Tox Unit) Mr S Robjohns (PHE Secretariat minutes)
Assessors:	Dr Lata Koshy (HSE)
Observers:	Dr M Cush (Delphic HSE Solutions Limited)
In attendance:	Dr G Hendriks (toxys - item 7) Mr K Okona-Mensah (PHE Tox Unit) Miss B Gadeberg (PHE) Ms F Pollitt (PHE)

ITEM 1: ANNOUNCEMENTS/APOLOGIES FOR ABSENCE

1. The Chair welcomed members, the secretariat and assessors. Dr D Gott was attending in place of Dr D Benford from the FSA. The Chair also welcomed Mr Ken Okona-Mensah (PHE Toxicology unit), Ms Frances Pollitt (PHE), Miss Britta Gadeberg (PHE), and Dr Meera Cush (observer – Delphic Limited). Dr Giel Hendriks (Toxys) would be attending later for item 7.

2. Apologies for absence were received from the members Dr S Doak, Dr B Elliot, Professor F Martin, and Professor M Rennie. Apologies were also received from the assessors Dr C Ramsey (Health Protection Scotland) and Dr H Stemplewski (MHRA).

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

ITEM 2: MINUTES OF MEETING ON 6th March 2014 (MUT/MIN/2014/1)

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

ITEM 3: MATTERS ARISING

5. The committee was updated on vacancies in the COM secretariat. The more senior post as the secretary to the COM had not been filled and would have to be re-advertised. This post may need to be amended to a more general toxicology role to make it easier to fill. The more junior secretariat post (previously Dr Lesley Hetherington) had been frozen. The COM administrative role was also vacant as Gill Fisher had recently left PHE. It was hoped that a replacement would be obtained.

6. The Chair would be having a meeting with Dr John Harrison the Director of CRCE Chilton to discuss matters relating to the COM and the support that the committee required.

7. Members were requested to update and send their declarations of interest to the secretariat and were informed that the COM 2013 annual report had been completed.

8. The committee was informed that the COM contact email address had changed to the more generic COM@phe.gov.uk.

ITEM 4: Update review of the mutagenicity of alcohol (MUT/2014/05)

9. 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.

10. This updated review of the mutagenicity of alcohol and its primary metabolite acetaldehyde was prepared following a request from COC to support its on-going review of alcohol induced carcinogenicity. This would help the COC regarding possible mechanisms of cancer causally associated with the consumption of alcoholic drinks.

11. A systematic review of the literature had been conducted to capture the available evidence on the genotoxic effects of alcohol and acetaldehyde. The COM previously evaluated alcohol in 1995 and 2000. The COM published a statement in 2000. Any revised advice from the COM would be fed back to the COC.

12. The chair suggested that the COM should consider the review in three sections for each chemical, focussing on the different genotoxic endpoints, followed by a general discussion at the end. There was also a need to consider whether any changes were required to the COM 2000 statement.

13. The committee noted that a substantial number of studies had been published since the COM's last consideration of the mutagenicity of alcohol. Members suggested that a number of potential confounding factors may also need to be considered in terms of potential cancer risk e.g. body mass index, type of alcoholic beverage, drugs, diet and protective mechanisms. It was also suggested that the concentration of alcohol in alcoholic drinks could be important, for example, for cancer of the upper aerodigestive tract. For alcoholics, it would be difficult to control for non-ethanol contribution to the total alcohol intake.

14. Regarding DNA adducts, the importance of cytochrome P450 2E1 (CYP 2E1) induction and its role in the generation of oxidative metabolites was emphasised (this was also considered in more detail in item 5). The COM also noted the potential importance of polymorphisms in the metabolism of alcohol and its effect on mutagenicity. The committee considered that there was some evidence suggesting an increased formation of DNA adducts and micronuclei in individuals deficient in acetaldehyde dehydrogenase.

15. Members noted how key papers on DNA adduct formation were difficult to compare. For example, two studies by Balbo et al (2008 & 2012) found an increase in DNA adducts in individuals following alcohol consumption, while another by Singh et al (2012) did not find an increase in DNA adducts. This discrepancy in the results could be due to differences in the sensitivity of the studies. The Balbo et al studies related to intermittent exposure and the Singh study related to acute exposure. However, after adjustment for the use of different units in the studies, the results appeared to be similar, despite the authors' differing interpretation over a positive or negative finding.

16. Members considered the adduct N2-ethyl-deoxyguanosine to be a good biomarker of acetaldehyde exposure, but noted that in general there were substantial differences in the way studies were conducted (e.g. sensitivity, duration of exposure, and understanding of background adduct

levels). The COM also agreed that there were not many good or informative studies on DNA adduct formation following alcohol exposure.

17. Regarding studies on micronuclei (MN) formation and alcohol consumption, the COM agreed that studies on alcoholics and drug users were difficult to interpret. It was also noted that MN formation in bi-nucleate cells occurs *ex vivo* and there may be a publication bias towards positive results. The committee was informed that the COM (in relation to a consideration of exposure to pesticides) had previously evaluated data on the background incidence of MN and chromosome aberrations when considering these endpoints as biomarkers of genotoxicity. A large variability in the background levels of these biomarkers was found, which complicated interpretation. The tabulated data (table 1.3 and 1.4) of studies on MN and chromosome aberrations in alcohol drinkers showed a mixture of effects with only about a ¼ of the studies reporting negative results. The *in vitro* data for acetaldehyde and induction of MN were more convincing than the *in vivo* data. The *in vivo* data were difficult to assess.

18. The COM considered potential modes of genotoxic mechanism in some highlighted papers. Kayani and Parry (2010) performed a cytokinesis-blocked MN assay with kinetochore staining *in vitro* which showed a dose dependent increase in kinetochore positive MN with ethanol treated cells, but not for MN in acetaldehyde treated cells. The authors contended that this indicated an aneugenic mode of action for ethanol and a clastogenic mode of action for acetaldehyde. Members considered that this was an interesting paper that could not be ignored. However, there were other possible explanations for a positive result (e.g. oxidation of spindle fibres or an artefact from the use of antibodies). Furthermore, it was only one study. Therefore, further investigation would be helpful before drawing conclusions. A study by Kotova et al (2013) investigated the mechanism of genotoxicity from sub-chronic ethanol exposure in rats. The study suggested that the genotoxicity (as detected by MN) was due to acetaldehyde induced DNA replication lesions in dividing cells. Again, the COM considered that this was an interesting paper, suggesting a plausible mechanism for genotoxicity, but further investigation would be required before conclusions could be drawn.

19. Members noted that in some studies, such as in gastric mucosa cells, exposure to relatively high concentrations of ethanol (e.g. 1M) could result in secondary or indirect DNA damage following irritation, inflammation or dehydration.

20. The committee also looked at a paper on ethyl sulfate by Mitchell et al (2014) that suggested that ethanol could also be metabolised to ethyl sulfate that can alkylate DNA potentially leading to mutation. However, sulfate compounds were difficult to test (e.g. sulphates do not pass through membranes very easily) and the COM considered that further data would be required before conclusions could be drawn on this hypothesis.

21. Overall the COM agreed that it was reasonable to assume that acetaldehyde was genotoxic from the available *in vitro* and *in vivo* data. The data for ethanol were not clear due to a number of other potential confounding factors, therefore it could not be concluded that ethanol is directly mutagenic in vivo.

22. The committee considered that the papers on genotoxic mechanism were interesting and plausible, but required further investigation before any conclusions could be drawn.

23. Members agreed that the alcohol metabolite acetaldehyde was the most concerning candidate for the observed genotoxicity arising from exposure to alcoholic beverages.

24. Regarding the recent paper by Mitchell et al (2014) suggesting that ethanol can also be metabolised to ethyl sulphate, which could alkylate DNA potentially leading to mutation, the committee considered that further investigation would be required to draw any conclusions on this proposed hypothesis.

25. The committee agreed that there were sufficient new data to suggest that mutagenicity following exposure to alcohol and its metabolites was biologically plausible, which would require a revision of the COM 2000 statement.

ITEM 5: ALCOHOL AND OXIDATIVE DNA DAMAGE – A PRELIMINARY OVERVIEW (MUT/2014/6)

26. 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.

27. MUT/2014/06 provided brief summaries of studies retrieved during the literature search for paper MUT/2014/05, which examined endpoints associated with alcohol-induced oxidative mechanisms (which may in turn contribute to its carcinogenic mode of action). The committee was asked to comment on oxidative damage to DNA as a potential mode of action for alcohol and whether it wished to see a detailed review of these papers on this topic.

28. Members noted that there was uncertainty over the use of the adduct 8-hydroxy-2'-deoxyguanosine (8-OH-dG) as a biomarker of effect. This is because it represents a pre-mutagenic lesion only. It is well repaired in normal circumstances, but it could lead to mutation under some circumstances. It was noted that a more accurate description or term was 'oxidative damage to DNA' rather than 'oxidative DNA damage'.

29. The impact of alcohol on DNA repair was a further factor to be considered, together with the oxidative pathways that would generate oxidative biomarkers i.e. following irritation or inflammatory responses to

alcohol. 8-OH-dG was considered to be a biomarker of oxidative stress in general and the most abundant DNA lesion.

30. Members agreed that it was too simplistic to consider that alcohol is just metabolised to acetaldehyde. Expression of CYP 2E1 could be induced, which is also involved in the metabolism of ethanol to acetaldehyde and the generation of reactive oxygen species. Therefore, there may be a need to separate papers that consider tissues where CYP2E1 is expressed or induced from others that don't i.e. there was a need to separate out circumstantial evidence of oxidative damage to DNA arising from secondary processes such as irritation etc. Studies using knockout mice may be useful in this regard.

31. It was agreed that acetaldehyde was likely to generate a plethora of effects, including oxidation, which could result in DNA damage. The papers on human subjects again reflected the difficulties in assessing results due to potential confounding factors – such as consumption of fruit and vegetables. There were a lot of studies where co-exposure to other substances in addition to ethanol would occur, which would make interpretation difficult.

32. It was agreed that a systematic or detailed evaluation of the literature in this area was not necessary, but the addition of an extra paragraph reflecting current hypotheses in the revised statement would be appropriate. The additional paragraph should focus on the importance of the induction of CYP2E1 in different tissues. This would also need to include the role of CYP2E1 in the metabolism of ethanol to acetaldehyde; the impact of oxidative damage induced by inflammatory or irritant responses; the relative contributions of alcohol and acetaldehyde on oxidation and carcinogenicity; and the influence of other dietary factors.

ITEM 6: STATEMENT ON THE USE OF MUTATION SPECTRA IN GENETIC TOXICOLOGY (MUT/2014/07)

33. A paper on chemically induced mutation spectra was reviewed by the committee at the previous meeting in March 2014. Members decided that it would be useful to produce a statement on mutation spectra following the discussion. A first draft statement had been produced and was presented to the COM for comment. The statement was drafted based on the previous committee discussion; a review of the literature; and on three specific chemical exposures with defined mutation spectra.

34. Members commented that overall the first draft statement was a fair evaluation of the topic. It was agreed that the paragraph describing why phenotypically neutral genes are most suitable for examining mutation spectra should be altered so as to not imply that the selectable genes used in mutagenicity tests are not useful indicators of mutagenicity.

35. The COM agreed that there are some examples where mutation spectra (MS) are conserved across test systems and species (e.g. for the food mutagen MeIQx.

36. With regard to the specific chemical examples – it was considered unnecessary to go into detail about which chemicals in tobacco smoke were responsible for the MS profile, because this was complicated and imprecise.

37. The statement should include the reasons why spectra in tumours differ in different sites, for example the role of repair locus effects. Furthermore it should be noted that the *Tp*53 mutation is a late mutation in colorectal cancers and therefore it may not be detected even when smoking contributed towards tumour progression. Members considered the MS of aflatoxin B1 to be clear and distinct in human liver tumours. It was noted that a number of factors could also affect mutation spectra for aflatoxin and other chemicals, such as the effect of viruses (e.g. hepatitis B for aflatoxin) and the time at which spectra are measured (i.e. mutation spectra may change over time after the initial chemical exposure).

38. Aristolochic acid was considered to generate a distinct MS and represent an example of an unusual tumour. It was considered to be the best example of a specific chemically induced MS. The picture may also be clear for smoking for example, when the sample or measurement was taken at the right time and in the right tissue.

39. Members requested that the statement should clarify how and when MS could be used. MS could be potentially useful as part of an overall tool box of non-standard methods, which could contribute towards the overall identification of genotoxic hazards. They could also contribute to a weight of evidence approach towards the generation of plausible, causative associations and in mode of action evaluation. However, it was also stated that lots of different mutagens could cause the same mutation and thus the applicability of MS for identifying mutagens would be very limited. A different change in sequence does not always mean a differing reactivity or potency of the mutagen.

40. The paragraph on next generation sequencing needed to be enlarged and be more specific to the evaluation of mutagenesis. Members offered to provide suitable references for this section. It was decided that the Table containing the references reviewed in the previous paper was not necessary for the current statement. The statement will be re-drafted to reflect Members' discussion and suggestions for the next meeting.

ITEM 7: TOXTRACKER – IN VITRO GENOTOXICITY TEST- DISCUSSION AND PRESENTATION BY DR GIEL HENDRIKS

41. The committee had considered a paper on the newly developed ToxTracker genotoxicity assay at its last meeting in March 2014. Members had expressed an interest in keeping up to date with the development of the assay when additional validation emerged.

42. Dr Giel Hendriks was invited to the COM and gave a presentation on ToxTracker. The ToxTracker genotoxicity assay comprised a system of reporter cell lines where 6 identified genes, reflecting key pathways, had been cloned into mouse embryonic stem cells. This assay could identify both genotoxic and pro-oxidant chemicals. Dr Hendriks said that one of the main advantages of this genotoxicity assay over standard in vitro test systems, was that it was able to provide some insight into the mechanism of genotoxicity e.g. oxidative damage to DNA or protein damage. Different types of genotoxicity could be detected by certain biomarker genes. These reporter genes could be related to certain cellular pathways and related biological damage (e.g. DNA damage detected by Bacl2 and Rtkn; oxidative stress detected by Srxn1 and Blvrb; and protein stress by Ddit3). Chemically induced genotoxicity, could be detected by the induction of Green fluorescent protein (GFP) determined by flow cytometry.

43. Following the presentation, there was a discussion and members had a number of questions. The COM heard that all compounds could be tested for the influence of metabolic activation by the addition of S9, which was found to be the most effective method for the inclusion of metabolic activation. The fold increase in GFP induction was used to determine an overall positive genotoxicity response. Using the designed software, GFP induction could be calculated for a certain degree of cytotoxicity (50% cytotoxicity was selected as the optimum value).

44. The sampling time was 24 hours after initial exposure. The exposure time was said to not markedly change the results. The time point for measurement had to be sufficiently long to detect aneugenic activity, which was a later event. The cut off point for a positive genotoxicity result was chosen as a 1.5 fold increase in the induction of GFP, which was 5 times the standard deviation.

45. For validation of the assay, the developers used the ECVAM suggested library for carcinogens and non-carcinogens and the USA Toxcast library.

46. The 6 chosen genes incorporated into mouse embryonic stem cells were the 6 best identified performers (i.e. Bscl2, Rtkn, Srxn1, Blvrb, Ddit3 and Btg2) for predicting genotoxicity. There were other reporter genes that could also be used. The results for methyl methanesulphonate did not predominantly indicate DNA damage, but had given a stronger signal for oxidative stress, which was unexpected. .

47. Regarding the role of this assay and where it might fit in a testing strategy, it was suggested that the current view was that it would be useful as an early screen before *in vivo* testing. There was a possibility that for a situation where there was a positive *in vitro* genotoxicity result, considered to be weak or a misleading positive, then results from ToxTracker may help with the overall interpretation.

48. Members suggested that there was a need to gain a better understanding of what the gene expression changes meant in terms of the mechanism of genotoxicity (i.e. what the genes were doing or reflecting). The COM were informed that this was on-going process and currently being examined e.g. the signalling pathways were being assessed and there was need to understand reporter gene activation and how this correlates with carcinogenicity.

49. It was pointed out, that if a 'heat map' (i.e. degree of GFP induction for each reporter gene following a chemical exposure) was examined for ECVAM model compounds then the profile for 'new' chemicals could be looked at to see what model chemicals they were closest to. For about two thirds of chemicals looked at so far, a primary activity could be identified.

50. The COM may consider this item further at the next meeting.

ITEM 8: OECD UPDATES

51. The COM were informed that there would no longer be an OECD test Guideline for the *in vitro* Syrian hamster embryo (SHE) cell transformation assay but a guidance document instead. This was due to the concerns that the COM and other countries had expressed over the development of a test guideline.

52. Members were asked to provide any comments to the secretariat that they might have on the draft update Dominant lethal test (TG 478) and the mammalian spermatagonial chromosomal aberration test (TG 483).

53. One member updated the committee on WNT meeting held in April 2014. Essentially everything that went to the WNT was approved. The *in vivo* tests would not have to provide a justification for sex differences. If there was no evidence for a difference then either sex could be used.

54. Regarding *in vitro* tests the request for wording to include duplicate cultures in preference to single replicates was agreed.

55. The latest draft revised guidelines would be circulated to members for comment i.e. mouse lymphoma assay and the mammalian cell gene mutation test (Thymidine Kinase and Hprt and xprt assays).

ITEM 9: ANY OTHER BUSINESS

56. Members were informed that there were some difficulties with the 'new' COM website now that its location had been moved to the .gov.uk site. Unfortunately, the secretariat had not been consulted over the changes. It was hoped that the minutes and statement etc. would be able to go on the new website in the future. Previous COM documents are no available from the main COM page, but could be assessed via links. The previous COM page

and documents were available from an archived site that cannot be changed over time. The secretariat was having on-going discussions with web publishing at PHE to try and improve the current site.

ITEM 10: DATE OF NEXT MEETING

57. 5th March 2015.