MUT/MIN/2014/1

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

Minutes of the meeting held at 10.30 am on Thursday 6th March 2014 in Room 125A Skipton House, Elephant and Castle, London, SE1.

Present:

Chairman:	Dr D Lovell
Members:	Dr G Clare
	Professor M O'Donovan
	Dr B Elliot
	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 H Stemplewski (MHRA)
Observers:	Dr A Scott

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 Dr A Scott from Unilever who would be attending from 12.30 pm as a member of the OECD Expert Group for Cell Transformation Assays (CTA).

2. Apologies for absence were received from the members Dr S Dean, Dr S Doak, Professor F Martin, and Professor M Rennie. Apologies were also received from the assessors Dr C Ramsey, Mr S Fletcher (VMD) and Dr S Dutton (HSE).

3. The Chair congratulated the COM member Dr Shareen Doak on the birth of her baby boy Riley born in January. The committee was also informed that Professor Guy M. Poppy had been appointed as the new Chief Scientific adviser to the Food Standards Agency.

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

ITEM 2: MINUTES OF MEETING ON 28th November 2013 (MUT/MIN/2013/3)

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

ITEM 3: MATTERS ARISING

6. The committee was informed that the post for the secretary of the COM (previously the role of Jon Battershill) had been re-advertised. This post would also involve the evaluation of pesticides and biocides as a regulatory toxicologist. Members were requested to inform colleagues who may wish to apply. The COM expressed its concern over the difficulty and delay in recruiting for this post.

ITEM 4: ANNUAL REPORT FOR 2013 (MUT/2014/01)

7. The aim of the Committees on Toxicity, Mutagenicity, and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment Annual Reports is to provide a brief toxicological background to the Committees' decisions.
Paper MUT/2014/01 provided draft summaries of the items and statements considered by the COM during 2013. This was intended to form the COM contribution to the joint COC/COM and COT 2013 Annual Report. The draft text had been summarised from the minutes and statements for 2013.

8. Insufficient time was available to consider this item at this meeting. However, members were asked to send any comments to the secretariat.

Members were also asked to send updated 'Declarations of Interest' statements to Gill Fisher the COM administrator.

ITEM 5: MUTATIONAL SPECTRA (MUT/2014/02)

9. The term 'mutation spectra' refers to the composite of the number, types and sites of all mutations observed in a given sequence. It is also more loosely used in referring to the number and types of mutation found or even the main type of mutation observed (e.g. GC to AT transversions).

10. The COM had previously advised on the significance of mutation spectra arising from a specific chemical exposure. In a 1999 statement, the COM reported on the high frequency of mutations at codon 61 of the K-ras gene in lung tumours from ozone exposed mice.

11. The topic of mutation spectra was also raised in the Horizon scanning exercise in 2006, when it was suggested that a review of studies examining mutational fingerprints and hotspots for mutation following carcinogen exposure could be conducted. The topic had also been raised at a subsequent horizon scanning exercise, but had not been undertaken due to other priorities.

12. Paper MUT/2014/02 presented an overview and summaries of a selection of studies retrieved from the literature, which analysed mutation spectra induced by different chemicals in different test systems. The paper was intended as an overview and summaries and findings of the reviewed papers were tabulated. A variety of test systems had been used. *In vitro* systems included bacterial, human, rodent and transgenic cell lines. *In vivo* systems identified were primarily transgenic models from which genes were more easily isolated and sequenced (i.e. Muta^{TM} mouse, Big Blue and gpt delta mice). A paper discussing the use of diagnostic mutations in establishing the mechanisms of carcinogenicity, presented to the Committee in 1999, was also appended at Annex 1.

13. Members considered that this was a very interesting area of research. Its main value lay primarily in evaluating a chemical's mode of carcinogenic action or in understanding cancer aetiology and types of adducts and mutation involved in cancer. Currently it was not suitable for regulatory purposes.

14. The committee advised that Ames tester Salmonella strains and the hprt locus are not suitable for use in mutation spectra analysis. Mutation spectra should be assessed in phenotypically neutral genes, which were not subject to selection. The *lacI or lacz* genes from transgenic rodents were considered to be examples of neutral genes that would not be selected for *in vivo*, however it was pointed out that the genes are selected for in the *ex vivo* part of the studies.

15. It was noted that the analysis of p53 across different models was of value in evaluating chemically-induced mutations as it has been shown that mutation patterns are conserved in different test systems (e.g. BaP induced GC →TA transversions). Mutations in p53 are seen following exposure to PAH's in animals and these are correlated with those seen in some human cancers, for example in smokers, as detailed in an IARC database.

16. The human p53 knock-in (Hupki) mouse model containing a human wild-type TP53 DNA sequence was considered to be useful for investigating experimentally induced mutations in the human TP53 gene. However, it was noted that not all clones will have the p53 mutation and that the acquired immortality could be due to a mutation in a gene other than p53. The nature of the transformed foci in *in vitro* Hupki cell lines are characteristic of the chemical tested and could be used for proof of principle evaluations. Important limitations in using *in vitro* systems include that DNA damage and mutation are more likely *in vitro* than *in vivo* due to the higher levels of oxygen and the greater potential of oxidative damage and results from cell lines may be unrepresentative of untransformed diploid cells. This could confound the results and interpretation.

17. Currently, there were only a few good examples of mutation spectra that could be associated with certain cancer causative agents e.g. UV light, aflatoxin B1, tobacco smoke and aristolochic acid. However, members agreed that the development of 'next generation sequencing' technologies, where the whole genome could be sequenced would provide a substantial amount of new data that could be very useful for evaluating and understanding the role of mutation patterns in cancer development. Current methods that looked at only a single reporter gene may only provide limited information. It would be important to distinguish between mutations in genes that drove the cancer process and mutations in genes that had no effect i.e. were only 'passengers' in the cancer process. Members agreed that an *in vitro* experimental test system (not Hupki) was needed in which the whole genome could be analysed in a non-selective model (representative of human cells), from which a mutation pattern seen in human tumours could be identified. Where possible it would be better to use human cells and a 3D model rather than a 2D model. It would also be important to identify key signal genes and pathways in the cancer process.

18. The committee also discussed the use of mutation spectra from transgenic animal models in interpreting the significance of a positive *in vitro* genotoxicity result and a negative *in vivo* genotoxicity test result where a chronic carcinogenicity assay was positive. It was suggested that in such cases, any differences in metabolism and target tissues exposure would be considered. Furthermore, the MHRA noted that mutation spectra had not been used in the regulation of pharmaceuticals and medicines. Rather, further tests would be conducted or a weight of evidence approach adopted and/or a risk/benefit analysis would be used.

19. Overall, the COM concluded that the identified and summarised papers provided a reasonable representation of the current methods used in

assessing mutation spectra. It was noted that 'next generation' sequencing and new technologies would soon provide substantial new data that would potentially be very useful. Members agreed that at present, mutation spectra could not be used for regulatory purposes, but would be useful in evaluating mode of action and understanding the link between mutation and cancer. The committee agreed that it would be useful to produce a statement on mutation spectra incorporating and building on the information from the 1999 COM paper on mutation spectra by Professor A Boobis. It was also agreed that the COM should maintain a watching brief on this topic and consider a joint meeting with the COC if there are important developments in this area.

ITEM 6: TOX TRACKER (MUT/2014/03)

20. At the previous November 2013 meeting, one member had informed the committee of development of a new genotoxicity test system called ToxTracker. This comprised a system of reporter cell lines where 6 identified genes reflecting key pathways had been cloned into mouse embryonic stem cells. It was suggested that this would be useful for the COM to review.

21. Paper MUT/2014/03 described the development of the test system and proof of concept exercises. Some validation data from the Bscl2-GFP and Srxn1-GFP reporter cell lines, as presented in two publications from Dr Giel Hendriks et al (2011, 2012) from Leiden University, was also included in the paper. These cell lines are considered to identify genotoxic and pro-oxidant chemicals respectively.

22. Members agreed that the assay appeared to be an interesting approach to identifying genotoxicants and would be potentially useful in evaluating mode of genotoxic action, although it was noted that the selection of the genes used in the test system could have been chosen on an empirical basis rather than on a mechanistic basis.

23. According to the Tox Tracker website the entire system comprising six cell lines would be required for the assay to be of sufficient value. Validation data from only two of the cell lines had been published namely the Bscl2 reporter cell line responding to genotoxins and the Srxn1 reporter cell line, responding to pro-oxidants. The COM was not aware of published validation data for the other cell lines, namely Rtkn, Blvrb, Ddit3 and Btg2. The small number of chemicals tested in the presence of S9; a lack of evaluation of the effects of S9 on the expressed genes; and the unexpected results for methyl methanesulphonate (i.e. did not indicate a predominantly genotoxic response); were all considered to be limitations of the data. Currently, the apparent high sensitivity of the test system indicated on the website could not be verified from the published data.

24. Members considered that pro-oxidants have genotoxic potential i.e. if the degree of oxidation is sufficient then genotoxicity may occur. It was noted that processes that lead to oxidative stress generated *in vitro* can be very

different to those generated *in vivo* (which may also be attributable to immune driven or inflammatory responses).

25. Members indicated an interest in a comparison between the response of pro-oxidants in the ToxTracker and the Green screen (GADD45 assay). However it was also considered that the ToxTracker may be able to identify non-genotoxic carcinogens which cause cellular stress independent of DNA damage and the system would also be useful to provide mode of action information. The committee considered that an inter-laboratory trial for the use of this assay would be useful, but queried how costly and resource demanding the assay would be to use.

26. Regarding the potential use of this assay within a genotoxicity testing strategy, it was considered that it would be more useful as a biomarker assay as it does not directly address one of the three mutagenic endpoints (i.e. aneuploidy). However, it may be potentially useful in a genotoxicity testing strategy where *in vivo* testing is not permitted, such as in the testing of cosmetics. Furthermore, the committee suggested that it would be very useful to invite the developers of this assay to provide a presentation at a future COM meeting where the unpublished validation data could also be presented.

ITEM 7: OECD UPDATES (MUT/2014/04)

27. The OECD Test Guidelines (TG) are a collection of the most relevant internationally agreed test methods used by government, industry and independent laboratories to determine the safety of chemicals and chemical preparations, including pesticides and industrial chemicals. Many of the OECD test guidelines for genotoxicity had not been revised since 1997 although one (for the *in vitro* micronucleus test) was more recent. Many are being reviewed and updated and there are additional TGs for new genotoxicity assays (cell transformation and *in vivo* comet). The committee was provided with draft updated OECD genotoxicity guidelines and members were asked to provide any relevant comments that could be presented at the next meeting of the OECD Working Group of National Coordinators to the Test Guidelines Programme (WNT).

7.1 Draft TG in vitro Syrian hamster embryo (SHE) cell transformation assay

28. The committee re-iterated its previous concerns over the cell transformation assay (CTA) i.e. it does not discriminate between genotoxic and non-genotoxic substances; that it was not ready for regulatory purposes; that there was a need for further validation; even with the development of a photo-catalogue to identify morphologically transformed cells there is still a need for peer review of morphologically transformed cells; and that the underlying mechanism of the CTA was not currently understood.

29. The COM considered that the endpoint detected and the applicability domain of the assay were not clearly defined. Members felt that it was not clear what criteria constitute a positive response and that there were

uncertainties over how to interpret a positive response. It was agreed that it should not be used as a core test, but may have some use as a supplementary test.

30. Some members also considered that not all colonies were derived from fully transformed cells therefore the assay detected 'morphological changes' rather than 'transformed cells'. There was also continued concern expressed over the use of two different pHs. It was suggested that the two pHs were not equivalent because of differing sensitivity and specificity. Ideally it would be better to have a TG for just one preferred option or a separate TG for each.

7.2 Draft TG 474 mammalian erythrocyte micronucleus test

31. The COM suggested that there should be editorial alignment across the TG's for the *in* vivo tests with regards to dosing and assessment of sufficient exposure of the target tissue. The description of how to achieve the top dose (extent of toxic signs at the MTD) is not consistent across different *in vivo* guidelines. It was also commented that the recommendation not to use the assay if the test chemical (or a metabolite) will not reach the target tissue is a strange recommendation because it requires use of animals to try to show that the target tissue is not exposed.

32. The importance of the use of plasma pharmacokinetics to establish exposure was emphasised by members. It was queried whether signs of toxicity were no longer sufficient to demonstrate exposure and whether measurement of exposure was required in every case. One member said that the TG comment on sampling time or treatment compared to the lifespan of erythrocytes was unclear.

7.3 Draft TG 475 mammalian bone marrow chromosomal aberration test

33 There were no substantial comments on this draft update other than to ensure editorial alignment across the TG's for the *in vivo* tests.

7.4 *TG 473: In vitro mammalian chromosome aberration test*

34. Establishing the rate of division of the target cells in any particular laboratory, maintenance of culture conditions to ensure a high proportion of dividing cells in the (negative control) cultures and knowing the background level of cytogenetic damage in the target cells is critical to ensuring valid outcomes of the *in vitro* cytogenetic assays (TG487 and 473).

35. The COM considered that for both TG 487 and 473, it is not acceptable to use single cultures with only 3 concentrations of test chemical unless there is a robust historical data base for a laboratory showing acceptable homogeneity between replicate control cultures. It is scientifically more reliable to use duplicate cultures.

7.5 TG 487 in vitro mammalian cell micronucleus test

36. There were no substantial comments (other than above as also applicable to TG473) on this draft update.

7.6 Draft TG in vivo mammalian alkaline comet assay

37. Some members considered that this draft TG was too prescriptive and was more like a protocol than a Guideline.

38. As above, the committee advised that there should be editorial alignment across the *in vivo* TGs regarding the requirements to demonstrate target tissue exposure i.e. the approach should be harmonised. Members added that signs of toxicity should be sufficient and there should be no need to go to lethality.

39. The COM agreed to accept the draft *in vivo* comet guidance, but to raise the issue for the potential for increased use of animals (see below).

40. Overall, for all of the *in vivo* guidelines (TG474, TG475 and the new comet assay guideline) the COM questioned the requirement to demonstrate lack of difference between males and females before deciding whether to test 5 males or 3 female with 3 male. It is rare that there are no differences between males and females, and even a small difference could be considered to represent sex differences. It was felt that this would lead to most laboratories erring on the side of caution and testing 6, or even 10 animals, which would be contrary to the 3Rs principles and animal welfare.

7.7 Draft TG genotoxicity testing for manufactured nanomaterials

41. It was noted that this document was currently not sufficient to be regarded as a TG as it was more of an introductory document with the main emphasis on the characterisation of nanomaterials to be tested. However, it was acknowledged as an important aspect of the guidance. Members considered that it will be important for this document to note that the Ames test is not suitable for the genotoxicity testing of nano-materials. It was suggested that individuals with expertise in this field not at the COM meeting (both internal and external to PHE) should be asked for comments on this document.

42. Members were asked to email any additional comments on the OECD test guidelines to the secretariat.

ITEM 8: ANY OTHER BUSINESS

43. There was no other business.

ITEM 9: DATE OF NEXT MEETING

44. 19th June 2014

Item	Actions	Responsibility
Item 5: Mutation spectra	Draft or update COM statement on mutation spectra	Secretariat
Item 6: ToxTracker	Invite speaker to provide a presentation to the Com on ToxTracker	Secretariat
Item 7: OECD TGs	Provide to the COM comments on OECD TGs to the WNT meeting.	Secretariat