

MUT/MIN/2011/1

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

Minutes of the meeting held at 10.30 am on Thursday 10 March 2011 at Terrace Room, Royal Society of Medicine, Chandos House, 2 Queen Anne Street, London, W1 9LQ.

Present:

Chairman: Professor P Farmer

Members: Dr B Burlinson
Dr G Clare
Dr B Elliott
Dr D Gatehouse
Professor G Jenkins
Professor D Kirkland
Dr D Lovell
Dr E Parry
Professor D Phillips

Secretariat: Mr J Battershill (HPA secretariat)
Dr L Hetherington (HPA secretariat)
Mr S Robjohns (HPA minutes)
Ms S Kennedy (HPA administration)
Dr D Benford (FSA)

Assessors: Dr R Shillaker (HSE CRD)

In attendance: Dr O Sepai (HPA)
Dr K Burnett (HPA – Tox unit)
Ms F Pollitt (HPA)
Dr P Edwards (HPA)

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ITEM 1: ANNOUNCEMENTS/APOLOGIES FOR ABSENCE

1. The Chair welcomed Dr D Benford (FSA secretariat), Dr K Burnett (HPA Tox unit), Dr L Hetherington (HPA secretariat) Mr S Robjohns (HPA), Dr O Sepai (HPA).
2. The Chair thanked Sue Kennedy for finding a new venue for the COM meeting at such short notice. This was due to the late cancellation of the meeting room which had been booked at the Department of Health.
3. Apologies for absence were received from the members Dr C Allen and Dr A Lynch. Apologies were also received from the assessors Dr A Smith (HSE) and Dr H Stemplewski (MHCRA).
4. Members were reminded of the need to declare any interests before discussion of items.

ITEM 2: MINUTES OF MEETING ON 17th June 2010 (MUT/MIN/10/2)

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

ITEM 3: MATTERS ARISING NOT COVERED BY LATER AGENDA ITEMS

Closed session

6. COM consideration of Chlorthal dimethyl.

Open session

ITEM 4: COM STRATEGY FOR GENOTOXICITY TESTING AND MUTAGENIC HAZARD ASSESSMENT OF CHEMICALS: CONSULTATION COMMENTS (MUT/2011/02)

7. Members were asked to consider the comments received as part of the consultation exercise. A brief overview of the main comments was presented according to the sections in the draft consultation document. A total of 18 responses had been received which originated from all regions of the world. The comments covered most of the draft consultation document and were appended at Annex 1. A copy of the consultation document was appended at Annex 2. Additional information relevant to some of the main comments were also appended to aid members consideration e.g. COM 2003 statement on high dose positive results (Annex 3); a number of papers cited by consultees (Annex 4); abstracts reporting updated information on the *in vivo* micronucleus (MN) test and the comet assay (Annex 5).
8. The secretariat had begun to include minor editorial comments into a post-consultation draft document. The Committee was asked to consider the major comments and to suggest changes to the consultation document. The secretariat suggested that a small working group of members could be convened in April 2011 to give a detailed consideration to these proposed changes and prepare a revised draft document. A revised post consultation document could then be considered with the objective of finalisation at the 16th June 2011.

9. The Chair noted there was approximately 100 detailed comments and agreed that a working group was an appropriate way forward. He asked for comments on the major issues raised by consultees.

10. Overall members commented that the structure of the consultation document which included testing strategy and mutagenic hazard evaluation had confused several consultees. Guidance on a strategy for testing chemical substances with no existing data should be the focus of the current COM consideration. It would be necessary to draft a separate document on the mutagenic hazard assessment of chemical substances with existing limited or inadequate genotoxicity data. Members agreed one comment asking for an executive summary to include consideration of potential future developments in genotoxicity testing strategies. Members also noted several consultees had raised the definition of genotoxicity and agreed to use a revised definition drafted by one member. Members also noted that genotoxic endpoints should not be used interchangeably with Mode of Genotoxic Action (MoGA), which was a description of the underlying events leading to a genotoxic effect. Members considered some of the text used in the glossary should also be used in the main text.

11. One member noted that several consultees had favoured the inclusion of the mouse lymphoma assay (MLA) in the core *in vitro* test battery. They had noted that the performance of this assay had been considerably improved and that the poor specificity was largely due to the results of the US NTP testing programme, which were now considered unreliable. Other consultees had noted that the performance of the combined Ames and *in vitro* micronucleus (MNvit) tests was similar to Ames and MLA. The Committee agreed that the rationale for the proposed strategy was based on the need to evaluate three levels of mutagenicity (namely gene mutation, clastogenicity and aneuploidy) and this needed to be emphasised more clearly. Members heard that there was no compound that induced mitotic recombination, which was not detected by MNvit. Members agreed there was a similar overall performance of *in vitro* chromosomal aberration and MNvit tests for detection of clastogenicity. The COM also agreed a suggestion that the initial *in vitro* strategy of using the Ames and MNvit tests would not identify all potential mutagenic endpoints was not supported by the existing data. The committee agreed to make no change to its strategy in this regard and noted that the US Environmental Mutagen Society (EMS) also favoured the use of the Ames and *in vitro* MN tests.

12. Members confirmed that QSAR evaluations should not overrule *in vitro* genotoxicity test data. The COM confirmed that it was acceptable to include tests where no OECD guideline existed where equivalent international guidance existed (e.g. IWGT).

13. Members agreed that either the plate incorporation or pre-incubation approaches to the Ames test could be used and that adequately conducted tests did not need to be repeated. It was agreed that choice of method could be considered on a case-by-case basis. Members agreed that a short section on the HPRT assay should be included in the non-core *in vitro* tests. This should also include conclusions reached by the COM on this test at the October 2003 meeting.

14. Members heard that four potential options were under discussion with regard to the top concentration to be used in mammalian cell tests and agreed it was unlikely that a clear conclusion would be reached within the time scale for the completion of the COM guidance.

15. The COM noted the diverse views of consultees on the use of the *in vitro* comet assay. There was also the suggestion that the *in vitro* comet assay should not be included in the core strategy because it was not fully validated and did not have an OECD guideline. However, the COM considered that they had fully addressed this issue and confirmed this test should be placed in the non-core *in vitro* test section. Members considered that it was not essential to only include tests with an OECD guideline in its overall strategy. The COM also noted the extended period of time that it takes to obtain an OECD test guideline, which would make the sole reliance on such tests impractical.

16. Members agreed that the order of tests in the text outlining the core- Stage 2 *in vivo* tests should be altered so that the *in vivo* MN test should come first. Members acknowledge that the additional data on the performance of the *in vivo* Pig-A test should be cited in the section on future developments. It was noted that Pig-A measured mutation in the erythropoietic system and could be included in regulatory toxicity tests. One member noted that current work to sequence Pig-A mutant DNA was underway.

17. The Committee agreed to reconsider the testing strategy document at the 16th June 2011 meeting.

ITEM 5: GENOTOXICITY TESTING OF IMPURITIES (MUT/2011/1)

18. The COM guidance on a testing strategy published for consultation makes specific reference to the development of guidance for the testing and evaluation of impurities. Test substances may also contain impurities at varying concentrations which may also be genotoxic. Separate guidance on the genotoxicity assessment of impurities was identified as a priority during the COM horizon scanning exercise in 2010.

19. The committee previously considered a number of aspects associated with genotoxicity testing of impurities at its June 2008 meeting (MUT/08/10). This paper was appended at Annex 1. Briefly, it reported that out of 454 mutagens tested in the Ames test; 87% were identified at < 250 micrograms (µg)/plate; 11% at 250 – 2,500 µg/plate; and 2% at 2500 – 5,000 µg/plate (Kenyon MO et al., 2007. *Regul Toxicol Pharmacol* 48, 75-86).

20. The COM had also considered a proposed rationale for determining the testing, and control of specific impurities in pharmaceuticals that are genotoxic. Muller L et al., 2006 (*Regul Toxicol Pharmacol*, 44, 198-211), identified five categories of compound with positive carcinogenicity and/or genotoxicity data and/or alerting structures. The authors proposed restricting exposure to the threshold of toxicological concern (TTC i.e. 0.15 µg/person day for genotoxic substances (with certain exceptions) as proposed by Kroes et al., 2004 (*Food Chem Toxicol* 42, 65-83) as the most appropriate risk management option.

21. The draft COM guidance on testing and evaluation of impurities (MUT/2011/01) suggested that the TTC can aid in ranking priorities for the testing of chemicals where there is little or no genotoxicity data. The proposed draft approach to the genotoxicity testing of impurities included three key elements:

- Use of TTC (0.15 µg/person/day (or 0.0025 µg/kg bw/day)) to select impurities requiring testing and assessment
- Use of QSAR for all impurities. Use of 5% incorporation rate to determine whether impurities should be isolated before testing
- Testing of impurities to include the Ames and *in vitro* MN test

22. It was also noted that for pharmaceuticals, QSARs plus an Ames test is considered sufficient.

23. The secretariat had sought the advice of Emeritus Professor A Renwick from the Faculty of Medicine at Southampton University, who considered that the use of the TTC is appropriate for chemical impurities where there are no carcinogenicity data and that this would represent a conservative approach (Annex 3 to MUT/2011/1) (Professor Renwick was one of the originators of the of the TTC approach to risk assessment and prioritisation for toxicity testing).

24. The committee was asked for its views on the above proposals.

25. Members considered it would be helpful to consider the proposed draft COM guidance on impurities in conjunction with the ongoing ICH consideration of this topic. Members agreed the TTC was a useful concept in identifying impurities requiring genotoxicity assessment, although reference needed to be made to the classes of concern, e.g. aflatoxin-like, azoxy and N-nitroso compounds.

26. Members considered the practical aspects of the application of the proposed incorporation limit of 5% for testing impurities in a test substance in the Ames test. For example, a dose of 1500 µg/plate was required to detect mutagenicity of ethyl methane sulphonate (EMS) in the Ames test which equated to 30% in a test material. Members agreed that a combination of Ames and MNvit would optimise the detection of mutagenic hazard of impurities. There were some data available regarding the sensitivity of the Ames test with regard to the lowest concentrations of mutagens that could be detected but there was no equivalent data available for the MNvit. Members felt the sensitivity of MNvit to detect low concentrations of mutagens would be lower than the Ames test. Thus, overall, the Committee agreed that impurities should be isolated and tested separately. QSAR data would be helpful, but would be limited in the case of novel structural moieties and in some instances where metabolic activation of pro-mutagens occurred.

27. Members agreed to consider this topic further under matters arising at the 16th June 2011 meeting.

ITEM 6: SIGNIFICANCE OF CHEMICAL INDUCED MUTATION FOR HUMAN HEALTH (MUT/2011/3)

28. At the June 2010 COM meeting, the committee considered a short paper on the significance of chemical induced mutation for human health. It was decided that the paper should be expanded to include more detail and that it would be a stand alone document available on the COM website i.e. as part of the Guidance series. Additionally, it was agreed that this was an opportunity to outline some basic concepts regarding the process of chemically induced mutagenesis. This would also explain the role of the COM and put its work into some context. The committee agreed that the document should be aimed at informed non-expert lay-readers. Paper MUT/2011/03 was intended to be quite broad and cover the basis principles potentially relevant to human health. It was intended that an executive summary and glossary could be produced once the main document had been finalised. Members were asked for their comments on the expanded document.

29. The committee agreed that this was a good document, which was at the correct level for the target audience. It contained a lot of detail, but this was considered to be necessary. Members suggested that there could be some re-ordering with a brief executive summary and some explanation of the significance for human health appearing towards the front of the document. It was also suggested that the explanation of mutation could also be moved nearer to the beginning. The beginning of the document could use more lay language with more details appearing later. Regarding referencing, members agreed that a numbering system could be used throughout the main body of text with the references listed at the end of the document.

30. Some explanation of the potential role of mutations in relation to the genetic component of human disease was requested, with an emphasis on the lag time between an initial mutation and subsequent disease. This was one reason why it was considered difficult to establish evidence for a link between an initial mutation and a resultant adverse health outcome. Also some mention of the potential for mutation to result in adverse developmental effects was requested i.e. rather than just cancer.

31. The committee agreed that the use of visual aids, such as figures and diagrams could help the understanding of the important principles and processes of mutagenicity. Appropriate sources for such information might be gleaned from the internet including 'Wikipedia'.

32. Members went through the document and made a number of more detailed specific comments. One member had already provided an explanation and definition of genotoxicity prior to the meeting for incorporation into the COM guidance, which could also be used in this document. Other specific points included appropriate citing of genotoxic endpoint and use of the term Mode of Genotoxic Action (MoGA) particularly in relation to references to DNA strand breaks, unscheduled DNA synthesis (UDS) and sister chromatid exchange (SCE) ; and further explanation of the term 'error prone repair'

33. Members considered that reference to germ cell mutagenicity of trichlorophen and ethyl nitroso urea (ENU) should be included. Members were asked to send other specific detailed comments to the secretariat after the meeting.

34. **Closed session**

ITEM 8: QSARS IN AN INITIAL ASSESSMENT OF THE POTENTIAL MUTAGENICITY OF DRINKING WATER CHEMICALS- ILLUSTRATIVE EXAMPLES (MUT/2011/04) RESTRICTED COMMERCIAL IN CONFIDENCE

35. **Open session**

ITEM 9: DRAFT ANNUAL REPORT FOR 2010 (MUT/2011/5)

36. Members were reminded that the draft COM annual report for 2010 had been circulated and that any comments should be sent to the secretariat.

ITEM 10: RISKS TO HEALTH FROM CLIMATE CHANGE (MUT/2011/06)

37. The COM was informed that the Committee on Toxicity (COT) had discussed the topic of the risks to health from climate change. Extracts from the draft COT minutes relevant to this item were circulated to COM members. The Chair asked whether there were any comments.

38. The COM did not have any substantial additional areas of concern in relation to short, medium and long-term climate change than those identified by the COT e.g. potential change in the exposure to mycotoxins and pesticides. The latter might change due to a greater use of pesticides to treat increases in pests such as mosquitoes. The Committee felt it was unlikely that new mutagenic chemicals would be generated as a result of climate change.

ITEM 7: ANY OTHER BUSINESS

UPDATE ON OECD TEST GUIDANCE CONSIDERATION

39. One member informed the committee of the various topics that had been discussed at a recent OECD expert meeting. There had been agreement to delete the test guidelines for Mouse Spot test, heritable Translocation, SCE, *in vitro* UDS. It was agreed the guidelines on yeast assays and *Drosophila* should be archived. The test guidelines for *in vitro* chromosome aberration, *in vitro* micronucleus and mammalian cell mutation tests would be updated. A new guideline for the mouse lymphoma assay has been proposed. There had been discussion regarding assessment of precipitation for setting the top dose level in the *in vitro* micronucleus test. It was not expected that dose levels within the precipitation range would be investigated. There was also a preference for P53 competent cell lines.

40. The guidelines for the *in vivo* micronucleus and chromosome aberration (bone marrow and spermatogonia) tests would be updated. The guideline for the Transgenic rodent mutation assay had been finalised. There was a suggestion from Japan to integrate the mutation assay with 28-day repeat dose toxicity assessment, which would mean sampling for mutations on day 29 instead of day 31. Allowance for this was included in the guideline, but with caution to demonstrate that mutations could be detected in slowly proliferating tissues by using a suitable weak positive

control mutagen. It was hoped that the transgenic mutation assay would be more widely used with the completion of the test guideline.

41. There was a request to prepare a guideline on DNA adducts which would be a very complex undertaking. In addition it was also agreed that the Introductory chapter to genotoxicity testing would be revised (last revised in 1986). This would include guidance on strengths and weaknesses of tests and how they could be used in a complimentary fashion, but would not include guidance on strategy for testing.

42. The meeting ended at 15.45

ITEM 8: DATE OF NEXT MEETING

43. 16th June 2011.

Item	Actions	Responsibility
Item 4: Revision of COM Guidance: Consultation on a strategy for genotoxicity testing and mutagenic hazards of chemicals.	A COM working group would consider the minor editorial comments. The document would also be amended in light of major comments, then circulate to members	Secretariat & COM working group.
Item 5: Genotoxicity testing of impurities.	The COM would consider this at a later date after the consideration of other expert groups had been published	
Item 6: Human health significance of mutagenicity	Revise document in light of members comments	Secretariat
Item 7: QSARS for illustrative chemicals	Provide more background to the JRC review of QSARs	Secretariat