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2 **MUT/MIN/2010/3**

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

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7 Minutes of the meeting held at 10.30 am on Thursday 21st October 2010 at
8 Room 136/137B Skipton House, Department of Health, London SE1 6LH.

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12 **Chairman:** Professor P Farmer

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15 **Members:** Dr B Burlinson
16 Dr G Clare
17 Dr B Elliott
18 Dr D Gatehouse
19 Professor G Jenkins
20 Professor D Kirkland
21 Dr D Lovell
22 Dr A Lynch
23 Dr E Parry
24 Professor D Phillips

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26 **Secretariat:** Mr J Battershill (HPA secretariat)
27 Dr D Mason (HPA secretariat)
28 Mr S Robjohns (HPA minutes)
29 Ms S Kennedy (HPA administration)
30 Dr D Benford (FSA)

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32 **Assessors:** Dr R Shillaker (HSE CRD)

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36 **In attendance:** Dr O Sepai (HPA)
37 Dr K Burnett (HPA – Tox unit)
38 Dr N Hajji (Imperial College)

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A G E N D A

	Paragraph
Open session	
1. Announcements/Apologies for absence	1
2. Minutes of the meeting held on 17 June 2010 (MUT/MIN/10/2)	5
3. Matters Arising (not covered by later agenda items)	6
4. Revision of COM guidance on a strategy for genotoxicity testing And mutagenic hazard assessment of chemical substances (4th draft) (MUT/2010/15)	8
5. Horizon scanning (MUT/2010/16)	24
6. Presentation on: ‘Cytokinesis-block micronucleus (CBMN) assay for measurement and comparison of carcinogenic and *in vivo* genotoxic potency estimates’	31
7. Any other business	36
8. Date of next meeting: 10th March 2011	37

DRAFT

ITEM 1: ANNOUNCEMENTS/APOLOGIES FOR ABSENCE

1. The Chair welcomed Dr D Mason (HPA), Dr D Benford (FSA secretariat), Dr K Burnett (HPA Tox unit), Mr S Robjohns (HPA), Dr O Sepai (HPA). The Chair also welcomed Dr Nabil Hajji from Imperial College who would be attending for item 6.

2. Apologies for absence were received from the members Dr C Allen and Mrs R Glazebrook. Apologies were also received from Dr P Edwards (HPA), Ms F Pollitt (HPA), and the assessors Dr A Smith (HSE) and Dr C Pease (EA).

3. The Chair announced that Dr D Mason (HPA) would soon be leaving the HPA and thanked him for the good work he had done for the COM, particularly in helping develop a new approach to the COM guidance, and wished him well with his new position.

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

DRAFT

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

5. Members agreed the minutes subject to some minor editorial changes. An addendum to the minutes would also be required to include minor amendments of the data contained in the presentation by Professor Kirkland.

ITEM 3: MATTERS ARISING NOT COVERED BY LATER AGENDA ITEMS

6. The committee was informed that the studies on the genotoxicity of fumagillin recommended by COM were expected to be completed towards the end of the year. Thus, a COM opinion on the testing results may be required at the March 2011 meeting.

7. The COM was also informed that the committee was unlikely to be significantly affected by the recent Government announcements regarding non-Departmental Public bodies and their various advisory committees. The independence of the COM was expected to be maintained. Members agreed to maintain a watching brief on developments regarding independent advisory committees.

ITEM 4: 4th DRAFT DISCUSSION PAPER: GUIDANCE ON A STRATEGY FOR GENOTOXICITY TESTING AND MUTAGENIC HAZARD ASSESSMENT OF CHEMICAL SUBSTANCES (MUT/2010/15)

8. The committee had previously reviewed the revised guidance document at meetings in March 2010 (MUT/2010/01) and in June 2010 (MUT/2010/09). Since the last meeting, the Secretariat and the HPA Toxicology Unit had incorporated additional amendments and suggestions from COM members. The resultant 4th draft discussion document was

presented for further consideration. Major corrections and amendments were detailed as track changes. Members were informed of the important changes which were as follows:

- Title change. There was advice from HSE that it was not possible to distinguish between ‘new’ and ‘existing’ chemicals under REACH
- Further amendment of the Annexes; including the addition of a third annex providing a rationale for the change of *in vitro* clastogenicity assay.
- Clarification of substance vs. compound
- Further review of the section on QSAR. An authoritative and comprehensive evaluation of different QSAR approaches to the identification of genotoxic potential had been produced for the European Food Safety Authority. The HSE had also provided helpful comments
- Clarification of the use of the in vitro comet assay. Members had provided helpful comments via email
- Review of section on the *in vitro* comet assay. HSE had requested clear guidance on what endpoints could be detected using the comet assay
- Inclusion of comments from Dr Ilse-Dore Adler on germ cell effects

9. Members were asked to comment on the 4th draft, to provide any outstanding/additional references and any proposals for organisations to be consulted. It was intended that the draft document could be progressed to the stage where it could be released for general consultation before the end of 2010.

10. The Chair informed members that Stage 2 of the revised strategy would be considered first as the committee had previously considered Stage 0 and Stage 1 in detail.

11. The committee considered that the guidance document should provide a clear statement on when specific germ cell testing was required. Members confirmed that an *in vivo* somatic cell mutagen is presumed to be an *in vivo* germ cell mutagen, unless there is evidence to the contrary. Thus, there was no need to conduct germ cell specific genotoxicity testing to assess potential heritable effects where there was a robust negative *in vivo* somatic cell test result. However, members acknowledged that there was some uncertainty, as there were some aspects of germ cell genotoxicity involving meiotic cell division which could not detected by somatic cell tests. The COM also acknowledged that there were relatively few germ cell specific data available.

12. Members made a number of comments regarding relatively minor amendments to figures 1,2 and 3, such as renumbering and rearrangements, to improve consistency with the text in the main document.

13. Members highlighted the need for further clarification on the rationale for conduction *in vivo* studies. The committee discussed whether *in vivo* genotoxicity testing was required for chemicals with high or moderate and

DRAFT

prolonged exposure where *in vitro* genotoxicity tests had given negative results. The committee considered that in vast majority of cases robust negative *in vitro* test results were sufficient for assessing mutagenic potential of a test substance. However, members acknowledged that there was a possibility that *in vitro* tests may not detect a few *in vivo* mutagens. It was recognised that some regulatory bodies required an additional *in vivo* test for reassurance, where there was high or moderate and prolonged human exposure.

14. Members commented on the suggestion in the 4th draft document that there was a need for further development of the comet assay protocol to detect weak mutagens. Members considered that there was a need to develop a case-by-case protocol when investigating tissues with a low rate of cell turnover. For the comet assay, members agreed that where possible it would be useful to take more samples of different tissues than initially examined as these could be frozen and assessed at a later date if required. Members commented that the comet assay responded to a wide range of DNA damage including single and double strand breaks, repair induced breaks, alkali labile lesions and abasic sites and agreed that this needed to be included in the sections on this assay. Members also noted that the in vivo comet assay could respond to gene mutagens. There was discussion regarding which tissues should be selected, in cases where no specific tumour tissue had been identified. Members considered that a site of contact (e.g. gastrointestinal system) and site of metabolism (liver) would be appropriate in such circumstances.

15. The chair noted that the secretariat had circulated a short summary on the uses of the *in vitro* comet assay (MUT/2010/17). A positive comet assay may be due to repairable DNA damage or lesions which lead to cell death and not necessarily mutations or micronuclei. Negative results from an Ames test and/or in vitro micronucleus test would reduce the level of concern associated with positive results from an in vitro comet assay.

16. Members were requested to provide any comments on Table 1 on supplementary *in vivo* genotoxicity tests via email after the meeting.

17. After considering stage 2 testing, the committee then reconsidered the revised draft text for stage 0 and stage 1.

18. Members considered that there was a need to be clear that information was obtained on three levels of genetic damage. The detection of chromosome aberrations did not measure mutation or heritable effects directly. Most cells with gross chromosomal damage would not be viable.

19. Overall, (Q)SAR approaches for the prediction of genotoxic activity can be a valuable tool to aid in the high throughput screening of compounds, the provision of assessments for chemicals for which no genotoxicology test data are available and also prioritisation for genotoxicity testing. Q(S)AR can also aid in the interpretation of genetic toxicology tests, although currently such predictions cannot replace the need to undertake the *in vitro* and *in vivo*

DRAFT

genotoxicity tests currently required to derive conclusions on mutagenic hazard. With regard to chemicals for which there are limited, inadequate, or no available genotoxicity test data,, a (Q)SAR prediction of mutagenicity (within the domain of applicability of the system(s) used) should be taken as preliminary evidence for potential or lack of potential mutagenicity. However expert judgement is needed when reaching conclusions on mutagenic hazard on the basis of (Q)SAR information alone. In reaching conclusions, data from well conducted *in vitro* genotoxicity tests should be attributed a much higher weight of evidence than (Q)SAR predictions, although all information should be assessed on a case-by-case basis.

20. Members considered that the document should be clear that the threshold of toxicological concern (TTC) approach is adopted for assessing low exposure and is not informative for moderate or high or prolonged exposure.

21. Regarding *in vitro* genotoxicity testing, members considered that it was important to emphasise that the testing strategy was optimised to avoid misleading positive results as well as misleading negative results. The Committee considered that negative historical control data can be a valuable aid in the interpretation of genotoxicity tests. Members noted that the recent publication by Hayashi M, et al Compilation and use of genetic toxicity historical control data. Mutation Research (accepted for publication 29 September 2010) should be cited. Members agreed that it should be possible to omit concurrent positive control administrations in micronucleus and chromosome aberration tests (but not for the *in vitro* comet assay) where the test facility had appropriate historical positive control data as positive control slides ‘banked’ from previous treatments and coded in with the experimental slides (i.e. to demonstrate the proficiency of the technicians).

22. In relation to the *in* vitro micronucleus assay for clastogenicity and aneugenicity, the committee agreed that the use of human cells either primary or established cell lines were preferable to the use of rodent cells.

23. The COM agreed that a small group of members would meet approximately 10 days after the current meeting to go through the document to make editorial changes before it could go out for consultation. Members would be consulted if there were any scientific changes to the document made by this group.

ITEM 5: HORIZON SCANNING 2010 (MUT/10/16)

24. A horizon scanning exercise is conducted every year, where new and emerging topics in the field of genotoxicity are identified that may require review. The horizon scanning process provides an opportunity for members and advisors from Government Departments and regulatory agencies to suggest topics for further work. This year, most of the committees’ work has involved updating the current COM guidance and resources had not been available to undertake all of the projects identified in the 2009 horizon scanning exercise. MUT/10/16 reviewed progress made on last years topics

DRAFT

and re-considered previously suggested reviews that had not yet been conducted.

25. Some topics raised during the 2009 horizon scanning exercise were considered as part of the drafting of the revised genotoxicity testing strategy and generation of new guidance documents. These included:

- Does the mouse lymphoma assay detect aneugens?
- Which mammalian cell test best compliments the Ames test in terms of detecting rodent carcinogens and *in vivo* genotoxins?
- An evaluation of the GADD45a-GFP ‘GreenScreen HC’ genotoxicity assay.

26. Additionally, the COM agreed a format for separating the guidance into separate statements. Progress was also made on consideration of the validation of the mutation assay using the PIG-A gene. A review of expanded simple tandem repeat (ESTR) mutation had been initiated, but not completed.

27. Topics not addressed in 2010 included a review of the mutagenicity of nanomaterials; mutational spectra in the investigation of chemical mutagenesis; the role of epigenetics in mutagenesis; mitochondrial mutagenesis; and exposure to mutagens via soil.

28. Members were asked to provide any views on how this work should be prioritised to the secretariat. The genotoxicity of nanomaterials was suggested as a priority topic. The Chair noted that the secretariat would have to complete a review of the significance of chemical induced mutation for human health and a review of genotoxicity testing of impurities.

29. The committee was also informed that some funding was available to the HPA for research projects relevant to public health. This included initial funding for a one year project of around 25K as well as larger two to three year projects of up to a maximum of 250K per year. There was also scope to fund PhD’s. Members were asked for suggestions for suitable projects which could also involve collaborative work between different organisations.

30. Some initial suggestions included research into low dose genotoxicity effects compared with higher doses; non-DNA target; systems biology approach to key gene suppression and expression; and the sequencing of whole genomes for different cancers.

ITEM 6: PRESENTATION ON ‘CYTOKINESIS-BLOCK MICRONUCLEUS (CBMN) ASSAY FOR MEASUREMENT AND COMPARISON OF CARCINOGENIC AND *IN VIVO* GENOTOXICITY POTENCY ESTIMATES’

31. Dr Nabil Hajji from Imperial College gave a presentation on developing a generic approach to ranking *in vivo* mutagens where there is no carcinogenicity data. An approach using only a single end point from an *in vivo* genotoxicity test was suggested to be preferable as this would be relatively simple and readily comparable. An approach to ranking *in vivo*

DRAFT

mutagens, which did not have carcinogenicity data, using the lowest effective dose (LED) had already been developed by Sanner and Dybing 2005 (Basic & Clinical Pharmacology & Toxicology 2005,**96**, 131 – 139)**).**

32. Dr Hajji had identified three potentially useful database sources. A published evaluation of the rodent MN tests undertaken as part of the US EPA Gene Tox program during the 1980s and 1990s was suggested as a useful source of information. Under this program, 506 chemicals had been assessed, but not all the current data was available in the public domain or readily accessible for the derivation of LED values. Another potentially useful data source was the 6th Collaborative Study Group on the Micronucleus Test (CSGMT) available from the Japanese Environmental Mutagen Society (JMS). This identified approximately 100 mouse MN assays predominantly undertaken by using the intraperitoneal dosing. A third suggested data source was the International Programme on Chemical Safety (IPCS) INCHEM database.

33. It was proposed that the data sources would be used to obtain *in vivo* genotoxicity potency estimates such as the LED or the Benchmark Dose (BMD) (i.e. where there were at least 3 dose-response data points). These could then be compared with available carcinogenicity potency estimates such as the TD50 (the chronic daily dose that will give rise to 50% of the test animals having tumours above background at a specific site).

34. Members considered that it was important to be aware that several groups were already undertaking similar work and that it may be useful to liaise with them. For example, RIVM and ILSI/HESI. Members noted that the ILSI/HESI group were looking at extrapolating from *in vitro* genotoxicity potency to *in vivo* potency. Whereas the RIVM group, were considered to be mainly looking at *in vivo* data for prioritising mutagens and to examine what could be learned about carcinogenic potential without carcinogenicity data. It was also suggested that it may be helpful to invite someone from the RIVM to a COM meeting to give a talk on their work.

35. The committee agreed that where possible, the use of BMD would be preferable to the LED, and that it would be important to define the biologically significant response level e.g. 1% or 10% above the control response.

ITEM 7: ANY OTHER BUSINESS

36. No other items of business were raised.

ITEM 8: DATE OF NEXT MEETING

37. 11 March 2011.

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Item	Actions	Responsibility
Item 4: Revision of COM Guidance: Draft discussion paper on a strategy for genotoxicity testing and mutagenic hazards of chemicals.	Revise draft in light of comments and prepare for external consultation Arrange editorial group meeting.	Secretariat

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DRAFT