Committee on MUTAGENICITY

MUT/MIN/2015/2

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

Minutes of the meeting held at 10.30 am on Thursday 18th June 2015 at the Department of Health in Room 137B Skipton House, Elephant and Castle, London, SE1 6LH.

Present:

Chairman:	Dr D Lovell
Members:	Dr C Beevers Dr G Clare Dr S Dean Professor S Doak Professor M O'Donovan Ms P Hardwick Professor G Jenkins Professor D Kirkland Professor D Phillips
Secretariat:	Dr O Sepai (PHE Secretary) Mr B Maycock (FSA Secretariat) Dr K Burnett (PHE Tox Unit) Mr S Robjohns (PHE Secretariat)
Assessors:	Dr Lata Koshy (HSE) Dr I Martin (EA)
In attendance:	Mr W Karani (DH) Mr D Dipple (DH) Mr P Holley Ms F Pollitt Miss B Gadeberg Ms C Mulholland

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ITEM 1: ANNOUNCEMENTS/APOLOGIES FOR ABSENCE

1. The Chair welcomed Members, the secretariat and assessors. The new Member Dr Carol Beevers was welcomed to the Committee. Mr B Maycock was attending for the FSA. The Chair also welcomed Paul Holly (DH), William Karani (DH) and David Dipple (DH) who were attending in relation to a triennial review of the COM.

2. Apologies for absence were received from the members Professor M Rennie, Professor A Lynch, Professor F Martin. Apologies were also received from Dr D Benford (Secretariat) and from the assessors Dr S Fletcher (VMD), Dr C Ramsay (Health Services Scotland) and Dr H Stemplewski (MHRA).

3. The Committee on Mutagenicity (COM) was asked for its view on a request from the secretariat to record the present and future meetings to aid in the production of the technical minutes. The recordings would not be shared outside the secretariat and would be deleted after agreement of the minutes at the next meeting. There were no objections to this suggestion and it was agreed that meetings could be recorded.

4. The COM was informed that as it was an Advisory Non-Departmental Public Body (ANDPB) it would be undergoing a triennial review. The COM is an ANDPB partly due to the composition of the Committee, having over 50% of its members from the private sector. The review would have two stages. The first would look at the functions to see if they could be delivered more efficiently. The second would look at its governance and performance, looking at efficiency and wider relationship with other stakeholders. Due to the COM's relatively small size and low running costs, a 'light touch' review would be undertaken. The review would be led by William Karani from the Department of Health (DH). The reviewers would be meeting with the Chair of the COM, Dr David Lovell, after the present meeting and would be contacting the other members of the COM individually for their views (e.g. for any suggestions for improvements to the COM). The review would seek three sources of evidence i.e. one to one with Members; an online call for evidence via a questionnaire; and via an open workshop to be held at Richmond House (Department of Health) on the 5th of August 2015. It was intended that most of the review would be completed by around September 2015.

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

ITEM 2: MINUTES OF MEETING ON 5th March 2015 (MUT/MIN/2015/1)

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

ITEM 3: MATTERS ARISING

7. The Committee was informed that following a re-structuring process at the Centre for Radiation, Chemical and Environmental Hazards (CRCE) in

PHE, there was still a vacant administrative post in the secretariat dedicated to the COM. Also following the PHE re-structuring process, the secretariat was still under pressure to balance committee work with their other non-committee workload. On-going efforts are still being made to improve the current COM website to make previous Committee papers more visible and more accessible.

ITEM 4: HORIZON SCANNING: 3D TISSUE MODELS (MUT/2015/06)

8. The COM undertakes an annual 'Horizon Scanning' exercise, which provides an opportunity for Members and assessors from Government Departments/Agencies to discuss and suggest topics for further work. A formal 'Horizon Scanning' exercise had not been carried out in 2014. At the last meeting in March 2015, there had been some initial discussion of potential topics and consideration of paper MUT/2015/5, which provided a brief outline of topics recently reviewed (e.g. cell transformation assay and mutation spectra) and some for consideration (e.g. gene expression profiling; quantification of genotoxic responses; epigenetics and epimutations; and 3D tissue models). Members had also been asked to suggest topics. In response to the initial discussion paper MUT/2015/06 had been drafted.

9. A number of topics had been suggested, such as the incorporation of genotoxicity studies into repeat dose toxicity studies; toxicokinetic data in relation to genotoxicity testing; mitochondrial DNA mutations; the in vivo PIG A assay; and the use of 3D models in genotoxicity testing. In response to the latter it was agreed that one member of the Committee Professor Shareen Doak from Swansea University would give a presentation on this.

Presentation on 3D models by Professor S Doak:

10. Professor Doak informed the COM that 3D models for genotoxicity had so far mainly been developed for the skin (e.g. 3D reconstructed skin micronucleus assay and the 3D reconstructed skin comet assay). The main drivers for the use of such 3D models was the Cosmetics Directive preventing the use of *in vivo* testing for cosmetics and the 3Rs principle requiring the reduction in the use of animal toxicity testing.

11. 3D models are likely to be more physiologically relevant than 2D assays. There are clear differences in the way cells behave (e.g. improved metabolic capacity and closer to *in vivo* gene expression and protein function). There are different types of models, ranging from single cell microtissues to multi cell types grown within scaffolds. It is hoped the use of such models will reduce the number of misleading positives and improve the accuracy of predictions. Most of the available data relates to skin models, which have the additional advantage of allowing topical application, more realistic metabolism, and use of a skin barrier. Other endpoints can also be evaluated, such as irritation. The genotoxicity 3D models allow both the assessment of micronuclei (MN) and comet evaluation. There is on-going international validation of 3D MN and comet assays. For the 3D reconstructed micronucleus assay, the first phase of evaluation involved optimisation of the

protocol, which has been completed. The second phase involved inter- and intra-laboratory reproducibility with 5 coded chemicals, which has also been completed. Phase 3, pre-validation, with 38 coded chemicals is underway.

12. The 38 coded chemicals included three groups i.e. known positive genotoxic substances; known misleading positive substances; and known non-genotoxic substances. The chemicals were tested in at least two laboratories. At least two valid experiments were conducted for each chemical and the results sent to ECVAM for decoding and evaluation. Inter-laboratory reproducibility was high. There was excellent specificity (>90%). Initially sensitivity was around 70%, but modifications have improved sensitivity to 88% (e.g. increased exposure time). As skin has very low phase 1 (normally bio-activating) capacity, an extended dosing period may allow more time for pro-mutagens to be metabolised to active substances. Over 90% of the negative substances were correctly predicted and the false positives were mainly correctly predicted as negative. A 3rd, phase investigating protocol improvements, had been entered.

13. It was suggested that in terms of regulatory testing of cosmetics, a tiered approach to genotoxicity testing could be used (including the use of the Ames test, *in vitro* mammalian cells and a 3D skin MN/comet). It was noted that the 3D MN test could also be used to determine whether a substance was clastogenic or aneugenic. A current disadvantage of the test system was that it did not currently have a high throughput method, although scoring had the potential to be automated.

14. Development and evaluation of the 3D comet genotoxicity test has lagged behind that of the MN 3D test. Phase 1 of development showed relatively poor sensitivity. A move towards full thickness skin models has improved the results. Phase 1 was now largely completed. Phase 2 has so far shown quite good reproducibility across laboratories, but there was some concern over the detection of pro-mutagens. A re-evaluation was underway with the use of aphidicolin (APC - a DNA-polymerase inhibitor), which increased sensitivity without reducing specificity. Every negative finding was now being re-tested with APC. A re-evaluation involving the Cosmetics Europe initiative merging with the German project "Comet" was intended to be completed in 2016.

15. Other 3D models were also being developed. Swansea University was currently investigating the use of human liver HepG2 cells grown as microspheres in hanging drops. These spheres of cells grow over time, and reach optimal size and cytochrome P450 enzyme levels after about 4 days. Some cells towards the centre of the mass undergo necrosis with time. Early studies have looked at benzo(a)pyrene induction of micronuclei in these 3D liver models.

16. Advantages of 3D genotoxicity models include the fact that a greater proportion of MN are produced than in 2D models (which could be due to a greater cytochrome P450 enzyme expression). They could be used to help bridge the gap between *in vitro* and *in vivo* assessment. Another advantage of

3D models is the potential to assess nano-material genotoxicity via dermal exposure (a section can be taken through the tissue to look at nanoparticle migration through the tissue and the skin barrier). Tissues other than the skin could be considered in the future. Disadvantages of 3D models are that they are currently relatively expensive; require expertise to conduct; and are at early stages of development requiring further work and validation. They will not replace the currently accepted tiered genotoxicity testing requirements.

17. In the discussion after the presentation, Members noted that the sponsors considered that it was too early to release the data for outside assessment (unlikely that the data would be made publicly available within the next 18 months). It was likely the 3D MN model would be considered by an IWGT working group in 2017. There was still a need to carry out further work e.g. an assessment of reproducibility. Further work was also required on the 3D comet model. Overall, the COM noted that progress had been made in the area of 3D genotoxicity models and would follow the developments with interest and provide any comment when necessary.

18. Other potential topics identified from 'horizon scanning' paper were discussed. The integration of *in vivo* genotoxicity assays into repeat dose toxicity tests was considered. It had been noted that incorporation of the micronucleus endpoint was already routinely undertaken for human pharmaceuticals and that ICH S2(R1) gave some guidance on dose selection based on toxicokinetic data. Such data were not widely available for other types of chemicals, such as food additives and industrial chemicals (it was noted that the comet assay was not routinely integrated into repeat dose studies). Therefore, there was a question whether the COM needed to provide some guidance on studies where toxicokinetic data were not routinely included. Members noted that there was a lot of focus on chemical levels in the plasma, but that this may not reflect target tissue exposure. It was suggested that some chemicals may give positive results following acute exposure and that negative results may be obtained if the top dose is reduced in 28 day studies. It was of key importance to know whether there was sufficient target tissue exposure, especially when negative genotoxicity results had been obtained. Members agreed that it would be useful to request a presentation to the COM by an expert in toxicokinetics, and then the Committee would be able to consider any appropriate advice.

19. Epigenetics had been considered by the Committee on Carcinogenicity (COC) in 2013 and the relevant overview paper had been provided to the COM for information. The COC had also considered a paper in 2013, which included specific examples of benzene, arsenic and endocrine disruption. It was concluded that it was possible that epigenetic changes could contribute to the carcinogenicity for arsenic and benzene, but more work was required before this could be proven. Epimutation is defined as abnormal transcriptional repression of active genes and/or abnormal activation of usually repressed genes caused by errors in epigenetic gene repression. From the 'horizon scanning' literature review, it was evident that there was considerable research on the role of epigenetic changes in carcinogenesis. However, no studies were identified that looked at the impact of specific

chemical exposure on epimutations. Members considered that this was an interesting topic and that epimutations in somatic cells were transient and not fixed. It was regarded as an important mechanism in cancer, but it may not be relevant to the COM. However, if epimutations occur in germ cells and can be heritable, then this would generate potential concern and would be worth investigating. It was difficult to find chemical specific data, with most evidence being anecdotal. The best available evidence related to arsenic. It was decided that Professor Tim Gant from PHE could be asked to give a presentation to the COM on this subject and that the Committee should keep a watching brief on this topic.

20. The 'horizon scanning' provided a brief overview of mitochondrial DNA mutations. Identified studies often focused on oxidative damage. The majority of the papers were used to generate hypotheses for a variety of health effects (e.g. neurodegenerative diseases, heart conditions, epilepsy and diabetes etc.). Limited data were identified on associations between environmental chemical exposure and mitochondrial mutations and disease. Members noted that there had been a lot of work on the impact of mitochondrial DNA damage and repair. Chemical exposure may have other non-mutagenic adverse effects on mitochondria leading to disease. The COM agreed that there were hundreds of mitochondria in a cell, and that the effect of mitochondrial DNA mutation in just one or a few mitochondria was not known. One member noted that there had been some work reporting that the DNA mutation rate was much higher (e.g. tens of times higher) in mitochondria than in the cell nucleus, but this work had not been followed up. It was also noted that genotoxicity testing in mitochondria would likely require genome sequencing and that this would be easier than for nuclear DNA, because mitochondrial DNA has far fewer base pairs. The Committee agreed to keep a watching brief on this topic.

21. Regarding the topic human DNA mutation rate and disease (e.g. in germ cells), the Committee acknowledged that were a large amount of data being developed on mutation rates with age. Members were aware that some work had previously been carried out on the effects of radiation on mitochondrial DNA mutation and therefore suggested that the secretariat contact PHE radiation colleagues for a view on this topic. It was decided that it was worthwhile for the COM to monitor developments in this area.

22. Regarding the PIG-A assay in human erythrocytes, only one study had been identified (Dertinger et al., 2014). This paper described further development of the method for evaluating PIG-A mutations in human erythrocytes. Members noted that most biomonitoring studies in humans had mainly involved cytogenetic analysis rather than gene mutation. Therefore, the PIG-A assay may be a useful tool for assessing DNA mutation rates in humans. It was relatively easy to use and had a high throughput. However, the use would likely be limited by the wide variability in background mutation rates and by the need to account for potentially important confounding factors e.g. folic acid levels. Due to the large variability in mutation rates, it would be difficult to compare different populations to investigate a particular chemical exposure. However, it may have more use in investigating changes in

mutations in individuals and following changes in mutation with chemical exposure over time. Again, members considered that this would be an interesting topic to monitor.

23. One member asked whether the COM should consider glyphosate in light of the recent International Agency for Research on Cancer (IARC) conclusion that glyphosate is probably carcinogenic to humans. The IARC conclusions conflicted with the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) view. In response to the IARC evaluation, the World Health Organization (WHO) decided to form an "ad hoc expert taskforce" to review the information available to IARC in order to determine whether a new evaluation of glyphosate was required by the JMPR. This was expected to report to the JMPR September 2015 meeting. It was agreed that the Committee would wait and see if any relevant information would be made available by the next COM meeting in October 2015. The COM could then consider the data at that time. If the data were not made available by then, the Committee could undertake its own evaluation i.e. in terms of genotoxicity. The Chemicals Regulatory Directorate of the Health and Safety Executive (HSE) may also be involved, as it is the lead organisation in the UK for the evaluation of pesticides.

24. One member also mentioned a recent article in the scientific journal Nature, relating to the re-incorporation of MN into the nucleus and causing DNA damage and cancer. Other suggested topics included methods for measuring germ cell mutation due to current lack of validated methods and in light of the developments in molecular biology.

ITEM 5: ALCOHOL STATEMENT. ALCOHOL AND OXIDATIVE STRESS (MUT/2015//07 AND MUT/2014/02 – UPDATED PAPER)

25. The COM was presented with both the revised draft statement on the mutagenicity of alcohol and its metabolite acetaldehyde (MUT/2015/07) and the revised COM paper on the potential role of oxidative damage in alcohol's mutagenic and carcinogenic mode of action (MUT/2015/02). Members provided a number of drafting and typographical amendments and were asked to provide any further comments to the secretariat after the meeting. It was intended that both the statement on alcohol and mutagenicity and the paper on oxidative stress would be agreed and finalised by the Chair before publication on the COM website.

ITEM 6: CYCLOASTRAGENOL (MUT/2015/08)

26. The Committee on the Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) had been previously requested to consider the potential carcinogenicity of cycloastragenol-TA65 by the Advisory Committee on Novel Foods and Processes (ACNFP). This was in relation to an application for a novel food use. Cycloastragenol is extracted from plants and intended for use in food supplements.

27. The COC concluded that the available data were inadequate to demonstrate a lack of carcinogenic potential and that a bioassay or other comparable further work might be needed, although the precise requirements were uncertain given the reported mechanism of the compound. The manufacturers considered that the results of the supplied genotoxicity data were either equivocal or negative. The COC requested the COM's view on whether the genotoxicity studies that had been conducted were appropriate to demonstrate a lack of genotoxic potential. The COC were particularly concerned over the *in vitro* chromosome aberration study, which indicated an increase in aberrations at one dose level. Following subsequent discussions with some members of COM, it was suggested that it may be useful to look at the effects of cycloastragenol in non-transformed cells (e.g. human lymphocytes) and that a pre-incubation Ames test may also be useful. The COC had advised that the COM should also be consulted regarding potential genotoxicity.

28. Cycloastragenol has been reported to reduce the number of critically short telomeres, but not to increase telomere length (possibly by activating telomerase).The submitted data indicated that cycloastragenol has low oral bioavailability and was metabolised to a number of oxidised and hydroxylated compounds. A number of genotoxicity studies were also submitted that were considered to be either equivocal or negative. No standard carcinogenicity studies were submitted. Treatment with cycloastragenol did not affect the growth of tumour cells xenografted on to mice. Additionally, a paper by Bernardes de Jesus et al., 2011, reported that it did not increase 'global cancer incidence'. The ACNFP had noted that there was a suggestion of a non-statistically significant increase in liver cancer incidence in treated mice. However, the study was limited due to a small number of mice used; a relatively short duration of exposure; and a relatively high background rate of tumours. This prevented any clear conclusions being drawn from the study.

29. Paper MUT/2015/08 provided information on the available genotoxicity studies as well as background data on telomeres and telomerase activity in normal and cancerous cells. The COM was asked to comment on the genotoxicity studies provided.

30. The Committee considered that cycloastragenol had given valid negative results in the Ames test and that the correct strains had been used (there was no requirement for a specific pre-incubation test). Members also agreed that there were no concerns over the *in vitro* chromosome aberration test results in V79 Chinese hamster cells. An equivocal finding had been reported due to an increase in chromosome aberrations in only one intermediate test group (i.e. at a concentration on 1.5 mM) in one of the two experiments. There was no positive dose-response relationship. Members also noted a 70% reduction in relative cell density for the intermediate dose group (i.e. 1.5 mM), which suggested that this dose group was associated with cytotoxicity. The increase in chromosome aberrations at this dose could have been an artefact due to cytotoxicity and was not considered to indicate any concern.

31. The data supplied had suggested that cycloastragenol could form oxidative metabolites. However, the addition of S9 to V79 cells was considered to have provided adequate metabolic capacity for activation to potentially reactive metabolites. The results of the available *in vitro* tests indicated that only one *in vivo* genotoxicity study would be required. The *in vivo* erythrocyte micronucleus test in mice via intraperitoneal administration had given a negative result. Members agreed that there were no concerns over this negative *in vivo* test result.

32. The Committee gave some consideration to whether the suggested effect of cycloastragenol on telomeres and telomerase would require any additional genotoxicity testing requirements compared to a standard regulatory genotoxicity test package. The cells used in the chromosome aberration assay (i.e. V79) were considered already to have disrupted telomeres. If telomeres had been disrupted, then it was more likely that chromosome aberrations would have been produced. Chinese hamster V79 cells were also considered to be sensitive to genotoxicity. But, a negative result had been produced. It was, however, suggested that a chromosome aberration test conducted in primary non-transformed human cells may have been more relevant biologically. Members agreed that any potential concern did not arise from the genotoxicity test results, but from the suggestion of an increase in liver tumours. If the COC had concerns, then the mode of action in the target tissue could be investigated further. This could be done by an *in vivo* comet or micronucleus study in the liver to investigate a possible genotoxic mode of action or by studies investigating evidence for a non-genotoxic mode of action for liver tumours. Furthermore, it was suggested that an expert on telomeres be consulted. However, overall, the COM considered that the genotoxicity tests that had been conducted did not indicate *in vivo* mutagenic potential.

ITEM 7: OECD GENOTOXICTY TEST GUIDELINES UPDATE

33. The Committee was provided with a brief update on the progress following the April 2015 OECD working group for National Co-ordinators for Test Guidelines (WNT) meeting. Members were informed that two revised *in vivo* germ cell genotoxicity OECD Test Guidelines had been agreed, namely the Rodent Dominant Lethal Test (TG 478) and the Mammalian Spermatogonial Chromosome Aberration Test (TG 483). A clause would be included in each to say that they are intended as supplemental and not primary tests (i.e. as both use a relatively large number of animals, which is not encouraged under the 3Rs principles). Also the expert working group would be asked to determine historical control ranges from the literature rather than individual laboratories. Additionally, there was a proposal to remove and archive the Test Guideline for the dominant lethal assay within the next two years.

34. Members were informed that the updated OECD Test Guideline (TG 476) for the mouse lymphoma and the new *in vitro* mammalian cell gene

mutation test new (TK, HPRT, and XPRT assays) had been approved. It was noted that there was no longer a recommendation for a prolonged treatment period in the absence of S9 in both tests, and that this differed to the ICH testing requirements.

35. The Bhas cell transformation assay would be a Guidance document rather than a Test Guideline.

36. Regarding genotoxicity testing for nanomaterials, the intention was to produce a Guidance document rather than amend each Test Guideline.

37. For the *in vivo* PIG A assay, a Standard Project Submission Form (SPSF) had been accepted into the OECD work plan. This would be led by the USA. A work plan had also been agreed for the development of *in vitro* methods to detect non-genotoxic carcinogens. This would be led by the UK.

ITEM 8: DRAFT ANNUAL REPORT FOR 2014

38. The Committee was provided with an initial draft COM 2014 annual report, which was mainly produced from the minutes of the relevant meetings. This included topics that were considered by the COM during 2014, such as mutational spectra, the review of the mutagenicity of alcohol, and the revision of various OECD Test Guidelines. Members were asked to provide any comments (via email) to the secretariat so that appropriate amendments could be made.

39. A few queries were made by Members, for example on the text relating to alcohol and OECD Guidelines. The secretariat would check that the wording in the draft Annual Report was consistent with the COM view at that time i.e. 2014.

ITEM 9: ANY OTHER BUSINESS

40. There was no other business.

ITEM 11: DATE OF NEXT MEETING

41. 15th October 2015.