Committee on MUTAGENICITY

MUT/MIN/2016/1

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

Minutes of the meeting held at 10.30 am on Thursday 25th February 2016 at the Department of Health in Room 136B Skipton House, Elephant and Castle, London, SE1 6LH.

Present:

Chairman:	Dr D Lovell
Members:	Dr C Beevers Dr G Clare Professor S Doak Professor M O'Donovan Ms P Hardwick Professor G Jenkins Professor D Kirkland Professor A Lynch Professor F Martin 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 L Koshy (HSE) (Item 3 via teleconference)
In attendance:	Mr Daniel Medlock (PHE)

ITEM 1: ANNOUNCEMENTS/APOLOGIES FOR ABSENCE

1. The Chair welcomed Members, the secretariat and assessors. Mr B Maycock was attending for the Food Standards Agency (FSA) and Mr D Medlock was attending as an observer (Public Health England). The Chair also welcomed the assessor Dr L Koshy (Health and Safety Executive) attending via teleconference.

2. Apologies for absence were received from Dr D Benford (Secretariat FSA), the Member Dr S Dean, and from the assessors Dr H Stemplewski (MHRA), Dr S Fletcher (VMD) and Dr C Ramsay (Health Services Scotland).

3. Members were reminded of the need to declare any interests before discussion of items and to ensure declarations of interests were kept up to date.

ITEM 2: MINUTES OF MEETING ON 15th October 2015 (MUT/MIN/2015/3)

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

ITEM 3: MATTERS ARISING

5. The committee was informed by the HSE assessor that EFSA (European Food Safety Authority) had published its conclusions on glyphosate in November 2015 and concluded that it was unlikely to be carcinogenic. The Joint FAO/WHO Meeting on Pesticides Review (JMPR) would be evaluating glyphosate in May 2016 and the US Environmental Protection Agency was currently conducting a review on glyphosate. One member informed the COM that IARC (the International Agency for Research on Cancer) was about to publish a letter on its opinion of glyphosate.

6. The secretariat informed the COM that it had received a request from a company regarding the pesticide impurity and metabolite, para-chloroaniline (also a precursor in the dye and pharmaceutical Industry). The COM had previously considered this chemical in 2009 at the request of the UK Advisory Committee on Pesticides (now the Expert Committee on Pesticides (ECP)). It was concluded that para-chloroaniline is an *in vitro* mutagen, but the COM could not conclude on the *in vivo* mutagenicity based on the data provided. A strategy for the conduct of further genotoxicity testing was proposed. The company had conducted the suggested studies and requested that the COM consider the new data. The secretariat liaised with the ECP who confirmed that they support the proposal for the COM to review the new data. It was intended that the company could present the new data to the COM at either the June or October 2016 meetings.

7. The COM also heard that the Triennial review of the COM was currently going through ministerial approval and that the final report was likely to be approved and published in March 2016. Following recommendations from the review, there would be ‘light touch’ annual appraisal of members and

consideration of further training for the secretariat. Additionally, closer working between various Chairs of advisory committees and with the Directorate of the Public Health England Centre for Radiation, Chemical and Environmental Hazards (CRCE) had been recommended by both the COM Triennial review and the recent CRCE review. Therefore, future meetings would be considered in line with these recommendations.

8. The Chair announced that it was the last meeting of Professor David Phillips who had come to the end of his term. It was also the end of his term as Chair of the COC. The Chair thanked Professor Phillips for all his excellent work over the years and the COM provided its best wishes for the future.

ITEM 4: ASSAYS USED TO EVALUATE GERM CELL DNA INTEGRITY IN HUMAN FERTILITY INVESTIGATIONS (MUT2016/01)

9. At the previous October 2015 meeting members considered a paper on germ cell mutagenesis and aging. A paper on radiation and transgenerational effects was also considered. Recent developments in germ cell mutagenicity in general had also been discussed, including the possibility that a chemical could be a germ cell mutagen, but not a somatic cell mutagen, and that air pollution should be classified as a human germ cell mutagen.

10. During a literature review and preliminary investigations for a further paper on whether air pollution was a germ cell mutagen, it was noted that assays for DNA integrity utilised in assisted reproductive technologies (ART) were often used as a marker of DNA damage in human sperm. It was therefore considered appropriate for the COM to evaluate these assays before evaluating in more detail the suggestion that air pollution is a germ cell mutagen. It was not clear what value the sperm chromatin structure assay (SCSA) and the TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labelling) assays may have in investigating potential germ cell mutagenesis in humans.

11. Some information on germ cell DNA damage in humans has been obtained from studies investigating infertility or those aiming to improve the outcome of assisted reproduction technologies (ART). A report by Yauk et al., 2015 on an IWGT workshop on germ cell assays described these two assays (i.e. the SCCA and the TUNEL) and noted that while they generally correlate well with each other they measure different aspects of DNA integrity and therefore will differ in sensitivity. Paper MUT 2016/01 provided an overview of both the SCSA and the TUNEL assays for consideration by the COM.

12. Members noted that both the SCCA and the TUNEL were primarily assays for the detection of DNA strand breaks. They should be considered only as indicator assays and not informative on the consequences of the DNA strand breaks or downstream events. For example, they were not informative on whether the DNA strand breaks lead to a mutation, to apoptosis, or whether they would be repaired. The papers referred to in MUT/2016/01 mainly looked at effects on fertility. A key question raised was whether the observed reduced

fertility was due to a genotoxic effect or a toxic effect. Members also agreed that although both assays measured DNA strand breaks, they measured different types of DNA stand breaks. The significance and cause of the detected DNA strand breaks was also unclear. The observed DNA fragmentation could have arisen due to different reasons, such as chemical induced oxidative stress, apoptosis, or from another process not involving genotoxicity. There also appeared to be a relatively high background level and range of DNA strand breaks present in sperm, which would make it difficult to detect a chemically induced increase in DNA fragmentation. Furthermore, it was not clear at what point in spermatogenesis the DNA damage occurs. Members considered that it may be useful for the COM to have some input from an expert in this field i.e. a reproductive biologist with some knowledge of genotoxicity.

13. The COM considered that that there was some lack of consistency and conflicting results reported in the data and papers provided (e.g. there were conflicting reports on the correlation between the SCSA and the TUNEL assays).

14. Overall, members considered that there were a number of reasons why the results of both the SCSA and the TUNEL assays would be difficult to interpret in terms of germ cell mutagenicity e.g. they were indirect methods for evaluating potential germ cell mutagenicity; there was a lack of consistency between some of the data and in the test methods used; uncertainty over the underlying biology leading to the formation of DNA strand breaks and downstream effects; a large variation in background levels and range of effects; and a lack of validation of the test methods.

15. The COM considered that these assays may be able to indicate or contribute to lines of evidence for potential DNA damage caused by genotoxic chemicals, but there were a number of uncertainties as outlined above. It would be useful to harmonise these methodologies and for the validation of these assays to be undertaken. The COM would wait for the outcome of an IARC working group and further scientific developments on germ cell mutagenicity and the suggested germ cell mutagenicity of air pollution before deciding whether to conduct its own detailed review.

ITEM 5: GERM CELL ADVERSE OUTCOME PATHWAYS (MUT/2016/02)

16. As part of the COM's ongoing review of germ cell mutagenesis, the secretariat were made aware of recent papers by a group from Health Canada (Yauk et al., 2015 and Marchetti et al., 2015) regarding adverse outcome pathways (AOP) for germ cell endpoints. An individual AOP is specific to a molecular initiating event (MIE) and is not chemical specific. Key events (KE) are identified for the toxicological effect, which should be measurable. The connection between KE's is referred to as a KE relationship (KER). Modified Bradford Hill criteria are used to evaluate the empirical evidence and biological knowledge and this evaluation establishes the KERs. The DNA alkylation AOP (Yaulk et al., 2015) focused on premeiotic germ cell DNA alkylation using ethylnitrosourea as a model alkylating agent. Unique features of germ cells

suggest that they should be considered separately from somatic cells. The AOP makes the assumption that the processes of DNA repair and damage are conserved across eukaryotic cells. The tubulin binding AOP (Marchetti et al., 2015) uses colchicine as a model example and says that the majority of evidence is generated from rodents. It was noted that benzimidazoles induce this AOP. Members were asked for their views on the AOP approach and whether this would have any impact on the COM's 2007 statement on benzimidazoles (COM/07/S3).

17. Members considered that AOPs were useful for capturing and clarifying information obtained from systems biology approaches and to provide frameworks to aid in the communication of mode of actions, but there was some way to go before they could be used to evaluate the safety of chemicals. They had the potential to help communicate and explain expert evaluations and scientific reports (i.e. to make them more accessible to lay individuals). The two examples provided were very specific and more qualitative than quantitative. The AOPs would likely build over time and could aid predictive toxicology. It was noted that they were intended to be chemically agnostic. The two papers provided were considered to illustrate how to develop AOPs with two already well understood mechanisms (e.g. tubulin binding leading to microtubule depolymerisation and alkylation). Currently, one of the main difficulties was the different terminology used by different specialist areas of toxicology. There was no consensus on terminology, which would need to be addressed. It was noted that systems biology may help with this, as it already had a number of agreed terms (e.g. for receptor binding, antagonism, agonist etc.).

18. There was some discussion of whether AOPs could be used to evaluate mixtures of chemicals or in risk assessment. The COM considered that they could not currently be used for either as there were too many uncertainties, alternative pathways and potential chemical interactions (e.g. each chemical could have more than one AOP). Regarding the COM 2007 statement on benzimidazoles, where a 'common mechanism' of toxicity had been identified (aneugenicity via inhibition of tubulin polymerisation), members considered that there was some similarity between the flowchart in the statement illustrating the benzimadazole 'common mechanism group' and the more detailed AOP. Although the terminology used was a little different to that used currently, the COM agreed that the statement still remained valid and did not need to be changed.

19. Overall, members noted that there was a lot of work and interest in the area of AOPs (e.g. ECVAM were interested due to the potential to reduce animal testing). There was likely some way to go before they could be used by the COM. However, in the longer term, AOPs could aid communication of expert opinions (e.g. in explaining the difference between hazard and risk). The committee agreed to keep a watching brief on the development of AOPs for mutagenicity.

ITEM 6: ANNUAL REPORT 2015 (MUT/2016/03)

20. A draft COM annual report for 2015 had been prepared. The committee was asked to provide any comments. Comments such as typographical amendments could also be sent to the secretariat. A revised version would be circulated following members comments and this would be cleared by Chair's action before publication.

ITEM 7: ANY OTHER BUSINESS

1) Dominant lethal assay

21. The dominant lethal test was discussed due to an email from the National Toxicology Program in the USA requesting that the OECD Test Guideline 478 not be deleted because it was still used (e.g. in the USA) and the assay was still considered useful for detecting germ cell mutagens.

22. The COM considered that it would support the current UK view to delete the OECD Test Guideline 478 for the dominant lethal test.

2) COC statement on alcohol and cancer

23. Professor Phillips, the Chair of the COC, thanked the COM for its advice on alcohol that had been used in the COC's detailed review and statement on the carcinogenicity of alcohol. The COC published its statement on alcohol and cancer in January 2016 at the same time as the Chief Medical Officer's (CMO) report on alcohol. They were both broadly compatible in terms of cancer.

ITEM 8: DATE OF NEXT MEETING

24. 16th June 2016.