Committee on MUTAGENICITY

MUT/MIN/2021/01

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

Minutes of the meeting held at 11 to 4pm on 11th February 2021 via teleconference.

Present:

Chairman:	Dr D Lovell
Members:	Mr A Bhagwat Dr C Beevers Dr G Johnson Professor D Harrison (Ex officio) Professor S Doak Dr S Dean Professor P Fowler Dr J Kenny Dr R Morse Dr A Povey Mrs M Wang
Secretariat:	Dr O Sepai (PHE Scientific Secretary) Mr S Robjohns (PHE Secretariat) Dr C Mulholland (FSA Secretariat)
Secretariat Support:	Dr R Bevan (IEH Consulting) Dr K Vassaux (IEH Consulting)
Assessors:	Dr F Fernandez (VMD) Dr H Stempleski (MHRA) Dr W Munro (FSS) Dr J O'Brien (The Food Observatory)

Observers	Dr Gill Clare
	Ms Sam Saunders - PETA International Science Consortium Ltd.
	Dr F Hill (FSA)
	Professor M Skinner (Washington State University)

Guest attendance for leaving presentation to Dr David Lovell And presentation by Professor M Skinner:

Dr Olivia Osbourne (FSA)
Professor David Kirkland (ex-COM member)
Dr Ray Kemp (COC)
Dr Ruth Dempsey (COC)
Ms Philippa Hardwick (ex-COM member)
Dr Meera Cush (COC)
Dr Lesley Stanley (COC)
Professor Gary Hutchinson (COT)

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ITEM 1: ANNOUNCEMENTS/APOLOGIES FOR ABSENCE

1. The Chair welcomed the COM members, assessors and secretariat. The Chair also welcomed Dr Kate Vassaux as a new member of IEH Consulting who provided secretariat support to the COM and COC. Apologies were received from the member Dr M O'Donovan who had resigned and from Dr Lata Koshy (HSE).

2. The COM was informed that interviews had taken place for the position of the new chair of the COM and that a recommendation had been sent for ministerial approval.

3. Members were requested to declare any interests before the discussion of any items.

ITEM 2: MINUTES OF MEETING ON 25th NOVEMBER 2020 (MUT/MIN/2020/3)

4. Members agreed the minutes of the COM meeting held on the 25th November 2020 (MUT/MIN/2020/3).

ITEM 3: MATTERS ARISING

5. There were no matters arising not on the agenda.

ITEM 4: COM OVERARCHING GUIDANCE DOCUMENT (MUT/2021/1)

6. Amendments to the overarching COM Guidance document as a whole have been ongoing and previously considered at Committee meetings in July 2018 (paper MUT/2018/09), October 2018 (paper MUT/2018/13), February 2019 (MUT/2019/01), October 2019 (MUT/2019/12), February 2020 (MUT/2020/03), June 2020 (MUT/2020/09) and November 2020 (MUT/2020/16). An additional sub-group meeting was held in January 2021 to complete review of comments left outstanding following the November 2020 meeting. This was attended by Dr David Lovell (Chair), Dr Carol Beevers, Dr Paul Fowler, Dr Ovnair Sepai (Secretariat) and Dr Ruth Bevan (Secretariat support).

7. The presented paper (MUT/2021/01) included agreed amendments made following the November 2020 and January 2021 meetings and members addressed final outstanding queries. In earlier discussions during revision of the overarching guidance document, the need for a stand-alone document for screening methods had been proposed by some COM members, due to the fast-moving developments in this area. Members were asked whether this should now be developed. Following discussion, it was considered that the screening assays of choice would be very specific to the type of substance being tested and, as such, it would be difficult for COM to give specific recommendations.

8. It was agreed that, following completion of any amendments discussed, the overarching guidance document would be signed off by Chair's action and published. In addition, it was agreed that a stand-alone document for screening methods should not be developed.

9. It was agreed that, following completion of any amendments discussed, the overarching guidance document would be signed off by Chair's action and published. In addition, it was agreed that a stand-alone document for screening methods should not be developed.

ITEM 5: HEALTH AND SAFETY EXECUTIVE PRESENTATION ON HSE REQUIREMENTS POST EU EXIT

10. This item was postponed to a future COM meeting.

ITEM 6: GUIDANCE STATEMENTS – GERM CELL MUTAGENS (MUT/2021/02)

11. Drafts of a stand-alone guidance statement on genotoxicity testing strategies for germ cell mutagens were considered at the Committee meeting in February 2019 (MUT/2019/05), in October 2019 (MUT/2019/12), in June 2020 (MUT/2020/11) and November (MUT/2020/17).

12. The paper presented (MUT/2021/02) included the changes suggested by members in comments received following the November 2020 meeting. Members were asked to address specific queries towards finalisation of the guidance statement, and necessary changes were agreed. During review it had been suggested that the document could be more prescriptive to facilitate use by risk assessors. Following discussion, it was considered that the more general approach adopted in the guidance statement was the most appropriate format, particularly as it was due to be finalised. However, it was recognised that a more targeted strategy may need to be developed with individual government departments.

13. Members agreed that following revision of the document to reflect agreed changes, the guidance statement should be sent to 2-3 members for final review, followed by sign off by Chair's action.

ITEM 7: 3D MODELS (MUT/2021/03)

14. Drafts of a stand-alone guidance statement on the use of 3D models for genotoxicity testing were considered at the Committee meetings in February 2019 (MUT/2019/04), October 2019 (MUT/2019/12), June 2020 (MUT/2020/11) and November (MUT/2020/18).

15. The presented paper (MUT/2021/03) included the changes received from members following the meeting in November 2020. Members were asked to address specific queries towards finalisation of the guidance statement, and necessary changes were agreed.

16. It was agreed that, following completion of the suggested changes, the guidance document would be signed off by Chair's action.

ITEM 8: GUIDANCE STATEMENT ON TESTING FOR IMPURITIES – UPDATE (MUT/2021/04)

17. COM published a guidance statement in 2012 on a strategy for genotoxicity testing and mutagenic hazard assessment of impurities in chemical substances. There have been a number of initiatives since 2012 in this area and as part of the ongoing update of the COM Guidance Statement series, members agreed that the document should be updated. A revised document was presented at the Committee meeting in November 2020 (MUT/2020/21). Due to the shortened length of the meeting, the paper could not be discussed, and members were asked to forward comments and suggested changes to the Secretariat.

18. The paper presented (MUT/2021/04) a revised draft of the statement that included all comments received, for discussion by members. A number of specific queries were addressed, and amendments agreed. During review it had been suggested that the impurities guidance statement and QSAR guidance statement could be merged as there was overlap between the two areas. Members discussed this possibility but agreed to keep the two as separate documents.

19. Following revision of the document to include suggested amendments, members agreed that a second revised interim draft would be presented for discussion at the COM meeting in June 2021.

ITEM 9: COM STATEMENT ON THE USE OF QSAR MODELS (MUT/2021/05)

20. A draft COM statement on QSAR models was discussed in February 2019. Members requested a more general statement including some evaluation of the OECD principles applicable to QSAR models rather than an evaluation or opinion on specific QSAR models. A draft statement had been produced (MUT/2021/05) in response to this suggestion and the COM were asked for its view on the revised statement.

21. Some comments had been provided in advance of the meeting, for example from the Health and Safety Executive (HSE). The HSE had highlighted that the revised statement did not provide clear guidance on how QSARs could be used or interpreted.

22. Members also commented that that the draft statement did not currently say how QSARs could be used or what they should be used for. It was unclear whether standard *in vitro* tests still needed to be conducted when two different types of model (i.e. a knowledge-based model and a statistical based model) both gave a positive prediction. Also, if an equivocal result was obtained from an in vitro test, could QSAR models be used to support a positive result if a structural alert was identified? Or perhaps, if a structural alert was not identified to indicate that an *in vitro* equivocal result was oversensitive? These types of

questions remained unclear. Questions were also raised over the data behind some of the rules and whether these were clear[SR1]. It was suggested that links could be provided to the various principles for validating QSAR models rather than listing them all.

23. It was suggested that further revisions were required to the guidance statement. A COM sub-group could be convened to redraft the Guidance document, which would involve capturing the current thinking, considering the potential roles for QSARs (e.g. evaluating impurities) and how the use of QSARs could fit into the overall COM guidance on a strategy for genotoxicity testing.

ITEM 10: TOXICOGENOMICS SCOPING PAPER (MUT/2021/06)

24. The paper presented (MUT/2021/06) was a first draft scoping paper summarising a preliminary set of literature focussing on toxicogenomics and risk assessment. The aim of the paper was to provide an overview of current activities for discussion across COM, COC and COT, towards development of a guidance document.

25. During discussions, members emphasised that this is a broad area that is developing rapidly and, as such, COM should keep the topic under review. However, as fast-paced developments are occurring in the field, discussions should, where possible, focus only on very recent literature. The need to include toxicogenomics-related work being conducted in the US and by Health Canada, in addition to that in Europe, in the guidance document was highlighted.

26. From a COM perspective, members agreed that guidance would be required relating to the potential use of toxicogenomics in the evaluation of genotoxicity. Of particular concern was the need to note the distinction between toxicogenomics (i.e. gene expression analysis) and next-generation sequencing, as next-generation sequencing may be more applicable to COM guidance. It was noted that COC had previously published guidance on toxicogenomics but that this would now be outdated. Members considered that a discussion of approaches to the drafting of the guidance document with the COC secretariat would be beneficial to help clarify.

27. It was agreed that, following discussions with COC, the scoping document would be developed for presentation to COM at the meeting in June 2021.

ITEM 11: Presentation by Professor Michael K Skinner – Washington State University, Washington, USA – Environmental toxicant induced epigenetic transgenerational inheritance of disease. Generational toxicology – open to COC and COT members

28. As an introduction, Professor Mike Skinner highlighted that it is difficult to explain all disease based solely on the genome and that that environmental factors also play a role on the occurrence of disease. What is observed is not completely explained by the paradigm of the genome affecting gene expression, which in turn affects physiology and the development of disease.

An example is the observation that Japanese men have a lower rate of prostate cancer than men in the USA, but men of Japanese heritage living in the USA experience a higher rate of prostate cancer than those living in Japan. Similar observations apply to heart disease. This indicates that environmental factors are involved rather than the DNA sequence. The development of disease in identical twins is reported to vary when identical twins live in different regions. This also indicates that other factors are involved in addition to individual genetic sequence.

29. Professor Mike Skinner summarised animal studies that showed adverse effects in future generations (i.e. F2 and later generations, where the germline was not directly exposed to the initial test chemical) arising from an initial chemical exposure in pregnant females. The observed adverse effects arose from epigenetic changes. Epigenetic effects could arise from chemical induced changes in DNA methylation, histone modifications and effects on RNA (i.e. not involving a change in the DNA sequence). Such chemical induced epigenetic changes can result in modification of gene expression.

30. If a gestating F0 female animal is exposed to a particular chemical, then the F3 generation would be first generation that did not receive a direct test chemical germline exposure. Chemical induced effects seen in the F3 generation and subsequent generation could be due do epigenetic effects or inherited changes in gene expression arising from the initial gestating exposure of the F0 female. This would be an example of transgenerational inheritance. If a non-pregnant female or a male animal was exposed to the test chemical, then the F2 generation would be the first generation that did not receive direct germline chemical exposure. Chemical induced effects in this generation could arise from inherited epigenetic changes (this would be an example of transgenerational inheritance).

31. A number of examples of results of chemical exposure in animals were reported where 90% of treated animals showed adverse effects in the F3 generation resulting from an initial F0 gestating female exposure. For example, vinclozolin (agricultural fungicide), TCDD/Dioxin, DDT, bisphenol A and diethyl hexyl phthalate produced adverse effects in the F1 generation and in the F3 generation. Flutamide (anti-androgenic pharmaceutical) produced adverse effects in F1, but not in F3 generation. However, atrazine (an agricultural herbicide) and glyphosate (a herbicide) did not induce adverse effects in F1 but did in F3 (transgenerational effect). Examples of chemically induced transgenerational disease effects included spermatogenic defects, male infertility, prostate disease, premature ovarian failure, ovarian polycystic ovarian disease, birth defects, kidney disease, obesity, behavioural effects and immune effects.

32. Other types of exposures can also induce epigenetic and transgenerational effects, such as extreme temperature, drought, high fat diet or caloric restriction, smoking and alcohol. Epigenetic transgenerational effects have also been observed in other species e.g. plants, worms, flies and fish. Studies were described where various transgenerational epimutations and clusters were detected in the sperm genome in the F3 generation following initial chemical exposure, such as with vinclozolin and DDT. During early development, the epigenome goes through a cascade of changes. When a

sperm and egg first come together, the methylation is removed. As development progresses, re-methylation occurs. The time of chemical exposure can be critical for the later development of inherited adverse effects mediated through epigenetic changes. One of the most sensitive periods of exposure is during fetal gonadal sex determination when the germ line is undergoing epigenetic programming and DNA re-methylation occurs. The suggestion that environmental toxicants can re-programme the germ line to induce epigenetic transgenerational inheritance of disease, is a new paradigm in disease aetiology, and indicates the need to assess generational toxicology in the future.

33. The potential for chemical exposure to alter the epigenetics controlling adipocytes was also highlighted as this may lead to transgenerational inherited increased susceptibility to obesity. Key take home messages from the presentation included: the germline (eggs and sperm) are where epigenetic changes are critical because they get passed on in a transgenerational manner; this epigenetic transgenerational inheritance does not involve an inherited change in the DNA sequence; and a recommendation that adverse transgenerational effects need to be investigated in chemical health risk assessment. It was suggested that animal studies would be required to do this because current *in vitro* studies would not be suitable.

34. In discussions following the presentation, clarification was sought by members around how assessment of intragenerational effects may be included in current testing regimes. At the present time this can only be achieved through laboratory animal studies where the third generation needs to be evaluated, with minimum study length of between 1 and 1.5 years. It is not feasible to assess the germ cells of affected individuals because the shifts in developmental programming need to be established before the effects of the exposure are seen. A large proportion of the changes seen in earlier generations are due to direct exposure.

35. At present, transgenerational effects have been shown for many toxic compounds and so such testing is likely to be needed on a routine basis. There are no *in vitro* approaches that are effective to replace *in vivo* assays. It was considered possible that thresholds existed for the level of DNA methylation sites, below which long-term disease was avoided.

36. Diet was discussed as a major factor that had previously been linked with epigenetic changes. For a generational impact to occur the dietary influences have to be quite severe (for example, calorific restriction or high fat diets), with small shifts in diet not having an impact. Timing of exposure was also found to be key, with exposure during the early fetal life period being critical. Environmental toxicants were considered to have an effect at similar levels to calorific restriction. The importance of epidemiology studies in supporting animal data and showing causality was also discussed. Epigenetic biomarkers are needed for use in epidemiological studies and these have not been developed.

37. The Chair thanked the speaker on behalf of the Committee for an interesting and informative presentation. In conclusion, it was agreed that the COM would keep an

active watching brief on developments in the area, particularly in relation to inclusion in toxicity testing regimes.

ITEM 12: ANY OTHER BUSINESS

38. This was the last meeting of Dr David Lovell, who came to the end of his term as Chair of the COM at the end of March 2021. The current members, previous members, secretariat and assessors expressed their gratitude to Dr Lovell for his expertise and all his excellent and hard work over the years.

ITEM 13: DATE OF NEXT MEETING

39. XX June 2021