Committee on MUTAGENICITY

MUT/MIN/2018/2

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

Minutes of the meeting held at 10.30 am on 26th June 2018 at the Animal and Plant Health Agency, Central Veterinary Laboratory, Woodham Lane, New Haw, Addlestone, Surrey, KT15 3NB.

Present:

Chairman:	Dr D Lovell
Members:	Dr C Beevers
	Dr G Clare
	Professor S Doak
	Dr M O'Donovan
	Dr S Dean
	Ms P Hardwick
	Professor G Jenkins
	Professor D Kirkland
	Dr A Povey
Secretariat:	Dr O Sepai (PHE Scientific Secretary)
	Dr D Hedley (FSA Secretariat)
	Mr S Robjohns (PHE Secretariat)
	Miss H Smith (PHE Secretariat)
Secretariat Support:	Dr R Bevan (WRc/IEH Consulting)
	Dr P Rumsby (WRc/IEH Consulting)
Assessors:	Mrs R Pearson (VMD)
	Dr L Koshy (HSE) (via teleconference)

In attendance:	Miss B Gadeberg (PHE COC & COT Secretariat)
	Dr Catherine Moodley (Arysta Life Sciences)
	Mr John Street (Arysta Life Sciences)
	Dr Claire Koenig (Arysta Life Sciences)

ITEM 1: ANNOUNCEMENTS/APOLOGIES FOR ABSENCE

1. The Chair welcomed members, the secretariat and assessors. Miss B Gadeberg (PHE) attended for the COC and COT Secretariat. Mr D Hedley attended from the Food Standards Agency (FSA). Mr John Street (Arysta Life Sciences), Dr Catherine Moodley (Arysta Life Sciences) and Dr Claire Koenig (Arysta Life Sciences) attended for item 4.

2. Apologies for absence were received from Professor D Harrison (COC Ex-Officio member), Dr C Ramsay (Health Protection Scotland), Dr I Martin (EA assessor), Dr H Stemplewski (MHRA assessor), Dr Will Munro (Assessor Food standards Scotland) and Dr D Gott (FSA – Secretariat).

3. The committee was informed that interviews had been completed for new expert and lay members. The proposed appointments were waiting to be approved and signed by the Secretary of State. Appraisals had been completed for all COM members.

ITEM 2: MINUTES OF MEETING ON 22nd FEBRUARY 2018 (MUT/MIN/2018/1)

4. Members agreed the minutes subject to minor changes.

ITEM 3: MATTERS ARISING

5. There were no substantial matters arising.

RESERVED BUSINESS

ITEM 4: para-CHLOROANNILINE PRESENTATION AND DISCUSSION (MUT/2018/07)

6. This item was considered as reserved business as it relates to commercially sensitive information.

OPEN SESSION

ITEM 5: E-CIGARETTES E(N)NDS GENOTOXICITY (MUT/2018/08)

7. The Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) is currently considering the potential toxicological risks of electronic nicotine (or non-nicotine) delivery systems (E(N)NDS). A paper (TOX/2018/16) was presented at the COT, in which a literature search and full lists of publications retrieved were presented. After follow-up analysis of the abstracts obtained, it was agreed that the COM and the COC should consider the available papers on genotoxicity and

carcinogenicity, respectively. The aim was for the COM (and COC) to assess absolute risks from E(N)NDS and relative risk compared to conventional cigarettes, and if available to heated tobacco products.

8. A limited number of standard tests conducted to OECD Test Guidelines had been identified. These consisted of bacterial tests and micronuclei assays in mammalian cells, which gave negative results for E(N)NDS, while positive results were observed for conventional cigarettes. Members commented that these available OECD Test Guideline studies were conducted by or for the tobacco industry.

9. Of the other available studies, two were *in vivo* animal studies and the remainder *in vitro* studies. The two *in vivo* studies were a 4-week study in rats investigating genotoxicity and oxidative stress in lung, blood and urine and a 12-week study in mice assessing DNA damage and oxidative stress in various organs. The *in vitro* studies utilised relevant target tissue cells such as lung and oral cell systems. As a group, these studies assessed a wide range of genotoxic endpoints, including oxidative DNA damage, increase in reactive oxygen species and effects on mitochondria. There was a wide use of the comet assay in these studies.

10. Different exposure methods were utilised in the three *in vitro* studies conducted to OECD guidelines. In one study, liquid products or filtered particulates and aerosols condensed from various E(N)NDS devices were added to cell cultures. In the remaining two studies, an aerosol-media interface was utilised for direct interaction with a controlled amount of the aerosol passing over the *in vitro* media (such as agar).

11. The ‘non-standard’ studies (i.e. not conducted to OECD Test Guidelines) described exposure to a variety of E(N)NDS products using a number of experimental methodologies, some of which were not described in sufficient detail by the authors, making comparisons across studies difficult. A number of different systems were used to define a standard concentration for exposure, including ‘puffs’ per hour, nicotine concentration and particulate number following collection on a filter. It was noted that there was an effect of the voltage used on the E(N)NDS device, which resulted in different components in the emission. The Committee considered that it would be important for test systems to reflect exposures of users or bystanders. In addition, Members considered that standardisation of a delivery protocol would be helpful to allow for comparisons to be made across studies.

12. Members noted that mainly high doses had been used in the studies involving the comet assay. The DNA damage seen in these studies was associated with relatively high levels of cytotoxicity and thus could have been a consequence of toxicity rather than direct interaction with DNA. Only one comet assay appeared to provide a robust positive result.
The COM also questioned the suitability of the methodology used for the measurement of 8-OHdG and the extended duration of exposure in some cell culture studies, e.g. for one study an 8-week exposure was used. Although this was associated with some genotoxicity, members considered that the

extended period of exposure may have contributed to this and was not representative of human exposure to E(N)NDS which would not be continuous.

13. It was noted that one of the studies indicated that the carrier substance, propylene glycol, may have influenced overall toxicity. It was also noted that flavouring substances could have affected overall toxicity in some studies; however members had methodological concerns in these studies. Members were aware that some flavouring substances used in E(N)NDS may have been assessed for potential mutagenicity by authoritative bodies in relation to food. It was unclear whether the evaluation of potential mutagenicity of flavouring substances for food use would be relevant to inhalation exposure from the use of E(N)NDS.

14. For the non-standard studies (i.e. not conducted to OECD Test Guidelines) as a whole, the COM considered that there was no consistency in the assessment of mutagenicity or exposure, which made it difficult to evaluate the potential mutagenicity of E(N)NDS. However, members did not identify any mutation specific to E(N)NDS that are not produced by tobacco products.

15. In conclusion, members considered that although there was a breadth of evidence reported, studies conducted to OECD Test Guidelines showed negative results and these had been sponsored by industry. The non-test guideline studies generally reported positive results, but did not show consistency and had not been repeated by other investigators. Members also expressed concern that some studies reported genotoxicity only when wider toxic effects were also observed. It was possible to conclude that this limited evidence base did not indicate any specific mutagenic risks from E(N)NDS that were not observed with conventional cigarette products. However, members considered that greater consistency and demonstrable reproducibility in both product, exposure and methodologies were needed before any view could be taken on absolute risks of E(N)NDS products.

ITEM 6: COM GUIDANCE STRATEGY UPDATE (MUT/2018/09)

16. In February 2018, the COM considered two papers relating to an update of the COM Guidance on a strategy for genotoxicity testing of chemical substances. These consisted of paper MUT/2018/02, on the use of (Q)SAR models to predict genotoxicity, and paper MUT/2018/03, on a COM Guidance update on strategies for *in vivo* genotoxicity testing. Members considered that there had been no significant changes to strategy developments or assay methodologies that merited a re-write of the COM Guidance document in terms of the overall strategy for genotoxicity testing, at present. However, it was suggested that the document needed to be updated in other aspects, such as references and available supporting data.

17. Paper MUT/2018/09 was presented, which provided an initial draft update of the full COM Guidance document on a strategy for genotoxicity testing incorporating amendments agreed in February 2018 on *in vivo* assays and the discussion on (Q)SAR models.

Members reviewed the initial draft update of the full Guidance document and suggested numerous changes and updates, up to Annex 1. To assist with capturing these, one member agreed to provide the Secretariat with an annotated copy of the document, which could then be sent to other members in turn, to add any additional updates. Once complete, the corresponding changes to the Annexes could be undertaken.

18. Due to the frequent updates for QSAR methodologies, it was considered that this section should be taken out as a stand-alone guidance document that could be updated more regularly. In addition, separate stand-alone guidance documents concerning specific topics were recommended e.g. nanomaterials.

ITEM 7: CRISPR GENE EDITING TECHNOLOGY (MUT/2018/10)

19. Paper MUT/2018/10 provided a brief overview of the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology, its application as a genome editing tool in human medicine and viral vector mediated genotoxicity in general.

20. The technology has been used therapeutically in humans to treat diseases including cancer and HIV. However, mutagenesis had been observed in some cases. Members were asked to consider whether the CRISPR technologies have the potential for vector mediated genotoxicity and, if so, whether this should be explored further.

21. It was commented that this was an interesting technique and noted that mutations leading to cancer had been reported with a commercial product. It would be informative to know if any one particular CRISPR technology is more prone to this happening. Members agreed that a presentation to the Committee by an expert in this field would be useful to update members before this topic could be considered further by the COM.

ITEM 8: COM ANNUAL REPORT (MUT/2018/11)

22. The COM was presented with a draft annual report for 2017 that would be included in the combined annual report for the sister committees, the Committee on Toxicity (COT) and the Committee on the carcinogenicity (COC) of chemicals in food, consumer products and the environment.

23. Members made a few relatively minor typographical and editorial amendments. The secretariat would amend the draft report accordingly, which would then be incorporated into the joint committees' final 2017 annual report for publication.

ITEM 9: OECD UPDATES (MUT/2018/06)

24. The COM heard that work was ongoing on the development of an OECD Test Guideline for the Pig-a in vivo gene mutation assay. It was

considered unlikely that an OECD Test Guideline would be finalised or published before 2020.

25. The Committee was also informed that there has been a request for data to support the development of the mini Ames test. Additionally, it was likely that there would be a revision to OECD Test Guideline 471 (Bacterial reverse mutation test) in relation to the selection and use of appropriate Salmonella typhimurium test strains.

ITEM 10: HORIZON SCANNING AND FORWARD PLANNING

26. As part of the COM ongoing 'Horizon Scanning' process, members were requested to make suggestions on topics for its future work plan.

27. Members suggested that it would be useful to invite suitable speakers from the European Food Safety Authority (EFSA) and the European Chemicals Agency (ECHA) to explain the views of these organisations in interpreting in vivo genotoxicity test data. The COM were aware of certain aspects where there may be differences in opinion and interpretation, for example, appropriate route of administration, demonstration of sufficient target tissue exposure, appropriate endpoint specific follow up in vivo studies following an in vitro positive, and requirements relating to tissues to be sampled following site of contact exposure. Members also suggested that it would be useful to invite speakers with relevant expertise in the use of CRISPR gene editing technology and potential genotoxicity and a speaker with expertise in the genotoxicity of nanomaterials.

28. Other suggested future topics of interest included: how to evaluate the weight of evidence from standard GLP studies and non-standard genotoxicity data using different methods and endpoints; predatory journals; appropriate terminology and definitions in relation to genotoxicity (e.g. non-genotoxic carcinogen, indirect mutation, mode of action etc.); and an update on the quantitative analysis of genotoxicity data. It was also noted that an update of the current COM Guidance on genotoxicity testing was an ongoing and future area of work.

ITEM 11: DATE OF NEXT MEETING

29. Date of next meeting 18th October 2018.