

# 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 26<sup>th</sup> 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)

DRAFT

	Paragraph
1. Apologies for absence	1
2. Minutes of the meeting held on 22 <sup>nd</sup> February 2018 (MUT/MIN/2018/1)	4
3. Matters Arising	5
<b>ITEM 4 RESERVED BUSINESS</b>	
4. Para-chloroaniline (PCA) and presentation (MUT/2018/01)	6
<b>OPEN SESSION</b>	
5. E-Cigarettes E(N)NDS genotoxicity (MUT/2018/08)	7
6. COM Guidance strategy update (MUT/2018/09)	16
7. CRISPR gene editing technology (MUT/2018/10)	19
8. COM Annual Report (MUT/2018/11)	22
9. OECD Updates	24
10. Horizon Scanning and forward planning	26
11. Any Other Business	29

1  
2 **ITEM 1: ANNOUNCEMENTS/APOLOGIES FOR ABSENCE**  
3

4 1. The Chair welcomed members, the secretariat and assessors. Miss B  
5 Gadeberg (PHE) attended for the COC and COT Secretariat. Mr D Hedley  
6 attended from the Food Standards Agency (FSA). Mr John Street (Arysta Life  
7 Sciences), Dr Catherine Moodley (Arysta Life Sciences) and Dr Claire Koenig  
8 (Arysta Life Sciences) attended for item 4.  
9

10 2. Apologies for absence were received from Professor D Harrison (COC  
11 Ex-Officio member), Dr C Ramsay (Health Protection Scotland), Dr I Martin  
12 (EA assessor), Dr H Stemplewski (MHRA assessor), Dr Will Munro (Assessor  
13 Food standards Scotland) and Dr D Gott (FSA – Secretariat).  
14

15 3. The committee was informed that interviews had been completed for  
16 new expert and lay members. The proposed appointments were waiting to be  
17 approved and signed by the Secretary of State. Appraisals had been  
18 completed for all COM members.  
19

20 **ITEM 2: MINUTES OF MEETING ON 22<sup>nd</sup> FEBRUARY 2018**  
21 **(MUT/MIN/2018/1)**  
22

23 4. Members agreed the minutes subject to minor changes.  
24  
25

26 **ITEM 3: MATTERS ARISING**  
27

28 5. There were no substantial matters arising.  
29  
30

31 **RESERVED BUSINESS**

**Comment [SR1]:** This reserved  
business item is provided separately.

33 **ITEM 4: para-CHLOROANILINE PRESENTATION AND DISCUSSION**  
34 **(MUT/2018/07)**  
35

36 6. This item was considered as reserved business as it relates to  
37 commercially sensitive information.  
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39

40 **OPEN SESSION**  
41

42 **ITEM 5: E-CIGARETTES E(N)NDS GENOTOXICITY (MUT/2018/08)**  
43

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

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

4  
5 8. A limited number of standard tests conducted to OECD Test Guidelines  
6 had been identified. These consisted of bacterial tests and micronuclei assays  
7 in mammalian cells, which gave negative results for E(N)NDS, while positive  
8 results were observed for conventional cigarettes. Members commented that  
9 these available OECD Test Guideline studies were conducted by or for the  
10 tobacco industry.

11  
12 9. Of the other available studies, two were *in vivo* animal studies and the  
13 remainder *in vitro* studies. The two *in vivo* studies were a 4-week study in rats  
14 investigating genotoxicity and oxidative stress in lung, blood and urine and a  
15 12-week study in mice assessing DNA damage and oxidative stress in various  
16 organs. The *in vitro* studies utilised relevant target tissue cells such as lung  
17 and oral cell systems. As a group, these studies assessed a wide range of  
18 genotoxic endpoints, including oxidative DNA damage, increase in reactive  
19 oxygen species and effects on mitochondria. There was a wide use of the  
20 comet assay in these studies.

21  
22 10. Different exposure methods were utilised in the three *in vitro* studies  
23 conducted to OECD guidelines. In one study, liquid products or filtered  
24 particulates and aerosols condensed from various E(N)NDS devices were  
25 added to cell cultures. In the remaining two studies, an aerosol-media interface  
26 was utilised for direct interaction with a controlled amount of the aerosol  
27 passing over the *in vitro* media (such as agar).

28  
29 11. The 'non-standard' studies (i.e. not conducted to OECD Test  
30 Guidelines) described exposure to a variety of E(N)NDS products using a  
31 number of experimental methodologies, some of which were not described in  
32 sufficient detail by the authors, making comparisons across studies difficult. A  
33 number of different systems were used to define a standard concentration for  
34 exposure, including 'puffs' per hour, nicotine concentration and particulate  
35 number following collection on a filter. It was noted that there was an effect of  
36 the voltage used on the E(N)NDS device, which resulted in different  
37 components in the emission. The Committee considered that it would be  
38 important for test systems to reflect exposures of users or bystanders. In  
39 addition, Members considered that standardisation of a delivery protocol would  
40 be helpful to allow for comparisons to be made across studies.

41  
42 12. Members noted that mainly high doses had been used in the studies  
43 involving the comet assay. The DNA damage seen in these studies was  
44 associated with relatively high levels of cytotoxicity and thus could have been a  
45 consequence of toxicity rather than direct interaction with DNA. Only one  
46 comet assay appeared to provide a robust positive result.  
47 The COM also questioned the suitability of the methodology used for the  
48 measurement of 8-OHdG and the extended duration of exposure in some cell  
49 culture studies, e.g. for one study an 8-week exposure was used. Although this  
50 was associated with some genotoxicity, members considered that the

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

3  
4 13. It was noted that one of the studies indicated that the carrier substance,  
5 propylene glycol, may have influenced overall toxicity. It was also noted that  
6 flavouring substances could have affected overall toxicity in some studies;  
7 however members had methodological concerns in these studies. Members  
8 were aware that some flavouring substances used in E(N)NDS may have been  
9 assessed for potential mutagenicity by authoritative bodies in relation to food. It  
10 was unclear whether the evaluation of potential mutagenicity of flavouring  
11 substances for food use would be relevant to inhalation exposure from the use  
12 of E(N)NDS.

13  
14 14. For the non-standard studies (i.e. not conducted to OECD Test  
15 Guidelines) as a whole, the COM considered that there was no consistency in  
16 the assessment of mutagenicity or exposure, which made it difficult to evaluate  
17 the potential mutagenicity of E(N)NDS. However, members did not identify any  
18 mutation specific to E(N)NDS that are not produced by tobacco products.

19  
20 15. In conclusion, members considered that although there was a breadth of  
21 evidence reported, studies conducted to OECD Test Guidelines showed  
22 negative results and these had been sponsored by industry. The non-test  
23 guideline studies generally reported positive results, but did not show  
24 consistency and had not been repeated by other investigators. Members also  
25 expressed concern that some studies reported genotoxicity only when wider  
26 toxic effects were also observed. It was possible to conclude that this limited  
27 evidence base did not indicate any specific mutagenic risks from E(N)NDS that  
28 were not observed with conventional cigarette products. However, members  
29 considered that greater consistency and demonstrable reproducibility in both  
30 product, exposure and methodologies were needed before any view could be  
31 taken on absolute risks of E(N)NDS products.

#### 32 33 **ITEM 6: COM GUIDANCE STRATEGY UPDATE (MUT/2018/09)**

34  
35 16. In February 2018, the COM considered two papers relating to an update  
36 of the COM Guidance on a strategy for genotoxicity testing of chemical  
37 substances. These consisted of paper MUT/2018/02, on the use of (Q)SAR  
38 models to predict genotoxicity, and paper MUT/2018/03, on a COM Guidance  
39 update on strategies for *in vivo* genotoxicity testing. Members considered that  
40 there had been no significant changes to strategy developments or assay  
41 methodologies that merited a re-write of the COM Guidance document in terms  
42 of the overall strategy for genotoxicity testing, at present. However, it was  
43 suggested that the document needed to be updated in other aspects, such as  
44 references and available supporting data.

45  
46 17. Paper MUT/2018/09 was presented, which provided an initial draft  
47 update of the full COM Guidance document on a strategy for genotoxicity  
48 testing incorporating amendments agreed in February 2018 on *in vivo* assays  
49 and the discussion on (Q)SAR models.

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

7  
8 18. Due to the frequent updates for QSAR methodologies, it was considered  
9 that this section should be taken out as a stand-alone guidance document that  
10 could be updated more regularly. In addition, a separate stand-alone guidance  
11 document concerning nanomaterials was recommended.

#### 12 13 **ITEM 7: CRISPR GENE EDITING TECHNOLOGY (MUT/2018/10)**

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

19  
20 20. The technology has been used therapeutically in humans to treat  
21 diseases including cancer and HIV. However, mutagenesis had been observed  
22 in some cases. Members were asked to consider whether the CRISPR  
23 technologies have the potential for vector mediated genotoxicity and, if so,  
24 whether this should be explored further.

25  
26 21. It was commented that this was an interesting technique and noted that  
27 mutations leading to cancer had been reported with a commercial product. It  
28 would be informative to know if any one particular CRISPR technology is more  
29 prone to this happening. Members agreed that a presentation to the Committee  
30 by an expert in this field would be useful to update members before this topic  
31 could be considered further by the COM.

#### 32 33 34 35 **ITEM 8: COM ANNUAL REPORT (MUT/2018/11)**

36  
37 22. The COM was presented with a draft annual report for 2017 that would  
38 be included in the combined annual report for the sister committees, the  
39 Committee on Toxicity (COT) and the Committee on the carcinogenicity (COC)  
40 of chemicals in food, consumer products and the environment.

41  
42 23. Members made a few relatively minor typographical and editorial  
43 amendments. The secretariat would amend the draft report accordingly, which  
44 would then be incorporated into the joint committees' final 2017 annual report  
45 for publication.

#### 46 47 **ITEM 9: OECD UPDATES (MUT/2018/06)**

48  
49 24. The COM heard that work was ongoing on the development of an  
50 OECD Test Guideline for the Pig-a in vivo gene mutation assay. It was

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

3  
4 25. The Committee was also informed that there has been a request for  
5 data to support the development of the mini Ames test. Additionally, it was  
6 likely that there would be a revision to OECD Test Guideline 471 (Bacterial  
7 reverse mutation test) in relation to the selection and use of appropriate  
8 *Salmonella typhimurium* test strains.

#### 9 10 **ITEM 10: HORIZON SCANNING AND FORWARD PLANNING**

11  
12 26. As part of the COM ongoing 'Horizon Scanning' process, members were  
13 requested to make suggestions on topics for its future work plan.

14  
15 27. Members suggested that it would be useful to invite suitable speakers  
16 from the European Food Safety Authority (EFSA) and the European Chemicals  
17 Agency (ECHA) to explain the views of these organisations in interpreting in  
18 vivo genotoxicity test data. The COM were aware of certain aspects where  
19 there may be differences in opinion and interpretation, for example, appropriate  
20 route of administration, demonstration of sufficient target tissue exposure,  
21 appropriate endpoint specific follow up in vivo studies following an in vitro  
22 positive, and requirements relating to tissues to be sampled following site of  
23 contact exposure. Members also suggested that it would be useful to invite  
24 speakers with relevant expertise in the use of CRISPR gene editing technology  
25 and potential genotoxicity and a speaker with expertise in the genotoxicity of  
26 nanomaterials.

27  
28 28. Other suggested future topics of interest included: how to evaluate the  
29 weight of evidence from standard GLP studies and non-standard genotoxicity  
30 data using different methods and endpoints; predatory journals; appropriate  
31 terminology and definitions in relation to genotoxicity (e.g. non-genotoxic  
32 carcinogen, indirect mutation, mode of action etc.); and an update on the  
33 quantitative analysis of genotoxicity data. It was also noted that an update of  
34 the current COM Guidance on genotoxicity testing was an ongoing and future  
35 area of work.

#### 36 37 38 **ITEM 11: DATE OF NEXT MEETING**

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40 29. Date of next meeting 18<sup>th</sup> October 2018.