Committee on MUTAGENICITY

MUT/MIN/2019/2

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

Minutes of the meeting held at 10.30 am on 10th October 2019 at Department of Health, Skipton House, 80 London Road, London, SE1 6LH.

Present:

Chairman:	Dr D Lovell
Members:	Mr A Bhagwat Dr C Beevers Professor S Doak Dr M O'Donovan Dr S Dean Professor P Fowler Professor D Harrison (Ex Officio) Dr R Morse Dr A Povey
Secretariat:	Mr S Robjohns (PHE Scientific secretary) Mrs H Nakeeb (PHE Secretariat) Dr O Osborne (FSA Secretariat) Ms C Tsoulli (FSA Secretariat)
Secretariat Support:	Dr R Bevan (WRc/IEH Consulting)
Assessors:	Dr L Dearsley (HSE)
Observes	Dr A Lorenzoni (EU Fora fellow) Dr M Elissavet Valanou (EU For a fellow)

ITEM 1: ANNOUNCEMENTS/APOLOGIES FOR ABSENCE

1. The Chair welcomed the COM members, assessors and secretariat. Dr O Osborne and Ms C Tsoulli were attending for the Food Standards Agency. Professor Paul Fowler (Fstox Consulting) was welcomed as a new member. Dr L Dearsly was attending as an assessor for the HSE. Dr Andrea Lorenzoni (EU Fora fellow) and Dr Maria Elissavet Velanou (EU Fora fellow) were attending as observers from the Food Standards Agency.

2. Apologies for absence were received from Dr O Sepai (PHE), Dr D Gott (FSA), Dr H Stempleski (MHRA), Mrs R Pearson (VMD).

3. The COM was informed that Dr D Gott had unfortunately been taken ill earlier in the year. He had now returned home from hospital and was making progress, although it was expected that it would be a few months before he could return to work. The COM offered him its best wishes for a successful recovery.

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

ITEM 2: MINUTES OF MEETING ON 28th February 2019 (MUT/MIN/2019/1)

5. Members agreed the minutes subject to minor typographical changes.

ITEM 3: MATTERS ARISING

6. Regarding the UK exit from the EU, the Food Standards Agency (FSA) had set up Joint Expert Groups for the assessment of regulated products. These groups were currently undergoing training and would be able to start work as required post UK exit from the EU. It was thought that items relating to the mutagenicity of regulated products would occasionally be referred to the COM.

7. The Chair informed the COM that Professor Dame Sally Davies had moved on from the post of the Chief Medical Officer (CMO) and that Chris Whitty had been appointed as the new CMO of England. Also, that Professor Guy Poppy was moving on from the post of Chief Scientific Adviser at the FSA. From a recent meeting at the FSA, the COM Chair had been made aware of the release of Government funding for relevant research and encouraged members of the COM to make applications for any FSA related research projects.

8. Regarding COM appointments, the Chair informed the committee that he had been reappointed as Chair of the COM until 2021. The COM were also informed that there had been three applications for the position of expert members and two applications for a vacant lay member post. The COM was requested to encourage suitable applicants to apply.

RESERVED SESSION

ITEM 4: RISKS TO HUMAN HEALTH FROM THE USE OF AZODICARBONAMIDE AS A FOOD ADDITIVE (MUT/2019/07)

9. This item was considered as a reserved in confidence item.

OPEN SESSION

ITEM 5: PRESENTATION – UPDATE ON THE VALIDATION OF TOXTRACKER BY DR GIELS HENDRICKS

10. The ToxTracker assay is a stem cell-based genotoxicity screening platform which utilises 6 unique reporter cell lines to detect genotoxicity and provide information relating to the mode of action for genotoxicity and non-genotoxic carcinogenicity. The COM first evaluated the technology in 2014 and since that time ToxTracker has undergone further validation and development. Giel Hendriks from 'toxys' in The Netherlands presented an update of this, with a specific focus on mutagenicity endpoints.

11. The assay responds to DNA damage (e.g. mutagenic lesions and DNA double strand breaks), activation of p53, oxidative stress and protein damage and indicates this via Green Fluorescent Protein (GFP) induction in the reporter cell lines determined by flow cytometry. ToxTracker ACE (Aneugen and Clastogen Evaluation) includes the detection of cell cycle block, aneugenicity and polyploidy. Toxtracker has been improved in terms of optimizing metabolic activation by diluting S9 and reducing the exposure period to mitigate against the cytotoxicity of pure S9. The assay can also be run in presence of reactive oxygen species (ROS) scavengers, such as N-acetyl cysteine and reduced glutathione. This approach can be used to demonstrate a positive response due solely to oxidative stress rather than direct interaction with DNA. To date, a large number (>1000) and range of substances have been tested using ToxTracker including: single molecules; polymers; complex mixtures; nanomaterials; and intermediates. There is a growing trend to include the assay for early screening and hazard identification purposes, in addition to its use in follow up testing, identifying mode of action, quantitative dose response modelling, TTC and for Weight of Evidence (WoE) considerations.

12. Technical in-house validation of ToxTracker indicated sensitivity and specificity to be around 90% and this was supported by the findings of a small inter-laboratory validation exercise (2 laboratories). A much larger inter-laboratory validation exercise (8 independent laboratories in the US, EU and Japan) is currently in progress involving 64 compounds, with the aim of assessing adoption of the assay by ECVAM and OECD, with findings expected to be reported in early 2020.

13. Three questions were suggested that could help the COM discussion following the presentation:

- Is there added value of ToxTracker in addition to the standard in vitro genotoxicity assays?
- Is ToxTracker primarily a screening assay or can it also be used for regulatory applications?
- Is ToxTracker as addition to the standard genotoxicity test battery or can it replace and assay?

14. Clarification was sought by members around the influence of the dose range chosen and the 'yes'/'no' categorisation of the assay. To this respect, safety measures are included in the choice of a maximum dose, and defined increases that signify a' true positive' and 'true negative' result. A two-fold increase in GFP induction was used to indicate a positive response and less than 1.5-fold GFP induction was regarded as a negative response, with responses in between considered as borderline. Members considered that border-line chemicals would not be straight forward to classify, and it was explained that dose-response analysis was crucial when categorising these, currently dependant on expert judgement. However, more sophisticated software that will enable learning for the classification of such chemicals, is a possible future development. In addition, it was recognised that as a greater number of compounds are run through the ToxTracker platform unexpected results will provide learning opportunities regarding the limitations of the platform. A cut-off for cytotoxicity of approximately 55% or 65% was used.

15. The sensitivity of ToxTracker in terms of being able to detect individual chromosome loss was also considered. In this regard, if the chromosome loss triggers an effect on cell cycle progression then it will be picked up in the assay, otherwise not. Members discussed the added value of using ToxTracker, particularly when equivocal data has been found using 'standard' *in vitro* testing methods. It was considered that information on the MoA provided by ToxTracker could help explain equivocal findings. In addition, ToxTracker could be used where *in vivo* follow up studies are not permitted following a positive Ames test (for example when testing cosmetics).

16. Increased or more widespread use of the assay was seen to be necessary to trigger its inclusion in the standard battery of genotoxicity assays and to gain regulatory acceptance. The outcome of the ongoing OECD process will decide if a guideline is needed for the screening assay. Although there has been much interest in using ToxTracker from industry, the question remains as to whether compounds can be accepted in a regulatory process if there is no OECD guideline attached.

17. The Chair thanked the speaker for an interesting update. In conclusion, it was agreed that the COM would keep a watching brief on developments with the ToxTracker platform, particularly with regards to regulatory acceptance of its use for genotoxicity testing.

ITEM 6: METING NOTES AND DRAFT SUMMARY OF OUTCOMES FROM THE "WORKSHOP ON THE INTERPRETATION OF GENETIC TOXICOLOGY DATA IN A REGUALATORY ENVIRONMENT", BIRMINGHAM, JUNE 2019 (MUT/2019/08 AND MUT/2019/09)

18. A workshop was held in June 2019 that brought together key people with an interest in developing views on the interpretation of genotoxicity data and discussed new methods and challenges for future testing strategies. From this workshop two papers were presented to COM members.

19. The first paper (MUT/2019/08) provided notes of the presentations given and discussion sessions. Members considered the paper to be an accurate record of the workshop. Further comments were invited by the 18th October, after which time the notes would be sent to other workshop participants for their review.

20. The second paper (MUT/2019/09) provided an assimilated summary of the workshop. Members considered that the paper provided a comprehensive summary of discussions. Further comments were invited by the 18th October, following which time the summary would be sent to other participants for their review. There was support for the publication of the workshop summary, once finalised. In addition, some members confirmed interest in helping to develop guidance to evaluate genetic toxicology data, one of the recommendations from the workshop. In addition, a further workshop, possibly in conjunction with UKEMs, was supported, with COM as the lead.

ITEM 7: REVIEW OF GENOTOXICITY OF CANNABIDIOL (MUT/2019/10)

20. No interests were declared.

Cannabidiol (CBD) is a type of cannabinoid found in the Cannabis plant. Research into the potential medicinal use of CBD has been conducted over a number of years including clinical trials for its use in treating seizures from epilepsy.

21. CBD has now been added to a number of food and beverages (e.g. beer, spirits, wine, coffee and soda style drinks), liquids (tinctures, drops, syrup and oils), chewables (gum drops) and chocolate. Claims have been made that the added CBD helps people to feel more relaxed and can help reduce anxiety. There are different methods for manufacturing CBD, which include: liquid solvents, oil extraction, and supercritical carbon dioxide extraction. As the method of extraction varies, so may the composition of the products and the extracts.

22. The amount of CBD in the various products also varies from approximately 2 to 200 milligrams in total. However, for tinctures, this may vary to a greater extent because the consumer has control over the dosage.

23. The Food Standards Agency has previously sought toxicity advice from the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT). The COT concluded in July 2019 that there was evidence for hepatotoxicity, immunotoxicity, reproductive toxicity, changes in organ weight and alterations in drug metabolising enzymes (e.g. P450s). The COT could not conclude on the safety of CBD products and requested advice on mutagenicity from the COM.

24. Regarding the available genotoxicity data, some *in vitro* studies in bacteria gave negative results, but some in vitro studies with mammalian cells indicated positive results. A recent oral *in vivo* micronucleus test in mice gave a negative result, while an earlier 1980s intraperitoneal administration MN test in mice gave a positive result. Due to the conflicting genotoxicity data, the COM was asked to review the available data presented in paper MUT/2019/10 and to give its opinion.

25. The COM considered that the Ames test reported by Marx et al., 2018 used high purity CBD, was conducted to OECD Test Guidelines and gave a clear negative result. It was noted that this negative result may not be applicable to other lower purity CBD extracts. Regarding the *in vitro* tests in mammalian cells, members noted the negative results reported for adverse chromosomal effects in V79 Chinese hamster lung cells (Marx et al., 2018) and the negative result for the comet assay conducted in Caco-2 cells by Aviello et al., 2011. However, members had concerns over the reported positive results in the comet and micronucleus test conducted in human cells (HepG2 and TR146) by Russo et al., 2019. A summary table provided MN data, but did not provide data for the comet assay. The unexpectedly high percentage of cells in necrosis and apoptosis (e.g. 33 and 37%, respectively at the highest tested dose) raised concern over whether the test had been conducted adequately and whether cytotoxicity was a potential cause of the observed positive result. Also, the fold increase in MN appeared to be higher than would be expected and positive control data were not presented. Additionally, evidence for oxidation was reported for the comet assay, which may provide an explanation for the observed positive result.

26. Regarding the *in vivo* data, members considered that there was insufficient information provided on the study that gave a positive result (i.e. the *in vivo* intraperitoneal micronucleus test by Zimmerman and Raj 1980) to interpret the positive result reported e.g. insufficient information on the extraction method and whether there were potentially impurities or metabolites present in the test material. The Marx et al 2018 *in vivo* MN was agreed to be well conducted and negative.

27. Overall, the COM considered that an appropriate range of genotoxicity studies had not been conducted (either *in vitro* or *in vivo*) to conclude on the mutagenic potential of CBD. Additional information would be required on extraction methods and CBD purity for the studies conducted. Each study would need to be evaluated on a case by case basis depending on the test material e.g. considering the presence of impurities and metabolites. A negative result in one test under a particular exposure condition or with one test material may not be sufficient for an overall evaluation on the mutagenicity of CBD.

ITEM 8: REVIEW OF GENOTOXICITY OF PATULIN (MUT/2019/11)

28. No interests were declared.

29. Patulin is a mycotoxin produced by certain species of the genera Aspergillus and Penicillium (i.e. it arises from common spoilage microorganisms present in apples).

30. The International Agency for Research on Cancer (IARC 1986) classified patulin in Group 3, i.e. not classifiable as to its carcinogenicity, due to limited evidence for carcinogenicity in experimental animals. A factsheet published by the World Health Organization in 2018, stated that patulin is considered to be genotoxic but has not demonstrated carcinogenicity.

31. The Joint FAO/WHO Expert Committee on Food Additives (JECFA 1990) evaluation of patulin established a Provisional Tolerable Weekly Intake (PTWI) of 7 micrograms per kilogram of body weight per day (µg/kg bw/day). In 1995, JECFA updated its opinion and recommended a Provisional Maximum Tolerable Daily Intake (PMTDI) of 0.4 µg/kg bw/day, which was subsequently endorsed by the EU Scientific Committee (SCF 2000).

32. The Scientific Advisory Committee on Nutrition (SACN) is undertaking a review of the scientific evidence that will inform the Government's dietary recommendations for infants and young children aged up to 5 years. A review of the potential risks of patulin in the diet of infants aged 0 to 12 months and children aged 1 to 5 was presented to the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) in May 2019. The COT concluded that the new toxicological data (excluding the genotoxicity data) available from 1995 to 2018 would not change the current health-based guidance value. However, the genotoxicity was considered to be variable and therefore a view from the COM on the available genotoxicity was requested by the COT.

33. Paper MUT/2019/11 presented a review of the available genotoxicity data on patulin and the COM was asked to provide its opinion.

34. Members agreed that although many *in vitro* studies had been conducted, they were mainly non-standard genotoxicity studies that were poorly described (i.e. insufficient details on how each study had been conducted) with many being quite old. This meant that the available *in vitro* data were difficult to interpret. However, a number of positive *in vitro* responses were reported (e.g. induction of micronuclei in human lymphocytes), which could not easily be discounted on a weight of evidence basis. There was also some evidence of oxidative stress, which may provide an explanation for the observed positive results. Members suggested that there was a possibility for the occurrence of publication bias, due to the large interest in conducting studies on potential anti-oxidative properties and mycotoxins, which was a popular area of investigation (i.e. a potential danger of a bias towards the publication of positive results compared to negative results).

35. Regarding the *in vivo* studies, these also consisted of non-standard genotoxicity studies that were poorly reported or inadequately conducted (e.g. involving single doses and intraperitoneal administration) and therefore could not be interpreted. Positive results were reported in *in vivo* comet assays, however there was no description of measures of toxicity or oxidative stress, so it was not possible to determine whether the positive response was due to

direct or indirect interaction with DNA. Again, for *in vivo* studies (e.g. MN, chromosome aberrations and comet), members agreed that there was an indication of a positive response in sub-standard studies, which were inadequately conducted or described, and often complicated by co-administration of anti-oxidants. Therefore, the *in vivo* studies could not be interpreted.

36. Overall, the COM concluded that the *in vitro* and *in vivo* genotoxicity studies were inadequate. There was some evidence of positive results (particularly *in vitro*, but also *in vivo*), but in non-standard tests with insufficient details on how they were conducted. Therefore, the observed positive responses could not be interpreted, but were also difficult to discount. It was suggested that a standard regulatory genotoxicity tests should be conducted to acceptable standards (i.e. Ames test and in vitro micronucleus test) and that it would also be useful to investigate whether any positive response was due to oxidative stress.

ITEM 9: COM GUIDANCE SERIES UPDATE (MUT/2019/12)

37. Amendments to the COM Guidance document as a whole, up to Annex 1, had been previously considered at Committee meetings in July 2018 (paper MUT/2018/09), October 2018 (paper MUT/2018/13) and February 2019 (MUT/2019/01). At the last consideration, the Committee reviewed and suggested amendments up to para 74 – 'Stage 2: *In vivo* genotoxicity tests'.

38. The paper presented (MUT/2019/12) contained all amendments made to date. The Chair addressed each page of the document from para 74 in turn, inviting suggested comments and/or amendments. Members were asked to separately consider whether the content of Table 1, 'Supplementary *in vivo* genotoxicity tests' was still appropriate and to pass comments to the Secretariat following the meeting. In addition, the author of Annex 1 of the Guidance, 'Sensitivity and Specificity Data Considered by the COM', would be approached with respect to making specific amendments to that section.

39. All changes received would be incorporated into a new version of the Guidance Document to be reviewed at the next COM Committee meeting in February 2020.

ITEM 10: OECD UPDATES

40. The committee was informed that work had been ongoing to adapt the *in vitro* mammalian cell micronucleus assay test guideline (TG 487) to be applicable for testing nanomaterials. The European Commission's Joint Research Centre (JRC) initiated a round robin activity, which had been handed over to Swansea University and BASF for completion. A meeting would be held at the Joint Research Centre (JRC) in January 2020 to discuss arrangements for either amending the guideline or producing a guidance document for nanomaterials to accompany the existing test guideline.

41. The *in vivo* transgenic rodent gene mutation assay test guideline (TG 488) was also being updated and had been subject to review for some time.

The guideline was last updated in 2013, but since then it was noted that the recommendation for sampling of sperm from the cauda epididymis at 28+3 days is inappropriate because the cells sampled at this time were exposed during periods of spermatogenesis with no DNA replication and progressive decline in DNA repair activities. Modelling of spermatogenesis had been performed and evaluated by Health Canada, the Germ Cell working group of the Genetic Toxicology Technical Committee (GTTC) for the Health and Environmental Sciences Institute (HESI) and an OECD working group, however, a consensus had not been reached on the appropriate sampling time for developing germ cells and how it could be integrated with the assay on somatic cells. In addition, concerns were raised about the lack of experimental data to support the modelling. Draft text for the revised guideline would be discussed at the next meeting of the OECD Working Group of National Co-ordinators of the Test Guideline Programme (WNT).

42. The Chair of COM had acted as an observer for peer review on the validation of the Pig-A Assay. Progress on the assay was going well and it was hoped that guidance would be developed by 2020.

43. The committee were also informed that a Standard Project Submission Form (SPSF) had been submitted to produce a guideline for a 3D Reconstructed Skin Micronucleus assay.

ITEM 11: ANY OTHER BUSINESS

44. There was no other business.

ITEM 12: DATE OF NEXT MEETING

45. Date of next meeting – to be arranged.