Committee on MUTAGENICITY

MUT/MIN/2020/1

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

Minutes of the meeting held at 10.30 am on 20th February 2020 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 Dr R Morse Dr A Povey
Secretariat:	Dr O Sepai (PHE Scientific Secretary) Mr S Robjohns (PHE Secretariat) Dr C Mulholland (FSA Secretariat)
Secretariat Support:	Dr R Bevan (WRc/IEH Consulting) Mr B Seery (WRc plc)
Assessors:	Dr L Koshy (HSE) Dr H Stempleski (MHRA) Mrs R Pearson (VMD)
Observers	Professor J O'Brien (FSA Scientific Council)

	Dr G Stoddart (PETA International Consortium limited) Dr H Thurston-Smith (GW Pharmaceuticals)
In attendance:	Dr R Foster (Lhasa Ltd)

ITEM 1: ANNOUNCEMENTS/APOLOGIES FOR ABSENCE

1. The Chair welcomed the COM members, assessors and secretariat. Dr C Mulholland attended for the Food Standards Agency. Professor J O'Brien (FSA Scientific Council); Dr H Thurston Smith (GW Pharmaceuticals); and Dr G Stoddart (PETA International Consortium limited) attended as observers. The Chair also welcomed Mr B Seery attending for WRc plc and Dr R Foster attending for Lhasa.

2. Apologies for absence were received from Professor D Harrison (Ex Officio), Dr R Morse, Dr D Gott (FSA), and Ms E Blenkinsop (DHSC).

3. The COM was informed that Dr D Gott is improving in health and hopefully will return to work in the next few months.

4. The Committee was informed that interviews would be conducted for the two vacant positions for expert members and one lay member. It was hoped that these vacancies would be filled in time for the next meeting in June.

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

ITEM 2: MINUTES OF MEETING ON 10th OCTOBER 2019 (MUT/MIN/2019/2)

6. Members agreed the minutes subject to minor typographical changes. Item 10 on OECD updates was not complete. This would be added and sent out for agreement.

RESERVED SESSION

7. The draft minute on the reserved business item on the risk to human health from the use of azodicarbonamide (MUT/2019/07) was approved.

OPEN SESSION

ITEM 3: MATTERS ARISING

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

ITEM 4: REVIEW OF THE GENOTOXICITY OF CANNABIDIOL UPDATE (MUT/2020/01)

9. Dr Carol Beevers noted a potential conflict of interest in that she may have been involved in some of the contract studies for a client while working for a previous employer. This was considered to be a non-personal specific interest. Dr Beevers was permitted to take part in the discussion as it was not possible to determine whether she had been involved in the specific studies presented.

10. The Food Standards Agency (FSA) previously asked for an opinion from the COM on the genotoxicity of CBD. This was to assist the FSA in developing its advice relating to the increasing number of requests for a risk assessment of CBD in consumer products. The Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) evaluated the potential adverse health effects of CBD products in July 2019. It concluded that that genotoxicity data were conflicting and requested a COM view of the genotoxicity data. Subsequently, the COM considered genotoxicity data relating to CBD at its previous meeting in October 2019. The COM concluded that the *in vitro* and *in vivo* studies were inadequate. In January 2020, the COT received an update on available data, which included additional genotoxicity data. The COT therefore referred consideration of the new genotoxicity data to the COM.

11. Paper MUT/2020/01 provided details of additional genotoxicity studies submitted to the European Medicines Agency (EMA) (available online) in relation to a medicinal form of CBD known as Epidiolex (used to treat seizures in certain medical conditions e.g. Lennox-Gastaut syndrome and Dravet syndrome).

12. The *in vitro* data consisted of pure CBD tested in the Ames test conducted to GLP (in *Salmonella typhimurium* strains TA98, TA 100, TA 102, TA 1535, and TA 1537). Members had no concerns over the reported data and agreed with the conclusion of a negative result.

13. Two *in vivo* studies were reported, a bone marrow micronucleus test and a comet assay for chromosome damage. Pure CBD was evaluated for its potential to increase the incidence of micronucleated polychromatic erythrocytes (MNPCEs) in rat bone marrow cells. Male rats received two oral gavage doses of 0 (sesame oil), 125, 250 and 500 milligrams per kilogram of body weight per day (mg/kg bw/day. The positive control group was dosed once with cyclophosamide (CPA 20 mg/kg) on the second day of dosing. In addition to animals tested for micronucleus formation, two groups of satellite animals were dosed with vehicle and pure CBD (500 mg/kg/day) for confirmation of exposure (this did not include toxicokinetic data). Clinical signs of exposure (e.g. lethargy, ataxia, piloerection, anogenital soiling and unkempt appearance) were observed on day 3. CBD treated rats showed mean MNPCE frequencies similar to those of the vehicle control group and fell within the laboratory's historical vehicle control range. Members noted that they could not see any information provided on whether the target tissue had been exposed (e.g. toxicokinetic or plasma levels) but assumed that because this study related to a medicinal product that appropriate toxicokinetic data would be available, which would be informative regarding bone marrow exposure. The COM agreed that from the information provided that the study appeared to be robustly conducted and gave a negative result.

14. In a rat alkaline comet assay, rats were given single oral gavage doses of 0 (sesame oil), 125, 250 or 500 mg/kg/day CBD oral solution. Liver samples were taken 24 hours after the initial dose. No clinical signs of toxicity were observed at any dose. Members agreed that from the information provided the study appeared to be robustly conducted and gave a negative result.

15. Overall, the COM concluded that from the information provided, the studies appeared to be well conducted and gave negative results. However, the COM asked whether it could see all the relevant data for the *in vivo* studies to confirm that there was sufficient target tissue exposure and to evaluate whether there was any important species difference in metabolism (i.e. between humans and rats) because the potential for this this was mentioned in the summary information provided.

ITEM 5. GUIDANCE STATEMENT ON QSAR MODELS TO PREDICT GENOTOXICITY (MUT/2020/02)

Presentation by Dr Robert Foster from Lhasa Ltd

16. Dr Robert Foster from Lhasa Ltd presented an update on the Lhasa Limited *in silico* prediction models for genotoxicity. Lhasa, was established in 1983, and has its Head Quarters in Leeds, United Kingdom. It is a Not-for-profit & Educational Charity. Licence holders become members of Lhasa, all members are encouraged to share and publish their data, working collaboratively with Lhasa scientists to improve their products.

17. Dr Foster introduced (Q)SAR systems, using Derek & Sarah Nexus as examples, before discussing the performance of (Q)SAR systems and model development with respect to genotoxicity.

18. Derek was one of the first pieces of software to include reactive groups in the form of structural alerts that flagged potential genotoxicity to the user. In the early years, alert refinement focused on alert specificity as the 'Ashby & Tennant' alerts were too general. Over subsequent years, improvements were made to the interface and efforts were made by the scientists at Lhasa to identify new toxicophores and structure activity relationships from the general literature. These were then encoded as new structural alerts in the system. For mutagenicity endpoints, the number of alerts increased from 58 in 1996, to 91 in 2010, to 132 alerts in the most recent release.

19. Derek Nexus is an expert rule-based model with a knowledge base incorporating 132 alerts for mutagenicity in 2018. It has been built using public and confidential data in collaboration with regulators and industry members. Thirty five percent of alerts for mutagenicity are based upon proprietary data. A new release to be published soon will comprise of 220 alerts for genotoxicity: 148 mutagenicity alerts; 99 chromosome damage alerts; and 5 non-specific genotoxicity alerts. Rules written by experts, incorporating chemical reactivity, metabolism, toxicology expertise make the SAR more relevant, this is an advantage of a rule-based system.

20. Sarah Nexus is a statistical system. Statistical systems are built on a large training set and use a computer algorithm to look for associations. In Sarah the compounds in the training set are fragmented, as DNA reactivity is associated with the chemical reactive group, Ames activity is then associated with each fragment.

21. In Sarah the training set contains 9882 individual structures from the public domain and member donations. This is made up of 4716 (48%) mutagens and 5166 (52%) non-mutagens These molecules are fragmented. Sarah then generates a *hypothesis* for a fragment. For a query structure the program carries out the same process, takes the fragments and assigns the activity as in the training set.

22. Although, Derek and Sarah are different types of models, both demonstrate a large amount of information to the user about the prediction for them to review. A key attribute of the Lhasa software is transparency of results to provide the reasoning behind the alert implementation (Derek) or prediction (Sarah) so users have the necessary information to review the prediction for their query. Lhasa systems are designed to be fully transparent to present the user with the information to show why the alert writer implemented the alert in Derek Nexus and the training set is shown in Sarah Nexus. These models perform better on public data as the compounds in the training sets are mainly public chemicals.

23. For mutagenicity it is accepted that these models perform very well, they are accepted for regulatory purposes. The ICH M7 guidelines state that one expert rule-based and a statistical-based model can be reviewed with expert knowledge to support the final conclusions for the mutagenic potential of impurities.

24. Improving the algorithm or display of results may be important for newer models but established systems such as Derek and Sarah may focus on improvements through increasing the chemical space coverage by addition of data from public and/or proprietary sources. Donation of proprietary data encourages collaboration to benefit scientific community. In Derek 35% of alerts for mutagenicity are constructed or refined using proprietary data. This also benefits members as this improves the models in their chemical space. The chemical structure is generalised and benefits other members. The data is used in Sarah will have to be published and be in the public space.

25. Dr Foster then went on to give examples of how public and private data have been used to develop Lhasa products.

26. The first examples looked at public data and genotoxicity for alylbenzenes. These compounds are relevant for food as well as pharmaceutical domains. For this group of chemicals three alerts were written: the Ames alert, was the most restrictive as S9 may not be metabolically capable, an alert for chromosomal aberrations and the UDS as a non-specific genotoxicity. An advantage of rules-based system they can be very specific and use expert knowledge.

27. The second example focused on the use of proprietary data. In a collaboration with Japanese Pharmaceutical Manufacturers Association which is a consortium of 11 companies. A large data set was shared which predicted with low sensitivity. The data were then curated and clustered, and alerts were refined, or new alerts were implemented. This led to a large increase in the predictive performance against the dataset.

28. Improving predictivity in Sarah: Sarah uses published literature. In 2019 Lhasa focused on increasing coverage of nitrosamines due to issues in pharmaceuticals. Lhasa's data team rapidly increased the coverage of nitrosamines form published data and added 95 added data sets to Vitic. All of these have gone into the Sarah training set.

29. Finally, Dr Foster discussed *in silico* predictions of genotoxicity in particular. He referred to the recent EFSA Supporting publication 2019:EN-1598. (Evaluation of the applicability of existing (Q)SAR models for predicting the genotoxicity of pesticides and similarity analysis related with genotoxicity of pesticides for facilitating of grouping and read across. doi:10.2903/sp.efsa.2019.EN-1598.)

30. This report concluded that QSAR predictions work well for mutagenicity but does not work so well for other endpoints. The reasons for this are given as the Ames test is very well accepted the protocols are standardised, for other genotoxicity tests the models are not as well established. There is also far greater Ames data available for model building compared to other tests for genotoxicity such as chromosome aberration and micronucleus tests.

31. A validation of Derek against chromosome aberration data showed that it performed well on chemicals which are expected to be DNA reactive. But Derek has low sensitivity for prediction of a set of compounds known to interact with either topoisomerase or tubulin. In Derek chromosomal damage (CD) alerts primarily cover NDA/protein reactive compounds. This is an issue with rule-based systems where creating a valid SAR is incredibly difficult for complex, poly(hetero)aromatic ring systems.

32. Dr Foster demonstrated how a statistical system may be able to complement the rule-based system by creating a Sarah model for the prediction CD. Data were taken predominantly from Vitic Nexus. Each time a compound is positive in both *in vitro* CA or *in vitro* MN data sets it is counted as positive in CD. This model is significantly more sensitive for prediction of chromosome damage compared to Derek. However, it is important to note that Sarah was designed for the prediction of mutagenicity *in vitro* and, in line with the report by EFSA, additional refinement would be required to the model should it be considered for use for prediction of chromosome damage *in vitro*.

33. Dr Foster Concluded his presentation with a summary:

34. *In silico* systems can provide predictions of genotoxicity. Very strong performers in mutagenicity. Performance accepted by regulators under ICH M7. The push towards other genotoxicity endpoints - this still requires more research. This is discussed in the EFSA report.

35. Lhasa is addressing the issues highlighted in the EFSA report. One way would be to increase the amount of data used to develop the models. QSARs don't necessarily need to predict the endpoint but could be used, for example, in adverse outcome pathways.

36. A member of COM asked if there are examples of requests for further tests such as CA, for Lhasa to fill data gaps. This does happen where data gaps

have been identified and Lhasa members will contribute further data. Members can also challenge the predictions. This dialogue helps to improve the Lhasa products.

37. In terms of the robustness of mutagenicity data there is a large volume of mutagenicity data but much less for chromosomal aberrations, the quality of the data can also be a factor with a number of false positives. The data is quality controlled before it is incorporated into the Sarah chromosome damage (CD) model, a reliability tag is added. These tags are used to quality control the data before it goes into Sarah. But there has to be balance between removing data which may be flagged and having sufficient data sets to build a model - ensuring the user is then aware of the quality of the data which has been used and therefore the reliability of the predictions is essential for review.

38. There has been a lot of debate as to whether *in vivo* data should be used instead of *in vitro* data if available However, there is a lot of *in vivo* MN but few *in vivo* CA data. So how can these data sets be combined? The CD model was built on *in vitro* MN and CA data. There are over 1500 compounds with *in vivo* data, this data could still be made available to a user for review but not used in a prediction. The predictivity of the models is only as good as the predictivity of the data upon which they are based. QSAR predictions can be used in a weight of evidence assessment and to guide testing.

39. QSARs which predict AMES positive results are basically showing compounds which can produce an electrophilic DNA reactive species. QSARs can be used to predict initiating events and not the endpoint this is a way to look at mechanisms in an adverse outcome pathway assessment. But this is outside the scope of Derek or Sarah.

40. There was discussion with regard to the availability of negative data. Pharmaceutical companies will submit both negative and positive data, that is proprietary data. It is often harder to find negative data in the open literature. This can cause some bias in the models.

41. The Chair thanked Dr Foster for a very informative presentation and discussion.

ITEM 6. COM Guidance Series update (MUT/2020/03)

42. Amendments to the 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) and October 2019 (MUT/2019/12). At the last consideration, the Committee completed their review and suggested amendments to the main text.

43. The paper presented (MUT/2020/03) contained all amendments made to date to the main text. Members were asked to separately consider the content of Table 1 and Annexes 1, 2 and 3 and outstanding questions regarding the main text. The Chair addressed each page of the document in turn, inviting suggested amendments to outstanding questions. The author of Annex 1 had been consulted by the Secretariat and had recommended removing the text as the

information was now historical in nature. This was agreed by the Committee with the suggestion that reference was made in the latest version of the Guidance to older versions with this information, as it provided valuable background. A decision was also taken to apply this approach to Annex 3.

44. With regards to Table 1 and Annex 2, the Committee agreed that these should remain. Members were also asked to provide updated references for a number of sections, and it was agreed that the specific areas needed would be identified by the Secretariat and sent to members.

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

ITEM 7: TWO DAY WORKSHOP IN BIRMINGHAM ON THE INTREPRETATION OF GENOTOXICITY DATA

46. At the previous COM meeting in October 2019 members were presented with two draft papers following the two-day workshop held in Birmingham in June 2019 on the interpretation of genotoxicity data in a regulatory environment. The first paper (MUT/2019/09) provided notes of the presentations and discussions. The second paper (MUT2019/09) provided an assimilated summary of the workshop. Following comments from members at the October 2019 meeting the two draft papers were sent out for comments to two ex-COM members who had been present at the workshop, external attendees from industry and participants from EFSA. Following the received comments, the two papers were updated. The amended papers (i.e. draft notes (MUT/2020/04) and summary document (MUT/2020/05)) were presented to the COM for any further comments.

47. Members considered that the various questions and the outstanding matters that needed to be resolved could better be addressed by a summary of the relevant questions being sent to the members by email. Regarding a future publication, it was suggested that this could be drafted by using the greater detail contained in the draft notes combined with some of the useful introduction and ‘setting the scene’ descriptions contained in the draft summary paper. The secretariat agreed to summarise the outstanding questions and circulate to members via email.

ITEM 8: HORIZON SCANNING

48. It was noted that the previous item on the two-day workshop on the interpretation of genotoxicity data contributed to horizon scanning. For example, there was a proposal to form a working group to develop a framework or guidance (perhaps, similar to that of the Bradford-Hill criteria) on how to evaluate genotoxicity data from different sources (e.g. unpublished GLP studies conducted to OECD test guidelines and non-GLP studies published in the scientific literature). A few members expressed an interest in contributing to this. It was also noted that an additional COM led workshop could be organised in the future to further discuss unresolved questions that came out of the Birmingham meeting.

49. The committee was informed of an email from the DHSC assessor that said the UK would start formal negotiations with the EU in March 2020. It was anticipated that the UK would publish its mandate for negotiations with the EU next week. This would include UK objectives for the chemical sector and rules/regulations relating to future trade. It was also anticipated that formal negotiations with the EU would start in March and that Defra would be developing a new chemical strategy. Additionally, it was expected that there would be a call for evidence in Spring relating to human health and chemicals in the environment.

50. The COM assessors considered that it was currently difficult to predict how the various government departments/agencies may require COM input in the future.

51. Members noted a few topics that the COM may need to consider in the future and these included the baseline for spontaneous inherited mutations; environmental DNA (eDNA) collected from environmental samples (e.g. soil, water or air), which could be informative for monitoring various aspects, such as biodiversity (via DNA sequencing without having to collect individual living organisms); and new techniques for evaluating DNA damage. Additionally, it was noted that horizon scanning needed to be targeted with a need to avoid duplication or unnecessary work (e.g. in terms of regulatory response to technological changes). The COM was also informed that the COT was holding a workshop on exploring dose-response analysis at Manchester on the 11th March 2020.

ITEM 9: OECD PIG-a UPDATE

52. The COM was provided with paper MUT/2020/06 relating to the PIG-a gene mutation assay, mainly for information. This included UK comments that had been submitted to the OECD on the development of its test guideline. Member were asked if they had any additional comments.

53. The Chair declared an interest in that he had been involved with an OECD working group on a development for a Test Guideline for the PIG-a assay.

54. The COM agreed this did not contain anything controversial and was generally content. It was noted that although there was nothing wrong with the assay, it did not appear to fill any useful gaps i.e. it did not enable anything to be investigated that couldn't already be done with existing methods. It would be useful if it could be developed further to examine other tissues in addition to peripheral blood.

55. Additionally, an update on the development of OECD Test Guideline 488 on transgenic rodent somatic and germ cell mutation assays was circulated to the COM (just a day before the meeting). Members were aware that there had been some disagreement between some countries over the text for sampling time in relation to rat germ cells. Members were also aware of reported evidence and modelling of rat spermatogenesis that suggested that a 28 day + 28-day (i.e. sampling 28 days later, after 28 days of dosing) designs was a better germ cell design than 28-day + 3-day (i.e. sampling 3 days later, after 28 days of dosing) for both the mouse and rat. The UK had previously commented that the

data on appropriate sample times were not as good for the rat as the mouse. The relevant paragraph had been reworded to create a ‘quick fix’ for TG 488.The COM was content with the new wording that had been circulated (e.g. regarding sample times).

ITEM 10: WHO JECFA RESPONSE TO CONSULTATION (MUT/2020/07)

56. The Committee was provided with comments from COM members that had already been sent to the Joint FAO/WHO Expert Committee on Food Additives (JECFA) secretariat on its draft revision of EHC 240 chapter on genotoxicity. Members were asked whether they wished to submit any additional comments. JECFA were expected produce a final version and provide responses to any not taken into consideration. The COM had no further comments.

ITEM 11: DRAFT ANNUAL REPORT (MUT/2020/08)

57. An initial incomplete version of the draft report was circulated for information. It was incomplete because items from the previous COM meeting in October 2019 could not be incorporated until the minutes had been approved. The items in the approved minutes from today’s meeting would be inserted into the draft annual report.

58. Members noted that the wording on Toxtracker needed to be amended to reflect that it detects two different responses to DNA damage rather than two different types of DNA damage (i.e. there are more than two types of DNA damage).

59. Members were requested to send any further comments on the draft annual report to the secretariat via email.

ITEM 12: ANY OTHER BUSINESS

60. There was no other business.

ITEM 13: DATE OF NEXT MEETING

61. 9 June 2020 – venue to be arranged.