Committee on MUTAGENICITY

MUT/MIN/2018/1

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

Minutes of the meeting held at 10.30 am on Thursday 22nd February 2018 at Public Health England, Wellington House, 133 – 155 Waterloo Road, Lambeth London, SE1 8UG.

Present:

Chairman:	Dr D Lovell
Members:	Dr C Beevers (via teleconference) Dr G Clare Professor S Doak (via teleconference) Dr S Dean Professor D Harrison Professor G Jenkins Professor D Kirkland Professor F Martin Dr A Povey
Secretariat:	Dr O Sepai (PHE Scientific Secretary) Mr B Maycock (FSA Secretariat) Mr S Robjohns (PHE Secretariat) Miss H Smith (PHE Secretariat)
Secretariat Support:	Dr S Bull (WRc/IEH Consulting) Dr K Burnett (WRc/IEH Consulting) Dr R Bevan (WRc/IEH Consulting) Dr L Rockett (WRc/IEH Consulting)
Assessors:	Dr L Dearly (HSE) Dr R Pearson (VMD) Dr H Stemplewski (MHRA)

Observers:	Wendy Dixon (FSA – item 4) Firth Piracha (FSA **–** item 4)
In attendance:	Miss B Gadeberg (PHE COC & COT) Secretariat – via teleconference for item 7) Dr F Hill (FSA for item 4)

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. Dr F Hill attended from the Food Standards Agency (FSA) for item 4. Wendy Dixon and Firth Piracha attended as observers from the FSA for item 4.

2. Apologies for absence were received from Dr Mike O'Donovan (member), Dr C Ramsay (Health Protection Scotland), Dr I Martin (EA assessor), and Ms T Netherwood (DHSC assessor).

3. The committee was informed that Professor Helga Drummond had resigned from the COM due to personal reasons and that a new lay-member would be sought. Dr Carol Beevers and Dr Steven Dean had been reappointed to the COM for a further 3 years and Professor David Kirkland and Professor Gareth Jenkins had been reappointed for a further year. An advert for a new expert member had been placed and an advert for a new lay-member would be submitted when it had gained ministerial approval. The Chair announced that it was the last meeting for Professor Frank Martin and thanked him for his hard work. The committee was informed that appraisals of its expert members would be carried out by the Chair.

4. The committee was informed that the new contract for scientific writing for the COM had been awarded jointly to WRc and IEH Consulting who introduced themselves to the committee.

5. The members were asked to review and provide any declarations of interest to the secretariat. Members were also reminded to declare any interests before discussion of items.

ITEM 2: MINUTES OF MEETING ON 23 FEBRUARY 2017 (MUT/MIN/2017/1)

6. Members agreed the minutes subject to minor changes.

ITEM 3: MATTERS ARISING

7. The COM was informed that the COT statement on Heat-not burn tobacco products had been published. The COM had been consulted and contributed to this evaluation. The COM statement on quantitative risk assessment of genotoxicity would also soon be published.

RESERVED BUSINESS

ITEM 4: CONSIDERATION OF EFSA SAFETY ASSESSMENT OF CERTAIN FLAVOURING SUBSTANCE (MUT/2018/01)

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

OPEN SESSION

ITEM 5: USE OF (Q)SAR MODELS TO PREDICT GENOTOXICITY: A SCOPING PAPER (MUT/2018/2)

9. The COM had previously agreed that when no genotoxicity data were available an initial assessment of potential genotoxicity could be based on publicly available Structure Activity Relationships (SAR) and Quantitative Structure Activity Relationships (Q)SAR models. An initial investigation was undertaken to determine whether Stage 0 (Preliminary Considerations prior to genotoxicity testing) of the COM 2011 Guidance on a Strategy for genotoxicity testing of chemical substances needed to be amended and updated in relation to developments in (Q)SAR models. A scoping paper (MUT/2018/2) had been prepared that provided a brief summary of ten (Q)SAR models, covering knowledge-based, statistical and hybrid models. For each (Q)SAR model considered, information was collated on a range of topics, such as the endpoints covered, the size of the data set and any statistics applied to test the robustness of the model.

10. Members raised concerns over the lack of transparency of the data on which the various models were based and the impacts on subsequent predictions (e.g. relating to the proprietary nature of the data contained within many (Q)SAR models, the quality of the data and the chemicals included). Members suggested that caution be applied in the use of (Q)SARs as a consequence, and that it may be appropriate to invite an expert to the committee to provide guidance on such issues.

11. A question was raised on whether the (Q)SAR models can predict the genotoxicity of metabolites. The Committee considered that if a structure of a particular metabolite is known, then a (Q)SAR model can be used to predict the mutagenicity of that metabolite (providing its structure falls within the model applicability domain). There are models e.g. within OECD Toolbox and LHASA Meteor (amongst others) that can predict the metabolites of a substance. One member suggested that metabolites should be identified first, and then a (Q)SAR model can be run on identified metabolites to predict mutagenicity.

12. Members had questions on the frequency at which (Q)SAR models were updated. The Committee was informed that some models were updated with regularity, whilst others had not been recently updated.

13. The Committee suggested that it is often necessary to run several models, which may have differing quality. Some regulations, such as the ICH M7 guidance, require the use of two (Q)SARs; one rule-based and one statistical-based model prior to acceptance. The Committee stated that this is also implied within the European Food Safety Authority (EFSA) guidance. However, it was unclear how many chemicals had been assessed by such an approach.

14. The Committee expressed a concern that different (Q)SAR models provide different outputs and utilise differing terminology. Therefore, there was a concern as to how multiple models are used and how the interpretations from these models are combined.

15. The Committee considered that whilst it would be useful to include information on the use of (Q)SARs as a negative predictor for screening purposes, the data on (Q)SARs were insufficient, at present, to warrant the COM reviewing their use in Stage 0 of the guidance document. It was agreed that currently there was no requirement to update the (Q)SAR section of stage 0 of the COM Guidance on genotoxicity testing. It was agreed to amend the wording in chapter G0 of the guidance document to reflect the fact that this section had been considered in 2018. Members recommended that the secretariat should consider the feasibility of producing a separate section on (Q)SARs on the COM website that could be updated more frequently than an overall Guidance document.

ITEM 6: COM GUIDANCE UPDATE – EVALUATION OF IN VIVO GENOTOXICITY (MUT/2018/03)

16. The COM Guidance on in vivo genotoxicity assays was last updated in 2011. Following on from preliminary discussions at the joint meeting horizon scanning exercise in October 2017, it was suggested that a brief overview of developments in *in vivo* genotoxicity testing would be useful to determine whether the Guidance on *in vivo* genotoxicity testing needed to be updated.

17. Paper MUT/2018/03 provided a summary of regulatory requirements relating to three *in vivo* genotoxicity assays, namely UDS, transgenic mutation and the comet assay and publications outlining significant changes since 2011. Two publications were specifically highlighted, an European Food Safety Authority (EFSA) Opinion on the UDS assay and a validation of the *in vivo* comet assay by the Japanese centre for the Validation of Alternative Methods (JaCVAM). Further ongoing developments were also noted via the International Workshops on Genotoxicity Testing (IWGT).

18. Members considered that there had been no significant changes to strategy developments or assay methodologies that merited a re-write of the COM guidance presently. However, there is a need to acknowledge that COM has considered the changes that have been made since 2011. For example, the Guidance document needed to contain a stronger statement about the use and applicability of the UDS assay.

19. Following discussion, the most appropriate way to do this was to keep the main body of the Guidance text to serve as a Framework document with generic guidance, and to have separate sections as stand-alone documents that could be updated as regularly as required. It was also considered that changing to a web-based version of the Guidance document may facilitate this.

Such a format would also allow information submitted in position papers to be linked to the website, for example on germ cell mutagenicity testing or the use of QSAR. A Member suggested yearly checks on the sectional documents with a re-badging of the year to ensure that the public can see it is up to date.

20. A Member updated the Committee on IWGT and Genetic Toxicology Committee (GTTC) discussions on whether the *in vivo* comet assay provides the same results (i.e. positive or negative) as a transgenic rodent gene mutation assay for chemicals that are positive in the Ames assay. Work is being conducted to determine whether the reliability of the comet assay to detect gene mutations can be qualified or quantified using existing data available on Ames positive substances. A revision to the OECD Test Guideline 488 on the Transgenic Rodent somatic and germ cell gene mutation assay has been proposed, however it make take some time before this is accepted. The possible development of test guidelines regarding the in vivo Pig-A assay were being discussed by IWGT and GTTC and whether the mini-Ames assay should be included in OECD 471 (Bacterial reverse mutation test). The ongoing evaluation of the appropriate sampling time for germ cells in the transgenic rodent assays (TGR) was also discussed.

21. Members did not consider that a detailed evaluation of the *gpt delta* TGR assay should be undertaken as it is not widely used. However it is still considered to be a valid assay. It was noted that the Lac Z (MutaMouse) and the Lac I (Big Blue) the most widely used for the TGR TG 488 assay.

22. With regards to the *in vivo* comet assay specifically, members considered that a statement regarding tissue selection should be included. Other significant developments to be included for review were the Pig-A assay and the liver micronucleus assays and germ cell mutagenicity assays.

23. It was suggested that the secretariat would consider the feasibility of producing separate sections on specific aspects of the Guidance on the COM website. These could subsequently be updated more easily and when necessary.

ITEM 7: STATEMENT FROM A JOINT COMMITTEE WORKSHOP ON THE USE OF EPIGENETICS – UPDATED FIRST DRAFT (MUT/2018/04)

24. In October 2017, the COC, COT and COM held a joint meeting. One of the topics discussed was “Whether epigenetics should be used in chemical risk assessment?” Paper MUT/2018/04 presented the first updated first draft statement from this joint committee meeting.

25. The statement was initially presented to the COC in November 2017 and amended following comments from Members and speakers at the workshop. The updated statement was then presented to the COT on 6th February 2018, and amended accordingly with Members comments, prior to presentation to the COM.

26. Members who attended the joint Committee workshop noted that one of the conclusions was that toxicological tests that are currently carried out are sufficient to detect toxicological changes, although it may be useful to further understand what tests would be available to investigate epigenetic changes. Members queried what endpoints would be covered, how these correlate with genotoxicity tests and how to extrapolate from in vivo data to humans.

27. Members had no further comments on the update first draft of the statement.

ITEM 8: FORWARD PLAN AND HORIZON SCANNING (MUT/2018/05)

28. The COM is a joint Department of Health/Food Standards Agency committee, which provides independent advice to government departments and agencies on the potential mutagenicity and genotoxicity of chemicals including natural products, synthetic chemicals, and chemicals used in pesticides and pharmaceuticals. It also advises on strategies and research for genotoxicity testing, and advises on the mutagenicity of chemicals in food, consumer products and the environment. The COM has a joint PHE/FSA secretariat, which is led by Public Health England. Every year the COM conducts a Horizon Scanning exercise, which feeds into the COM forward work plan.

29. Paper MUT/2018/05 summarised the current issues and some of the topics that had been suggested my members of the committee, Government Department/Agency assessors and through the joint committees (COT/COC/COM) discussions held in October 2017.

30. Members were asked to review the paper provided and to make comments in terms of developing a COM work programme for 2018.

31. The COM noted that E-cigarettes were currently being considered by the Committee on Toxicity in food, consumer products and the environment (COT) and that the COM may be consulted during the year on genotoxicity aspects.

32. Members noted the previous discussion at the joint COT/COC/COM meeting in October 2017 where concern had been expressed over publication bias (i.e. where there was a reluctance by journals to publish negative results); the increase in predatory journals resulting in the publication of poorer quality studies; that some agencies appeared to give greater emphasis to positive results in non-validated test systems using non-standard protocols compared to negative results from standard regulatory studies conducted in accordance to OECD test guidelines and Good Laboratory Practice (GLP). It had been suggested that these concerns could be addressed by the Committees jointly writing to the relevant authoritative organisations, such as ECHA and EFSA and/or to a high profile journal. It was noted that consideration of how to assess biological and statistical significance was another area of work that could be addressed jointly by the committees (e.g. COT/COC).

33. Members were aware of the recommendation to incorporate genotoxicity testing in standard 28 day toxicity tests to reduce the overall numbers of animals tested. However, this was considered to depend on the logistics of the study and planning/timing of tissue sampling, requiring collaboration between toxicologists and genetic toxicologists, rather than a scientific question. Other topics that been suggested, included genotoxicity associated with non-cancer endpoints and how high the maximum tested dose should be (e.g. in terms sufficient sensitivity); and the increase of genetic damage with age in terms of the extent was due to intrinsic aging and how much due to a greater duration of exposure to genotoxic substances.

34. A lack of clarity over an appropriate *in vivo* test following a positive *in vitro* gene mutation test result was highlighted, however, it was noted that an International Life Sciences Institute/Health and Environmental Sciences Institute (ILSI/HESI) Working Group was already addressing this. Members did not consider that evaluation of expanded simple tandem repeat (ESTR) mutation induction in the male germ line was a priority, at present.

35. The COM considered that it would need to have a further look at developments in the Quantitative dose-response analysis of genotoxicity data relatively soon and that it would be useful to investigate potential genotoxic effects arising from the use of CRISPR or other DNA damaging technology. Consideration of OECD genotoxicity Test Guidelines would be included as a regular item. A watching brief would be kept on the genotoxicity testing of nanoparticles and developments in epigenetics. The forward plan would also include an annual requirement to consider whether there were any developments that required an update of the COM Guidance on genotoxicity testing.

36. Members were requested to send any additional comments to the secretariat.

ITEM 9: ANNUAL REPORT 2017 (MUT/2018/06)

37. Members were informed that a draft of the annual report for 2017 would be produced for them to comment on.

ITEM 10: ANY OTHER BUSINESS

i) Update on International Workshops on Genotoxicity (IWGT)

38. One member provided the COM with an update on the recent IWGT Meeting:

3D models

39. 3D Models have been suggested as representing a more '*in-vivo* like' behaviour and for use as 2nd tier assays to follow up a positive result from standard *in vitro* assays and to provide a more realistic test system to study

particulate materials (e.g. nanomaterials), compared to 2D test systems. However, the IWGT considered that it is important that the full range of mutagenicity (e.g. gene mutations, clastogenicity and aneugenicity) can be detected in each tissue model.

40. The IWGT agreed that a micronucleus (MN) assay could be applied to 3D liver spheroids. The inability to detect substances that induce gene mutation was considered to be a gap. The comet assay could be used in this respect and it was recommended that this be investigated. Initial data indicated that the comet assay could be applied to 3D lung models. The 3D lung comet assay could detect chemicals that induce DNA damage leading to gene mutation and chromosomal damage. But, the inability to detect aneugenicity was considered to be a gap and the limited proliferation of the cells makes the MN assay problematic. More information on the metabolic competence of the cells was also considered important. The use of robust protocols and validation according to OECD Guidance document 34 was recommended.

41. It was agreed that a position had been reached, where standard protocols for the 3D skin comet assay and the reconstructed skin MN assay could be defined. Transferability of the assays to a large number of laboratories across 3 continents had been demonstrated. The assays are now available at several Contract Research Organisations and are performed under Good Laboratory Practice (GLP). International validation studies with coded chemicals have demonstrated good intra- and inter-laboratory reproducibility of the methods. The IWGT Working Group considered that the 3D skin comet and MN assays are sufficiently validated to move towards the development of individual OECD Test Guidelines.

Risk of aneugens for human health (cancer and hereditary diseases)

42. Adverse Outcome Pathways (AOPs) had been developed for 1) tubulin binding leading to somatic cell aneuploidy, and 2) aurora B inhibition leading to aneuploidy. In terms of germ cells, the IWGT considered that there was limited evidence that exposure to aneugens induced heritable diseases in humans. The IWGT agreed that some aneugens induce cancer in humans and animals. However, all of these compounds possess other genotoxic and non-genotoxic properties linked to carcinogenesis. The role of aneuploidy in carcinogenicity in these cases had not yet been established. Tubulin disrupting aneugens that do not possess other properties linked to mechanisms of carcinogenesis were not considered to be carcinogenic in rodents. Similarly, the extensive use of pharmaceuticals with tubulin disrupting properties was considered not to be associated with increased incidences of cancer humans.

Ames test revisited

43. The IWGT agreed that the bacterial strain TA1535 could be removed from the standard Ames test battery with no loss in sensitivity. Also that there was a disadvantage to including TA1537 compared to TA97/97a in the standard Ames test battery, as a higher sensitivity is achieved when TA97/97a is used instead. It was noted that there were noticeable differences in historical

negative and positive control ranges among laboratories world-wide. Each laboratory was recommended to develop and maintain its own historical control database. Data evaluation criteria, demonstration of laboratory proficiency and the role of *in silico* evaluations were not fully discussed.

In vitro mammalian cell gene mutation assays

44. The IWGT considered that mammalian cell gene mutation assays should have the ability to detect a range of heritable genetic changes including point mutations, small insertions and deletions (indels), large deletions, loss of heterozygosity (LOH) and/or recombination, and changes in chromosome structure and number. Mammalian cell gene mutation assays have the ability to address aspects arising from bacteria-specific metabolic capabilities (e.g. presence of nitro-reductase and absence of CYP2E1) as well as the inability of bacterial assays to assess some test articles. It was agreed that mammalian cell gene mutation assays can complement bacterial gene mutation assays by providing additional information for the overall assessment of mutagenic hazard. Human TK6 based systems (including WTK-1 and various mutant lines) can detect numerous genetic toxicity endpoints (e.g. *TK/HPRT* gene mutations, MN frequency, Chromosome aberrations, DNA damage, *PIG-A/PIG-L* gene mutations, gene mutations, DNA damage responses assessed using toxico-genomics and reporter-based systems). They can also detect agents that act via a variety of mutational mechanisms including base pair substitutions, indels, large deletions, recombination, LOH and non-disjunction.

45. The IWGT considered cell test systems from TGRs or cells containing recoverable transgenes. Over 20 TGR cell-based test systems had been developed and had been used to evaluate over 150 substances, but there was a lack of consistency in published protocols. It was agreed that major advantages included: use of established scoring protocols; avoidance of clone selection; use of metabolically competent primary cells and/or cell lines; ability to detect different types of genetic damage; large dynamic range; and complementarity with *in vivo* TGR endpoints. Major disadvantages included: lack of validation and little consistency in protocols and interpretation of methodology; use of costly specialised reagents; mutant enumeration is relatively slow and laborious; most cells lack metabolic capacity; no single test system could detect all mutational mechanisms. Efforts were being made to miniaturise and improve throughput. *In vitro* systems based on MutaMouse and *lacZ* plasmid mouse, which included immortalised cell lines as well as metabolically competent primary hepatocytes were considered to be the most advanced, with respect to assay validation. The IWGT agreed that if these assays were validated more thoroughly, then there was a potential that they could be used in routine mutagenicity testing.

46. IWGT agreed that cell lines for use with *in vitro Pig-a* assays needed to be adequately characterised i.e. characterisation of GPI anchor-associated genes implicated in the test system response – methods based on L5178Y/$Tk^{+/-}$ -3.7.2C cells appear to specifically measure mutations in *Pig-a*, while those in TK6 cells measure mutations in both *PIG-A* and *PIG-L*. It was

considered that that incorporation of methods for cytotoxicity assessment was needed. IWGT considered that data was needed on acceptable baseline/spontaneous mutant frequency, the number of cells that should be treated, maintained throughout the study and scored.

In vivo strategies

47. Analysis of the GTTC TGR/comet database was reviewed. Also based on liver and GI tract response, the IWGT considered that analysis of the data did not support a preference of one assay over the other for detecting Ames positive chemicals in *vivo*. However, it was considered that for genotoxic effects in the bone marrow that the analysis did not support the use of the comet assay.

48. Based on the analysis of tumour responses, it was agreed that there was no difference between TGR and comet in terms of positive results with IARC carcinogens.

49. The IWGT considered the need for site-of-contact tissues in the comet assay when MN in bone marrow and comet in the liver was already being investigated. Data from 95 chemicals indicated that for routine assessment of genotoxicity, that if there is no reason to investigate a specific tissue (other than the liver) and where adequate systemic exposure had been confirmed, then a site of contact assay was not necessary. However, some circumstances may warrant site of contact testing (e.g. low systemic exposure, chemical instability, and bacterial metabolism). A minority view was that the liver and two sites of contact (GI tract) may be needed. But, from multiple chemicals evaluated and for orally exposed substances, the data did not support the need to test more than one section of the GI tract.

50. Regarding route of administration, it was agreed that a physiologically relevant route should be used and that other routes would need to be justified. Whether intraperitoneal (i.p.) or oral administration was used, there was likely to be appropriate exposure to the liver. When high quality data are available from both i.p. and a physiologically relevant route for risk evaluations, then more weight should be given to the data from the physiologically relevant study.

51. With respect to evidence of bone marrow/tissue exposure, the IWGT recommended that multiple lines of evidence should be considered, which is consistent with recent EFSA Guidance (EFSA 2017 Clarification of some aspects related to genotoxicity assessment).

52. The IWGT agreed that the repeated dose liver MN test was sufficiently validated for an OECD Guideline in terms of numbers and types of chemicals. But, there was a need to evaluate the impact of dosing animals of different ages (6 and 8 weeks old).

53. The IWGT considered that the *Pig-a* assay was a useful follow-up test for positive *in vitro* mutagens and for investigation of *in vivo* mode of genotoxic

action. It was also noted that it could be routinely integrated into repeat-dose general toxicity and other studies and that repeat dosing allows detection of additive effects. Frozen stored blood from control animals could be used rather than a concurrent positive control. It was recommended that both reticulocytes and erythrocytes should be assessed wherever possible.

Use of high dimensional data

54. The IWGT did not have a clear consensus on what, when and how to use high dimensional mechanistic data. Presentations on adductomics, whole genome transcriptional profiling, single-molecule mutation analysis and high content phenotype-based assays had been given. SWOT analysis had indicated many opportunities, but potential threats and weakness had yet to be considered.

ii) EFSA Guidance on genotoxicity testing of nanomaterials

55. The COM was informed of an EFSA consultation on its draft Guidance document on the genotoxicity testing of nanomaterials. Members were asked to provide any comments on this to the secretariat so that a COM view could be submitted to EFSA by the deadline of the 4th March 2018.

ITEM 11: DATE OF NEXT MEETING

56. Tuesday 26th June 2018.