MUT/MIN/2024/02

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

Minutes of the meeting held at 10.30 on 20th June 2024 at UKHSA, 10 South Colonnade, London, E14 and via MS Teams.

Present:

Chairman:	Professor G Jenkins
Members:	Mr A Bhagwat Dr C Beevers Dr A Doherty Dr P Fowler Dr N Goldsmith (Associate member) Dr G Johnson Professor D Harrison (Ex officio) Professor S Doak (Co-opted member) Ms J Kenny Dr A Povey Mrs M Wang Mr P Rawlinson Dr Rob Smith Dr Robert Foster
Secretariat:	Dr O Sepai (UKHSA Scientific Secretary) Mr S Robjohns (UKHSA Secretariat) Dr N Raja (UKHSA Secretariat) Dr C Mulholland (FSA Secretariat) Dr C Potter (FSA Secretariat) Mr T Fraser (UKHSA) Dr D Gott (FSA Secretariat) Ms Chara Tsoulli (FSA Secretariat)
Secretariat Support:	Dr R Bevan (IEH Consulting) Dr Sarah Bull (IEH Consulting) Dr A Bernal (IEH Consulting)

Assessors: Ms Krystle Boss (FSS)
Ms Ann Baker (VMD)
Ms Fay Conry (HSE)

Observers: Mr Lewis Rogers (HSE)
Dr Meera Cush (COC member)
Ms Hannah Jones (OPSS)

		Paragraph
1.	Welcome and Apologies for absence	1
2.	Announcements	2
3.	Minutes of the meeting held on 29th February 2024 (MUT/MIN/2024/01)	4
4.	Matters Arising	5
5.	Horizon Scanning. A) Presentation from Alexander Kalian, King's College London, on work related to computational methods and mutagenicity.	10
	B) Presentation from Paul Rees, Swansea University, on Artificial Intelligence and mutagenicity data.	15
6.	Guidance on the use of QSARs – draft paper for discussion. (MUT/2024/03)	21
7.	OECD Updates	28
8.	AOB UN GHS Germ Cell Mutagenicity – for information. (MUT/2024/04)	29
9.	Date of next meeting	32

ITEM 1: WELCOME AND APOLOGIES FOR ABSENCE

1. The Chair welcomed the COM members, assessors, and secretariat. Three new members of the committee, Dr Ann Doherty, Dr Robert Foster (Lhasa) and Dr Rob Smith (LabCorp) were welcomed.

ITEM 2: ANNOUNCEMENTS

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

3. The induction process for new members was discussed. It was proposed that one-to-one meetings would be arranged to discuss roles, expectations, and provide guidance on committee procedures. A mentorship programme would also be established where experienced members would mentor new members, providing additional support and answering any questions they may have. The proposal to implement a formal induction process was supported and would be actioned.

ITEM 3: MINUTES OF THE MEETING HELD ON 29th February 2024 (MUT/MIN/2024/01)

4. The minutes of the COM meeting held on the 29th of February 2024 were agreed subject to minor typographical amendments.

ITEM 4: MATTERS ARISING

5. The Chair informed the committee that the final version of the COM's titanium dioxide review, including feedback from the March COM meeting, had been submitted to the COT. The Food Standards Agency (FSA) provided an update that an executive summary of the COT evaluation of titanium dioxide had been prepared, but the COT's statement on titanium dioxide was not yet ready for publication. The plan was to release the COT executive summary as soon as possible, followed by the COT statement. The intention was to publish both the COM and COT statements simultaneously to ensure transparency. The executive summary was with the Comms team at the FSA. The UKHSA would be kept informed of any updates. The COM's executive summary final version could be shared with COM members.

6. The Chair informed the COM that a few *in vivo* papers on titanium dioxide published in 2024 had been identified after the March COM meeting and would be reviewed by the subgroup and added as an addendum without delaying the publication of the main review. IEH Consulting had screened the papers and had identified four relevant papers. These papers would be distributed to the COM subgroup for review. After the review had been completed, a narrative summary would be written. This work was planned to be completed before the end of July.

7. FSA provided an update on the evaluation of smoke flavourings. The second round of the Joint Expert Group for Additives, Enzymes and Other Regulated Products (AEJEG) review on smoke flavourings had been completed. Requests for information had been sent out and responses were expected

during the summer. The AEJEG would conclude on genotoxicity in the third round in autumn. Safety advice documents for smoke flavourings testing positive for genotoxicity would be written based on the AEJEG's opinion and were expected to be cleared by Q1 2025. For those testing negative, the AEJEG would review relevant developmental toxicity studies, with final conclusions on their safety expected by late 2025. Positive genotoxicity cases would be passed to risk management. Risk managers had requested a risk versus benefit analysis of smoke flavourings in comparison with traditional smoking of food products.

8. The AEJEG approach involved the consideration of whole mixture testing and the use of QSAR data on individual components as supplementary and supporting information. In contrast, EFSA's approach focused on QSAR information for individual components and if a single component of a complex mixture was confirmed as genotoxic, then the whole mixture would be considered as genotoxic.

9. A weight of evidence document compiled by the AEJEG was expected to be circulated, outlining the AEJEG's evaluation of existing evidence on smoke flavourings, comparing old versus new studies, *in vitro* versus *in vivo* data, and the weighting of QSARs during the evaluation. This document would be included as an annex to the advice document to clarify AEJEG's scientific approach. COM members were requested to review this document and provide comments by the 2nd of July for the AEJEG and to respond to the comments during its meeting on the 25th of July. Members expressed the need for clarity on what is expected in the comments and the potential for an introduction to the document to aid understanding.

ITEM 5: HORIZON SCANNING

A) Presentation from Alexander Kalian, King's College London, on work related to computational methods and mutagenicity.

10. No interests were declared for this item.

11. The presentation reported findings of a PhD, supported by the UK Food Standards Agency, which aims to develop AI-driven models to improve the assessment of toxicity related to food. Of interest to COM is the use of such technology to predict mutagenicity. At present, food safety hazard assessments are carried out using experimental, analytical, and computational approaches but all of these have potential limitations including scientific validity, ethical considerations, and cost effectiveness. Of the currently available computational approaches, QSAR models are widely used to predict activities of molecules without data as they are very broad and versatile, however the models are very data intensive.

12. An AI-driven QSAR model utilising SMILES and deep learning (neural networks) was developed by the speaker which determined mutagenicity in a binary classification (YES/NO) with 78% accuracy (checked against Ames data). The model was further developed to use a convolutional neural network approach, which looks at aspects of images. As molecules are graph structured data, and may not fit into image analysis easily, graph convolutional neural networks (GCN) were developed to achieve this. In addition, the speaker evaluated the use of Explainable AI (XAI) with the

model to determine the reasoning behind the mutagenic predictions made, and to mine structural alerts. The model (incorporating node enrichment) was used to predict the mutagenicity (YES/NO) of 5625 molecules, for which Ames data is available, and the output compared to that obtained using a language-based transformer model. An accuracy of between 74% and 78% was achieved (depending on node features used). This represents 85% AUC (area under the curve) which is comparable to other available models, with the transformer model also having 84% AUC (now retrained to give 90% AUC).

13. When XAI was used to mine structural alerts from the GCN model, an accuracy of 85% was achieved (using a threshold of 0.7). Very similar identification of fragments (mutagenic and non-mutagenic) was obtained using the language-based transformer model, but not using the QSARpy model and this requires further investigation. In addition, some identified structural alerts did not make complete sense and this also needs investigation. Prior to releasing the model for public use, the OECD guidelines require formalisation of the identity of its applicability domain, and it will also need to be applied to different toxicological endpoints.

14. During discussions, a COM member asked whether the model could assess the possibility of positional (stearic) hinderance, which may be the reason why some fragments that are initially identified as DNA reactive are not so. The speaker replied that many of the fragments identified are very similar and while it is theoretically possible to look at positional hinderance, the false positives may also be due to other fragments being present, so the reasons are likely to be multifaceted. A member also asked whether the 3D structure of the molecule was important in determining whether it is DNA reactive. The speaker replied that the model developed here utilised fragments rather than 3D structure, however, there are examples where stearic chemistry is important to DNA reactivity and that the influence of stearic chemistry is often neglected as it is difficult to study and would need a more advanced model. Suggestions were made to the speaker by a member of COM to address some of the potential issues with the model.

15. The Chair concluded that these approaches are not used at a regulatory level at the moment. However, these tools show how current approaches may be replaced in the near future and it is important that COM is prepared and understands them.

B) Presentation from Paul Rees, Swansea University, on Artificial Intelligence and mutagenicity data.

16. No interests were declared for this item.

17. The speaker outlined a case study to show how traditional machine learning is used to evaluate the cell cycle using a set of label free flow cytometry images. CellProfiler is used to extract the cell features following training of the model (supervised machine learning) with features from annotated images obtained using biomarkers for different parts of the cell cycle. AI models can provide a classification for the cell without adding cell stains (label free) with an accuracy of around 90%; it is important for some

applications that cell biomarkers are not used. In addition, regression analysis has been used to predict DNA content from label free cell images.

18. Deep learning (neural networks) is a key concept in AI, but this does not have the same knowledge base as traditional learning. AI and deep learning are built around an artificial neuron which forms a neural network, and artificial weightings are given to determine how well they are connected. Although these have been around for 60 years, it is only now that computers are fast enough to develop deep learning. A commonly used network is the convolution neural network and the speaker outlined how this is used to synthesise an array (matrix) of numbers from the input image to allow matching with matrices from training images. Deep neural networks have been used to score micronucleus images for nine different phenotypes (from mononucleate to tetranucleate) with an accuracy of 96% (compared to human scoring). Label free detection has also been applied to leukaemia cells which reduces analysis (diagnostic) time considerably, to look at the change in morphology of red cells on storage, and to classify pollen grain in Arctic ice.

19. Another type of neural network is object detection, and this has been used to identify binucleated cells with micronuclei with 100% accuracy, following minimal training (175 binucleated cells with micronuclei images). Without retraining, the system detected tera- and tri-nucleated cells, with and without micronuclei, with an accuracy of 90%. Other developments include the evaluation of cell painting to detect genotoxic events in cells, which is an unbiased cell profiling method. The greatest use of the technique has been for drug discovery, but it has now been applied to look for genotoxic changes. Detection of micronuclei, gH2AX foci, fragmented nuclei etc., was achieved using CellProfiler (previously trained) in the same CellPainting pipeline. This has important advantages as very large, freely available datasets for chemical structure, imaging and gene expression have been developed using cell painting and these will be able to now be an available resource to support future work.

20. During discussions, a COM member asked how independent the variables are in the model and can additional ones be added easily. The speaker replied that you do not have to start from scratch as the variables are independent and so you just introduce the new ones. A member also asked how to ensure that the available classifiers have been validated. The speaker replied that expert scientists need to produce annotated data sets, so we have known valid sets to use. It is also possible that, in the future, the datasets will need to be regulated to help regulatory submissions where this data is used. A comment was made that it is likely there will be an OECD guideline for using AI for genotoxicity assessment in the future. A point of clarification was also given that, at present, Cell Painting data is only being used at the early stage of drug discovery and is not being seen by regulators. A member also asked what level of accuracy has been obtained with the deep learning approach and the speaker replied that it has not been taken past the 90%, obtained with machine learning, as the availability of images to develop a classification set is limited at the moment.

ITEM 6: Guidance on the use of QSARs – draft paper for discussion (MUT/2024/3).

21. Dr Robert Fraser (Lhasa) declared an interest because he works for an organization that develops QSAR models. This declaration does not represent a conflict of interest, as his expertise is necessary for developing the COM guidance document on the use of QSAR.

22. Paper MUT/2024/3 was presented by a member of COM and IEH Consulting.

23. (Q)SAR is recommended in the overarching COM 'Strategy for testing of chemicals for genotoxicity' within Phase 0 and for the assessment of impurities. However, COM guidance outlining best practice in the use of (Q)SAR is lacking. This paper was intended to provide information to support the production of COM recommendations for an evaluation of genotoxicity using (Q)SAR model(s), including the prioritization of compounds, selection of the (Q)SAR model, reporting of (Q)SAR predictions, considerations of expert knowledge, read-across approaches, and integration of findings into a weight-of-evidence evaluation.

24. The approach taken so far to produce this discussion paper had been to evaluate existing guidance on (Q)SARs (e.g., OECD, ECHA, ICH and SCCS). It was determined that current guidance is limited in its specificity for genotoxicity, especially clastogenicity. Therefore, to address this information gap, the primary literature was also reviewed.

25. During discussions, COM members considered that the current paper outlines the 'state of the science' and a more specific set of COM recommendations on how to use QSARs for evaluating genotoxicity should be established, based on the synthesis of information in the paper. The inclusion of case studies from Government departments and agencies in the final COM guidance was thought to be useful, but these may be hard to define. Members noted that the COM recommendations should have improved narration of data quality and a clear identification of the strengths and weaknesses of the different (Q)SAR models. In addition, incorporation of a summary of recommendations at the start of the document, possibly in the form of a flow-chart as per the overarching COM Guidance, was considered important for the accessibility of information to users.

26. Following discussions, it was agreed that a meeting should be held with the subgroup to discuss in more detail the document edits received to date, and to agree next steps to progress the document.

27. The target timeline for the guidance document on the use of QSAR models to predict genotoxicity is now set for the March 2025 meeting, allowing sufficient time for thorough preparation.

ITEM 7: OECD UPDATES

28. The committee was informed during the last meeting that the OECD test guideline TG487 (*in vitro* micronucleus assay) was being proposed for

adaptation to nanomaterial evaluation. The process of generating data is currently in progress and is anticipated to take some time. The committee is expected to hear more information about this in the upcoming meetings.

ITEM 8: AOB

UN GHS Germ Cell Mutagenicity – for information.

29. It has been noted that UK REACH Independent Scientific Expert Pool (RISEP) had comments regarding the classification of mutagens. In response to these comments, the Health and Safety Executive (HSE) drafted a document on the classification of germ cell mutagenicity and had requested this to be considered by the COM. The COM had recommended that a background document on CLP and UK REACH be prepared to provide context before engaging in detailed discussions. In response, a background document had been produced to inform those unfamiliar with CLP and UK REACH processes and circulated to the members. A more detailed discussion paper would be presented by HSE at the next COM meeting.

30. The Chair informed the COM about a vacancy for one or two associate member positions. This opportunity allows young scientists to gain experience by attending meetings and understanding the workings of the COM, with the potential to become full members after gaining sufficient experience. All members and the secretariat were requested to use their networks to inform interested individuals about this opportunity. Interested candidates can submit their curriculum vitae to the committee, which will review the applications, conduct interviews, and finalize the selection process.

31. The committee formally thanked Dr David Gott for his years of service and contributions and wished him well for the future, as this was his last meeting.

ITEM 9: DATE OF NEXT MEETING

32. Date of next meeting – The October 2024 meeting was cancelled; the next COM was planned for March 2025.