MUT/2023/06

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

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Foreward by Gareth Jenkins – Chair

I am pleased to present this report on the work of the Committee on Mutagenicity (COM) during 2021. I was honoured to be asked to take over the role of Chair of COM in May 2021 and I would like to begin by paying tribute to my predecessor (Dr David Lovell) for his stewardship of COM during the preceding years and Chairing the February 2021 meeting.

The Committee on Mutagenicity (COM) provides advice on potential mutagenic activity of specific chemicals at the request of UK Government Departments and Agencies. Such requests generally relate to chemicals for which there are incomplete, non-standard or controversial data sets for which independent authoritative advice on potential mutagenic hazards and risks is required. Recommendations for further studies are, on occasions, made.

The Committee also advises on important general principles and on new scientific work related to the assessment of mutagenic risk and makes recommendations on wider aspects of mutagenicity testing. The membership of the Committee, declarations of their interests, agendas and minutes of meetings, and statements are all published on the internet. https://www.gov.uk/government/organisations/committee-on-mutagenicity-of-chemicals-in-food-consumer-products-and-the-environment

In 2021, the updated COM guidance on genotoxicity testing strategy was published (MUT/2021/01). This update, begun in 2020, sets out the suggested strategy for genotoxicity testing of chemicals and updates our position to consider advances in the field of safety testing. COM also updated guidance on testing of germ cell mutagens (MUT/2021/02) and the use of 3D tissue models as alternative approaches to animals in testing (MUT/2021/03). The documents will be published on the COM website. The 3D tissue strategy responds to the growing focus on animal alternatives driven by the

production of novel sophisticated tissue models which can recapitulate aspects of human biology.
In 2021, COM discussed the safety testing of impurities (MUT/2021/04) and the use of QSAR and toxicogenomics in testing (MUT/2021/05 and MUT/2021/06).
In 2021, COM started a discussion of the genotoxicity of titanium dioxide (MUT/2021/07 and MUT/2021/12), following the updated opinion published by EFSA in 2021. This review of titanium dioxide will be continued and concluded in 2022.
In 2021, COM further discussed the use of toxicogenomics in safety testing (MUT/2021/08), separating out the transcriptomics aspect from the next generation sequencing (NGS) approaches. Given the advances in NGS in general, it is likely that over the coming years, NGS approaches may replace some traditional mutation testing platforms. COM also published guidance on a testing approach for nanomaterials, with a focus on considerations of the fact that key physico-chemical aspects of nanomaterials render some traditional genotoxicity tests not suitable (MUT/2021/09).
COM also discussed the potential genotoxicity of specific compounds as requested by Government departments and agencies. For example, COM reviewed the genotoxicity of Hydroxyanthracene Derivatives (MUT/2021/12) and associated human health risks.

The Committee carried out its annual Horizon scanning exercise, identifying potential topics for future work. The COM continues to be interested in hearing from Government Departments and Agencies on how its advice is acted upon.
The COM maintained its awareness of the implications of EU EXIT on its work and remained alert to the continuing uncertainty as to how the UK's regulatory environment and its relationships with international organisations will develop in 2022 and onwards.

I would specifically like to thank the COM secretariat for their exceptional support to the COM and to the WRc/IEH team for the excellent work they delivered in 2021. As always, I am grateful for the support of the individual members of the committee for their expert advice, the effort and time they put in and their support throughout the year.

[SR1]

Professor Gareth Jenkins

ONGOING WORK

COM GUIDANCE SERIES UPDATE

GUIDANCE STATEMENT THE USE OF BIOMARKERS IN GENOTOXICITY IN RISK ASSESSMENT

At the request of the COC, the COM considered a revised version of the COC Guidance Statement G04 'The use of biomarkers in carcinogenic risk assessment' at the COM March 2022 meeting (MUT/2022/03). Particular focus was given to the 'DNA' and 'genotoxicity biomarkers' sections, both of which had had been shortened in the current version of G04 as part of a document revision process.

It was agreed that COM would produce a guidance statement that provided a more comprehensive overview of these areas, which could then be referred to by the other Committees. A draft scoping document outlining the proposed content of guidance statement was presented to the COM at its meeting in June 2022 (MUT/2022/06).

Several modifications to the scoping document were suggested by members and these were incorporated into a first draft document presented at the COM October 2022 meeting (MUT/2022/11). Members considered that the focus of the COM document should be *in vivo* biomarkers of DNA damage, with greater distinction from the COC Guidance Statement G04. Work is ongoing to progress a second draft document.

GUIDANCE ON HOW THE COMMITTEES EVALUATE THE RELEVANCE AND RELIABILITY OF DATA WHEN ASSESSING A CHEMICAL OF CONCERN

At the COM March 2022 meeting, the COM considered a draft document outlining the Committee evaluation process focussing on the relevance and reliability of data written specifically to inform the lay person (MUT/2022/03). This document evolved from a scoping paper on the topic of 'biological relevance and statistical significance', presented to the Joint COC/COM meeting in November 2020 (CC/MUT/2020/03) also attended by some COT members, which outlined some of the more relevant and significant work that has been published on this issue in recent years. It was agreed that two documents should be progressed. The first document should be aimed at the lay audience about the process used by the Committees to evaluate evidence and reach conclusions and a second document aimed at a more informed audience on statistical significance testing and consideration of biological relevance.

Paper MUT/2022/04 presented an updated version of the draft document, amended following comments from COM members at the March 2022 meeting. The draft document would also be discussed by COT and COC at their July 2022 meetings.

COM members asked for a small number of additional changes to be made prior to the document being evaluated by COC and COT. This included emphasising the public-facing role of the document.

NON-EXPERT SUMMARIES FOR COM WEBSITE

At the COM meeting in June 2022, it was agreed that the general public could benefit from the addition of non-expert summaries to the start of each COM guideline document.

A draft non-expert summary for the overarching COM guideline, 'Guidance on a strategy for genotoxicity testing of chemicals (MUT/2022/13) was presented at the COM October 2022 meeting. Members considered that some text could be removed, as this was available on the COM website, and a link provided to that website. In addition, it was recommended that links to the glossary should be utilised fully as this provided an immediate and understandable definition for readers. Specific comments on the paper were requested to be sent directly to the Secretariat so that the paper could be updated.

COM EVALUATIONS

EFSA ASSESSMENT OF THE GENOTOXICITY OF ACRYLAMIDE

Following a request by the European Commission (EC), the European Food Safety Authority (EFSA) published a statement on the assessment of recent publications on the genotoxicity of acrylamide (EFSA, 2022).

The request by the EC followed the publication of a review article by Eisenbrand (2020a) and its erratum (Eisenbrand, 2020b). However, as EFSA did not consider the review and erratum to be comprehensive, a literature search of the recent data on the genotoxicity and mode of action of acrylamide was also undertaken.

EFSA concluded that the majority of the new studies published since 2015 confirmed and extended the clastogenic properties of acrylamide/glycidamide. In addition to genotoxicity, non-genotoxic effects may contribute to the carcinogenicity of acrylamide. There was further substantial evidence for the genotoxicity of acrylamide mediated by the formation of its metabolite glycidamide. Overall, the new studies evaluated extend the information assessed

previously and support EFSA's conclusion on the risks to human health related to the presence of acrylamide in food. EFSA further considered the Margin of Exposure (MOE) approach to still be appropriate and concluded that an update of its 2015 opinion is currently not required.

The COM considered the recent EFSA assessment and agreed that the information/data considered in the assessment confirmed and strengthened most aspects of EFSA's previous opinion.

The review paper by Eisenbrand 2020 argued against a genotoxic mode of action for the carcinogenicity of acrylamide and that genotoxic effects were only seen above normal physiological levels of exposure. Members had reservations about the paper by Eisenbrand and considered that it had limitations. The COM agreed that exposure to acrylamide induced gene mutation and was clastogenic in mammalian cells. The genotoxic mode of action appeared to occur via CYP2E1 metabolism to the mutagenic and clastogenic metabolite glycidamide. The role of acrylamide itself was unclear. Members considered that the genotoxicity arising from acrylamide exposure may also involve the generation of reactive oxygen species (ROS) and oxidative damage.

Overall, the COM agreed with EFSA's conclusion that the MOE approach would still be appropriate.

DISCUSSION PAPER ON A COATING IN CANNED FOOD PACKAGING MATERIALS

This item was presented as a reserved item.

Members discussed the information provided to the Committee on a can coating as well as the assessment and discussions of the Joint Expert Group on Food Contact Materials (FCM JEG). Following the COMs assessment, the discussion paper was presented to the Committee on Toxicity, together with the discussions of the FCM JEG and COM. The work is ongoing, but a final assessment is expected in 2023.

DRAFT STATEMENT ON THE GENOTOXICTY OF HYDROXYANTHRACENE DERIVATIVES IN FOOD

The genotoxicity of hydroxyanthracene derivatives (HADs) used in foods had been discussed at the COM meeting in October 2021. Following a request from UK-wide Nutrition Labelling Composition and Standards (NLCS) policy group, the UK Food Standards Agency (FSA) commissioned an independent view from the COM on the mutagenicity of HADs based on consideration of the European Food Safety Authority (EFSA) 2018 opinion on HADs and any additional new data that have become available.

This discussion of the COM was held in March 2022. At this meeting, COM Members were asked by the FSA Secretariat to consider whether they agree with the following overall conclusions of the EFSA ANS Panel, i.e. i) emodin, aloe-emodin, and dantron are genotoxic *in vitro*; ii) HADs should be considered as genotoxic *in vivo* unless there are specific data to the contrary (such as for rhein); iii) there is a safety concern for plant extracts containing HADs (although there is some uncertainty); and iv) it is not possible to provide advice on a daily intake of HADs that does not give rise to health concerns (for both the general population, and vulnerable subgroups of the population). Furthermore, the COM was asked to consider whether any of these conclusions would be affected by the results of the studies published since the 2018 EFSA opinion.

Overall, the COM agreed that that the available evidence indicates that emodin, aloe-emodin, and dantron are genotoxic *in vitro*, namely from Ames tests.

The COM agreed that the negative results from the *in vivo* bone marrow micronucleus assay are valid and concluded that there is reasonable evidence that there is no genotoxic effect or mechanism *in vivo*. Subsequently, a new *in vivo* genotoxicity study would not be helpful. The COM considered that the reported carcinogenic effects of HADs, including those seen in the comet assay of colon cells, are caused by the high levels of irritation, inflammation, and diarrhoea.

The Committee agreed that it should in theory be possible to establish a daily intake of HADs that does not give rise to health concerns using carcinogenicity data. However, more *in vivo* carcinogenicity data are needed to carry out dose response modelling and to identify a point of departure. The Committee agreed that a specification for supplements regarding HADS contents would be useful for comparison against this potential ADI.

The Committee agreed that the studies published after 2018 are mostly negative *in vivo* data, which weaken the evidence that there is a genotoxic effect *in vivo*. Following the COM consideration and conclusions, a draft statement was produced (MUT/2022/01) and Committee Members were asked to provide any comments on the structure and content of the draft statement. The COM were content with the draft statement, and this was agreed with no significant amendments.

REVIEW OF TITANIUM DIOXIDE GENOTOXICITY

Following the publication of the European Food Safety Authority (EFSA) opinion on titanium dioxide in 2021, which concluded that titanium dioxide could no longer be considered to be ‘safe’ for use in food, the Food Standards Agency (FSA) initiated a review of the EFSA opinion.

The EFSA opinion was presented to the COM in June 2021 (MUT/2021/03) and to the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) in July 2021 (TOX/2021/36). The COM had a number of concerns over the EFSA opinion on the genotoxicity of titanium dioxide. Due to this and following the advice of the COT the FSA initiated an independent evaluation of the safety of the use of titanium dioxide as a food additive.

In October 2021, paper MUT/2021/08 was presented to the COM, which summarised the available genotoxicity on titanium dioxide. Members considered that it was not possible to evaluate the genotoxicity of titanium dioxide at that stage. The COM suggested a sifting approach to the available genotoxicity should be adopted as a first step before evaluation. The Chair of the COM, a subgroup of the COM and the secretariat subsequently attended meetings to discuss and agree the criteria and methodology for sifting to identify suitable papers for the evaluation of titanium dioxide.

At the COM June 2022 meeting, paper MUT/2022/05 provided information and papers on approaches relating to the sifting and evaluation of the quality genotoxicity studies and evaluating data on nanomaterials. As an update since that meeting, members were informed that a sub-group of the COM had met to discuss the process to select relevant and appropriate studies to be reviewed by the committee. A proforma had been produced, which would be shared with members. This considered two levels, namely, whether the characteristics of the test material had been sufficiently described (e.g., micro or nano sized particles) and the quality and reliability of how the genotoxicity studies had been conducted.

UPDATE ON THE COM REVIEW OF THE EFSA EVALUATION OF BISPHENOL-A

The Food Standards Agency (FSA) provided an update on the EFSA consultation on its draft opinion proposing a lowering of the Tolerable Daily Intake (TDI) for bisphenol A. EFSA published a consultation on its draft opinion, which closed on the 22nd February 2022. In response to this consultation the FSA requested that the Committee on toxicity of chemicals in food consumer products and the environment (COT) provide a view to EFSA. The COT had a number of concerns over the approach used by EFSA in its evaluation, which the COT considered made it difficult to assess the toxicity database as a whole and had a number of concerns relating to the studies used to derive the new EFSA proposed TDI. The COT had requested the opinion of COM members on the EFSA evaluation of the genotoxicity data on bisphenol A and thanked the COM for its contribution. COM members were generally content with the EFSA review of the genotoxicity data and agreed with the overall EFSA conclusion that DNA strand breaks, clastogenic and aneugenic effects seen in mammalian cells *in vitro* following exposure to bisphenol A were very likely due to oxidative stress related mode of genotoxicity and that bisphenol A was not mutagenic *in vivo*. The combined COT and COM comments had been submitted to EFSA.

Following the publication of the finalised EFSA opinion the FSA would need to consider whether it needed to be referred to the UK expert advisory committees again. It was considered unlikely that there would be a need to consult the COM further on the genotoxicity aspect and would more likely be referred to one of the other expert committees, such as the Committee on the carcinogenicity of chemicals in food consumer products and the environment (COC).

HORIZON SCANNING: MEETINGS AND WORKSHOPS

A summary paper was presented outlining some of the current issues being discussed at a recent meeting and workshop covering issues that may be of interest to COM for future horizon scanning (MUT/2022/12). The first summary gave a brief overview of topics discussed at the UKEMS Next Generation Sequencing Workshop, held in May 2022 in London. The second provided a summary of some sessions of the UK Environmental Mutagen Society (UKEMS) Annual Meeting, held in July 2022 in Harrogate.

A few suggestions were made by members during discussion of the paper. These included consideration of: iPS organoids as model systems (COM and COC); the use of genomics in toxicity testing strategies; and whether epigenetics should/can be incorporated into standard toxicity testing.

OECD

Members were informed of a proposal from Norway to update OECD Test Guideline 489 on the in vivo alkaline comet assay to include the investigation of germ cells. Currently any modifications have not been sufficiently validated, but it was early stages for the OECD.

The COM also heard that the OECD Test Guideline 488 Transgenic rodent somatic and gene mutation assays had been updated and published.