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

REVIEW OF GENOTOXICITY OF TITANIUM DIOXIDE : COM Sub Group and methodology

Referral to COM

1. The Food Standards Agency (FSA), on the advice of the Committees on Toxicity and Mutagenicity of Chemicals in Food, Consumer Products and the Environment, has launched an independent evaluation of the safety of titanium dioxide for use as a food additive. The Committee on Mutagenicity has been asked to offer their opinion on the genotoxic potential of titanium dioxide

Background

2. Titanium dioxide is an authorised Food Additive (E171) in the EU in accordance with Annex II to Regulation (EC) No 1333/2008 in both its anatase and rutile forms (Commission Regulation (EU) No 231/2012) and under GB Food Law (retained EU law Regulation No 1333/2008 on food additives). Titanium dioxide is used in food as a colour to make food more visually appealing, to give colour to food that would otherwise be colourless, or to restore the original appearance of food. It is also widely used in cosmetics and medicines (EFSA, 2016).

3. Following the publication of the European Food Safety Authority's (EFSA) Opinion on titanium dioxide in 2021 which concluded that titanium dioxide could no longer be considered safe for use in food, the FSA initiated a review of the EFSA Opinion. Identifying a number of concerns, it was decided that the Opinion should be referred to the UK's Scientific Advisory Committees for independent expert review. The Opinion was presented to the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) (MUT/2021/03) in June of 2021 and to the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) (TOX/2021/36) in July of 2021.

4. Members of both Committees were asked to evaluate the EFSA Opinion and comment on whether they agreed with EFSA's conclusions and, if not, provide further guidance on the next steps that should be taken by the FSA.

5. The paper presented to the COM summarised the EFSA evaluation and particularly focused on the endpoints relating to genotoxicity.

6. The COM questioned the quality of the dataset and robustness of some of the studies used by the EFSA panel to draw its conclusions and noted that the overall data considered by EFSA were heterogenous (e.g. the range of particles evaluated was diverse, there were different types of experimental approaches taken and

assays used; different doses were used; some studies were published in obscure journals or in journals not specialising in genotoxicity and non-GLP studies were included, which all contributed to the difficulty in making comparisons between studies and an overall evaluation). Members were also concerned about the potential for publication bias in the studies evaluated by EFSA (since negative studies were less likely to be published). It was also noted that until relatively recently, the specification of E171 was poorly defined, which contributed to uncertainty in evaluation.

7. Regarding the mode of action for genotoxicity, the COM agreed that the evidence indicated an indirect interaction with DNA with a threshold for genotoxicity. The Committee noted that although some in *vitro* tests reported positive results, these appeared to mainly relate to nanoparticles with tests using the micro-sized titanium dioxide particles mainly reporting negative results. The relatively low nano-fraction in E171 (often less than 3.2%) and its low bioavailability, could be important factors when considering risk assessment.

8. In conclusion, COM Members considered that the evidence did not allow definitive conclusions to be drawn and therefore they did not agree with the overall EFSA conclusions on the genotoxicity of E171 titanium dioxide. They considered that a more reliable and robust dataset would be required before any conclusions could be drawn on the on the mutagenicity of TiO2 particles. Members noted that EFSA made no clear distinction between the genotoxicity of nano-sized and micro-sized titanium dioxide particles. EFSA also seemed to have put a lot of emphasis on the evidence from nano-sized particle evidence when nanoparticles made up only a small fraction of E171. The COM suggested that that if practicable, restricting the amount of nanoparticles in the specification for E171 might reduce any potential genotoxicity risk. Additionally, the COM considered that the wording of EFSA's conclusion was not helpful from a risk communication perspective. Due to the heterogenous data and equivocality of the evidence further refinement of the data evaluated may be needed before definitive conclusions on the genotoxicity and safety of titanium oxide could be made. Currently, the EFSA conclusions were not justifiable based on the available evidence and this could create unnecessary concern for the public.

9. As a result, the FSA, on the advice of the COT and the COM has launched an independent evaluation of the safety of titanium dioxide for use as a food additive.

10. In October of 2021 a paper (MUT/2021/08) was presented to the COM summarising all the available studies on the genotoxicity of titanium dioxide for the COM's consideration. The Members were asked to comment on the paper and the approach proposed by the secretariat for the evaluation of TiO2.

11. The Members acknowledged that although the paper presented a good overview of the available data on this endpoint, the database is extensive and they considered that it would not be possible to evaluate all of the available information if it was presented in its entirety in different papers. Therefore they proposed a strategy by which the sifting of the studies would occur as a first step and then all of the studies that met the criteria for consideration should then be presented for evaluation. It was noted that such methodologies for filtering the databases have previously been utilised and it was agreed by members that the details of those

examples should be circulated to the Committee in order for them to be adapted to the purpose of this evaluation. The formation of a sub-group that would undertake the work was also proposed by the COM. The Secretariat alongside the Chair of the COM and Members of the subgroup have subsequently held meetings to discuss the proposed methodology and approach for the evaluation of the genotoxicity of Titanium Dioxide.

12. The papers that formed the discussions of the subgroup meeting are presented in Annexes A-D (Annex B is confidential as it contains unpublished data).

- Annex A presents a paper by Fernández-Cruz *et al.,*2017 on the use of GUIDEnano approach on the quality evaluation of human and environmental toxicity studies performed with nanomaterials.
- Annex B contains information from an unpublished study that offers a case study of pragmatic use of the GUIDEnano approach in evaluating available data.
- Annex C contains offers recommendations on alterations of existing methodologies and the best practices as proposed by Elespuru *et al.,*2018 with regards to the standard battery of genotoxicity tests.
- Annex D presents a table meeting as an illustration of the genotoxicity assay specific criteria for quality control of available databases.

Questions to the Committee

13. Members are asked to consider the information provided and:

I. Comment on the papers presented and offer consider the questions at the end of each Annex.
II. Do the Members have any other comments?

Secretariat
May 2022

Annex A

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

REVIEW OF GENOTOXICITY OF TITANIUM DIOXIDE : COM Sub Group and methodology

Quality evaluation of human and environmental toxicity studies performed with nanomaterials – the GUIDEnano approach (Fernández-Cruz *et al.,*2017)

This paper discusses the challenges with regards to investigating and evaluating the effects of nanomaterials on human health based on the overall quality of the available publications. The authors note that the main challenge in the hazard assessment of nanomaterials is that their specific characteristics (which are dependent largely to their physiochemical properties) result in a high number of different nanoforms even if they are identical in their chemical composition.

The paper presents an approach in evaluating the available database which combines:

- the Guidenano quality assessment approach that consists of two scores, reliability of the study (K) and substance characterisation (S). These are combined to determine the final quality (Q) score.
- A modification on the ToxRTool approach (evaluating the reliability of the study- K score) to allow for both human and ecotoxicity studies
- and the S- score which is related to the substance characterisation as considered in the GUIDEnano hazard strategy.

It should be noted that the authors mention that the nano-specific considerations are included in a separate module, and therefore the method could be used for any chemical substance.

To evaluate the approach the authors used a combination of 137 toxicology/ecotoxicology studies with particular focus on: toxicity by inhalation, mutagenicity, carcinogenicity, toxicity to the freshwater compartment and toxicity to the sediment compartment. The database was used to identify any difficulties in the practical implementation of the proposed strategy and to assess the restrictiveness and comprehensiveness of the parameters used for the quality approach.

The paper is presented to the COM for information and also for discussion around the approach that is proposed by the authors and its relevance/suitability with regards to the evaluation of the available database on Titanium Dioxide.

Questions to the Committee
Members are asked to consider the information provided and:

I. Comment on the suitability of the proposed approach with regards to the work on Titanium Dioxide.
II. Do the Members have any other comments?

Reference:

Fernández-Cruz M.L, Hernández-Moreno D., Catalán J, Stockmann, Juvala, H.., Cabellos, J.,Lopes, Viviana R., Matzke, M., Ferraz, N., Izquierdo, J. J., Navas, J. M., Park, M., Svendsen, C., Janer, G. 2018. Quality evaluation of human and environmental toxicity studies performed with nanomaterials – the GUIDEnano approach. *Environ. Sci.: Nano*, 5, 381-397.

Annex B

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

REVIEW OF GENOTOXICITY OF TITANIUM DIOXIDE : COM Sub Group and methodology

CONFIDENTIAL

This paper was shared confidentially for discussion with Members, as the paper contains unpublished data.

Questions to the Committee

I. The COM are invited to consider the proposed criteria and comment on their suitability for the purposes of sifting the database on TiO2.
II. Do the Members have any other comments?

Annex C

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

REVIEW OF GENOTOXICITY OF TITANIUM DIOXIDE : COM Sub Group and methodology

Genotoxicity Assessment of Nanomaterials: Recommendations on Best Practices, Assays, and Methods (Elespuru *et al.,* 2018).

The paper discusses the challenges with regards to safety evaluation of nanomaterials, and specifically a critical review of the published data and offers recommendations on alterations of existing methodologies and the best practices for the standard genotoxicity assays: bacterial reverse mutation (Ames); in vitro mammalian assays for mutations, chromosomal aberrations, micronucleus induction, or DNA strand breaks (comet); and in vivo assays for genetic damage (micronucleus, comet and transgenic mutation assays). For each assay the authors analysed the available literature and considered recommendations for exclusion criteria that were based around issues with:

- Test substance (NM evaluated): Type and size, characterisation and sample preparation, test system uptake
- Test system used: OECD compliance/ adherence to standard methods, positive controls, metabolic activation, test validity appropriateness of system for assessment of NM, need for method alteration for NM assessment
- Results: critique of result based on acceptance criteria and comparison with a concurrent positive and negative control, dynamic range of effect (for positive results) consistency with particular NM in diverse published papers, insight into potential modes of action.

The paper is presented to the COM for information and also for discussion around the exclusion criteria proposed by the authors and their relevance/suitability with regards to the evaluation of the available database on titanium dioxide.

Questions to the Committee

Members are asked to consider the information provided and:

I. Comment on the suitability of the proposed approach with regards to the work on Titanium Dioxide.
II. Do the Members have any other comments?

Reference:

Elespuru R, Pfuhler S, Aardema MJ, Chen T, Doak SH, Doherty A, Farabaugh CS, Kenny J, Manjanatha M, Mahadevan B, Moore MM, Ouédraogo G, Stankowski LF Jr, Tanir JY. 2018. Genotoxicity Assessment of Nanomaterials: Recommendations on Best Practices, Assays, and Methods. *Toxicol Sci.* Aug 1;164(2):391-416. doi: 10.1093/toxsci/kfy100. PMID: 29701824.

Annex D

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

REVIEW OF GENOTOXICITY OF TITANIUM DIOXIDE : COM Sub Group and methodology

Example table

This was provided in the sub-group meeting as an illustration of the genotoxicity assay specific criteria for quality control of available databases. It should be noted that the table is specific to *in vitro* assays.