COM Evaluations 2021

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Review of the EFSA Opinion on Titanium Dioxide (E171) Presented by the Food Standards Agency

2.33 The Food Standards Agency requested advice from the COM on the genotoxicity of Titanium Dioxide, following a re-evaluation by the European Food Safety Authority (EFSA) published in 2021.

2.34 Titanium dioxide is an authorised Food Additive in the EU and under GB Food Law (retained EU law Regulation No 1333/2008 on food additives). It 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.

2.35 Titanium dioxide has been the subject of multiple safety evaluations. Following a review of Titanium dioxide specifications in 2019 and based on the fraction of nanoparticles present in E171, it was considered that the food additive fell under the scope of the EFSA guidance on nanotechnology and a recommendation for re-assessment of the safety of Titanium dioxide was proposed.

2.36 In the most recent evaluation published in 2021, data evaluated was for the food additive Titanium dioxide E171 as well as titanium dioxide other than E171 containing a fraction of nanoparticles <100nm or nano titanium dioxide. Concerning the genotoxicity studies, combining the available lines of evidence, the EFSA Panel on Food Additives and Flavourings (FAF) concluded that Titanium dioxide particles have the potential to induce DNA strand breaks and chromosomal damage, but not gene mutations. No clear correlation was observed between the physico-chemical properties of Titanium dioxide particles – such as crystalline form, size of constituent particles, shape and agglomeration state – and the outcome of in vitro or in vivo genotoxicity assays (i.e. a cut-off value for Titanium dioxide particle size with respect to genotoxicity could not be identified). The EFSA FAF Panel concluded that several modes of action **(**MOA) may operate in parallel and the relative contributions of the different molecular mechanisms resulting in the genotoxicity of Titanium dioxide particles are unknown. Based on

the available data, no conclusion could be drawn as to whether the genotoxicity of Titanium dioxide particles is mediated by a mode (s) of action with a threshold(s). Therefore, the EFSA FAF Panel concluded that a concern for genotoxicity of Titanium dioxide particles cannot be ruled out.

2.37 The COM were requested to consider paper MUT/2021/03, which summarised the EFSA 2021 evaluation and included a number of questions that the COM were requested to consider.

2.38 The COM had concerns over the quality and robustness of some of the studies considered by EFSA to draw its conclusions and noted that the overall data considered by EFSA was heterogenous (e.g. the range of particles evaluated was diverse; different types of approach and assays; different doses; different cell models; some studies were published in obscure or non-genotoxicity journals and the inclusion of non-GLP studies, which all contributed to the difficulty in making comparisons and an overall evaluation). Members were also concerned over the potential for publication bias in the studies evaluated by EFSA (i.e. where 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 and difficulty in evaluation.

2.39 Regarding mode of genotoxic action, the COM agreed that the evidence indicated an indirect interaction with DNA with a threshold for genotoxicity. Some positive results were found with a mixture of nano and micro particles. It was impossible to interpret which fraction was responsible, although pure micro sized particles generally were negative. The in vivo studies tended to be of better quality and negative. The nano-fraction in E171 is thought to be low but the fraction of nanoparticles (<100nm) can be over 50%. The percentage of the nano-fraction and its bioavailability are important factors when considering risk assessment.

2.40 Members considered that the lack of quality in the evidence (e.g. mixed particle sizes (micro and nanoparticles) and a wide variety of testing approaches) did not allow definitive conclusions to be drawn and therefore did not agree with the EFSA overall conclusions on the genotoxicity of E171 Titanium dioxide. A review of more reliable and robust dataset may be required before conclusion could be drawn on the mutagenicity of titanium dioxide particles. Members noted that EFSA made no clear distinction between the genotoxicity of nano-sized and micro-sized titanium dioxide 2.41 particles. EFSA seemed to have put a lot of emphasis on the evidence from nano-sized particle studies when nanoparticles made up only a small fraction of E171. The COM suggested that if practicable,

restricting the amount of nanoparticles in the specification for E171 may 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 may create unnecessary concern for the public.

2.42 The COM agreed to develop an approach to evaluating all the available data (e.g., sifting for relevant and suitable studies) before continuing its review of the genotoxicity of titanium dioxide and before it could derive any firm conclusions or opinion.

Hydroxyanthracene derivatives

2.43 On the request of the UK-wide Nutrition Labelling Composition and Standards (NLCS) policy group, the UK Food Standards Agency (FSA) commissioned an independent view from the COM to advise on the genotoxicity of hydroxyanthracene derivatives (HADs) based on the 2018 EFSA opinion and any new data that have become available. Paper MUT/2021/11 provided a summary and discussion of the EFSA 2018 scientific opinion on the safety of HADs for use in food. Relevant literature studies published after 2018 were also described, including studies by Galli et al. (2021a,b) and Hu et al. (2021). Members were asked to consider whether they agreed with the EFSA 2018 conclusions and whether any of the EFSA conclusions would be affected by the results of the additional studies published since the EFSA 2018 opinion.

2.44 The Committee agreed that that overall, the available evidence indicated that emodin, aloe-emodin, and dantron are genotoxic in vitro, namely from Ames tests. Mixed results for in vitro genotoxicity had been reported in the literature. This was sometimes due to a lack of clarity on the preparation used for testing. Decolourised extracts (which were generally negative as they contain a far lower concentration of HADs), and whole extracts (which were positive as they contain greater concentrations of HADs). However, more information was needed to be confident that there was also genotoxicity in the mammalian cell assays, because i) the mouse lymphoma and micronucleus data summarised in the EFSA opinion were published in 1996 (since then, changes have been made to how genotoxicity is evaluated, for example to make sure excessive doses are not used), and ii) Müller et al. (1996) did not perform statistical evaluation of the data. Therefore,

overall, it was not clear to the COM if the positive results in the mammalian cell assays were reflective of mutagenicity, or rather reflective of toxicity from the use of excessively high concentrations.

2.45 In terms of in vivo genotoxicity, one member questioned how much weight should be placed on negative mouse data published after 2018, as EFSA agreed that mice appear to be less sensitive than rats to the gastrointestinal effects caused by HADs. 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.

2.46 While EFSA concluded that results from the in vivo bone marrow micronucleus assay were irrelevant (due to insufficient bone marrow exposure), the COM noted that plasma analysis was conducted and the active compound was detected or quantified in plasma, indicating there was sufficient bone marrow exposure (albeit at a low level). COM further noted that a US National Toxicology Program (NTP) study included an assessment of plasma levels and micronucleus formation in rats and mice with acute intraperitoneal exposure (to ensure adequate systemic exposure) and these results were also negative. Therefore, the COM agreed that the negative results from the in vivo bone marrow micronucleus assay were valid and concluded that there is reasonable evidence that there is no genotoxic effect in vivo.

2.47 The COM considered that the carcinogenic effects of HADs, including those seen in the comet assay of colon cells, were caused by the high levels of irritation, inflammation, and diarrhoea. The 2-fold increase in tail moment (present at all dose levels) in colon cells under the comet assay was not caused by DNA reactivity, but rather an indirect mechanism involving ROS generation and/or topoisomerase II inhibition (mechanisms that were indicated from in vitro data). Since the Committee concluded that HADs do not show a genotoxicity mechanism, a new in vivo genotoxicity study would not be helpful.

2.48 The COM 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 were needed to carry out dose response modelling and to identify a point of departure. The COM agreed that a specification for supplements regarding HADS contents would be useful for comparison against a potential Acceptable Daily Intake (ADI).

2.49 The FSA Secretariat agreed to provide an update to the COM in due course.