COT Statement on the Safety of Titanium dioxide (E171) as a Food Additive - Executive Summary

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Introduction - (E171) Executive Summary

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1. Food grade titanium dioxide (TiO_2) was an authorised Food Additive (E171) in the EU but from the 7th of August 2022 it is no longer permitted following the publication of Commission Regulation (EU) 2022/63, amending Annexes II and III to Regulation (EC) No 1333. Since August 2022, manufacturers in Northern Ireland have not been permitted to produce goods containing

titanium dioxide according to EU legislation. It currently remains authorised in Great Britain. Food grade TiO2 comprises a mixture of micro and nanosized particles and is used in food as a colour (white pigment) 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 commonly used in products such as bakery products, soups, broths, sauces, salad dressings, savoury based sandwich spreads, processed nuts, confectionary, chewing gum, food supplements and cake icing. Titanium dioxide is also widely used in cosmetics and medicines.

2. Titanium dioxide has been the subject of multiple safety evaluations including three recent evaluations by the European Food Safety Authority (EFSA) in 2016, 2019 and 2021.

3. In their most recent Opinion (2021), EFSA considered that some findings regarding immunotoxicity, inflammation and neurotoxicity with respect to TiO2 nanoparticles, which are present in food grade TiO2, may be indicative of adverse effects. On the basis of the currently available evidence and the uncertainties, in particular a concern regarding genotoxicity which could not be resolved, the EFSA Panel concluded that E171 could no longer be considered as safe for use as a food additive.

4. Following this, in 2021 the COT published an interim position on titanium dioxide (COT, 2021) capturing the outcomes of discussions and outlining the next steps. A review has now been undertaken by the COT, which includes the conclusions on mutagenicity from the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM), to assess the safety of TiO2 as a food additive. This review is summarised below.

5. Since the EFSA and COT publications in 2021, reviews of TiO2 have also been carried out by Health Canada (2022), Food Standards Australia New Zealand (FSANZ) (2022) and most recently by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (FAO/WHO, 2024).

Characterisation and ADME considerations

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6. Specifically in food, the primary function of TiO2 is as an opacifier and white pigment. To achieve this function, it is critical that food grade TiO2 (E171) exists as an aggregate of smaller primary particles with a median particle size of 200 – 300 nm. Engineered nano-TiO2 has all (100%) of its particles less than 100 nm in diameter and is colourless and would therefore be unsuitable for use as a pigment in food applications.

7. The COT concluded that there is uncertainty over the toxicological effects of TiO2 nanoparticles. The Committee therefore considered that if animals and/or humans are exposed to test substances which contain higher levels of nanoparticles than normally found in food-grade TiO2, that could change the toxicological profile and potentially the risk, but it is unclear by how much or in what way.

8. The COT concluded that the physical form of TiO2 will affect its absorption and distribution. The focus of the COT was on food-grade TiO2 but the wide variance of test materials (nano, micro and mixtures of nano and micro) used in experimental studies was noted. Due to this large variability, as well as the potential impact of the matrix of administration, the Committee could not ascribe a specific percentage for the absorption of food-grade TiO2. However, the Committee considered that absorption of food grade TiO2 (E171) is low.

Review of toxicity for the endpoints identified by the COT

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9. The COT has reviewed toxicological studies which have been conducted using any form of TiO2, including nanoparticles, but its conclusions are primarily based on those which have used food grade TiO2 (E171).

10. The following endpoints were reviewed initially by the COT and then in more detail by a sub-group of COT Members: Aberrant crypt foci (ACF) (as a potential marker for carcinogenicity), inflammation and immunotoxicity, reproductive and developmental toxicity and neurotoxicity. The COM reviewed the genotoxicity data and reported their findings to the COT in May 2024.

11. The Committees gave greater weight to studies in which TiO2 was administered orally, particularly in the diet, as they were considered to be the most relevant for human exposure to TiO2 through consumption of food.

Reproductive and Developmental Toxicity

12. The COT reviewed the report of the Extended One Generation Reproductive Toxicity (EOGRT) study provided to the Food Standards Agency (FSA) by the Titanium Dioxide Manufacturers Association (TDMA) (Leuschner, 2020) as part of their safety assessment of TiO2. This study was carried out in response to a conclusion by EFSA regarding the uncertainty around TiO2 (E171) and reproductive and developmental toxicity. In addition to reproductive and

developmental toxicity, the EOGRT study also included cohorts for induction of aberrant crypt foci (ACF) in the colon, developmental immunotoxicity and neurotoxicity.

13. The COT considered the EOGRT report to be detailed and that the study was well conducted and carried out according to the relevant scientific guidelines, with no obvious deficiencies. It was noted that there were some minor effects observed in the study including focal effects on the testes and epididymides and a change in weight of the right testes. However, the COT agreed with the authors' conclusions that these changes reflected background variability (not attributable to the treatment) and were not of toxicological relevance.

14. The COT agreed that there was no evidence of reproductive or developmental toxicity up to and including the highest dose tested (1000 mg/kg bw per day). Therefore, this dose level was the no observed adverse effect level (NOAEL) for the study.

15. Analysis by the COT of the peer reviewed literature on reproductive and developmental toxicology identified two additional studies of appropriate quality (Warheit et al., 2015a; Lee et al., 2019). The COT concluded that these studies provided no significant evidence that TiO2 is reprotoxic and the NOAELs were consistent with that from the EOGRT study.

Aberrant Crypt Foci (ACF)

16. The Committee considered that although small numbers of ACF were observed in some animals exposed to TiO2 alone administered via drinking water in a single study (Bettini et al 2017), these could not necessarily be attributed to TiO2, as ACF were also present in animals in control groups not exposed to TiO2 in other dietary studies (e.g. Blevins et al, 2019; Leuschner, 2020). In addition, there was no evidence of the development of proliferative lesions of the colonic mucosa in any studies including the carcinogenicity study performed with Unitane (test material comparable to the E171 specification) (NCI, 1979; TDMA, 2022). The Committee concluded that there was no evidence that TiO2 induced ACF and no evidence to support progression to proliferative lesions in the colon.

Inflammation and immunotoxicity

17. The COT noted that only three studies on inflammation or immunotoxicity, including the EOGRT, (Riedle et al., 2020; Blevins et al., 2019; and Leuschner, 2020) used E171 TiO2 administered in the diet. These studies showed no adverse effects resulting in inflammation or immunotoxicity.

18. Five studies using food grade TiO2 (E171) administered to rats or mice in water (Talamini et al., 2019; Pinget et al., 2020; Bettini et al., 2017; Han et al., 2020; and Mortensen et al., 2021) on inflammation and immunotoxicity were considered by the COT. In some of the studies, differential inflammatory cytokine and host defence gene expression was observed but this was neither consistent across studies, nor ubiquitous in terms of pathway activation, making interpretation or formulation of conclusive statements challenging.

19. Other potential immunotoxic effects have been reported which include, but are not limited to: immune cell mediated inflammatory foci in the spleen and gut, including in Peyer's patches; effects on broader host defence mechanisms, including antimicrobial peptides; effects in the gut microbiota; effects on gut dendritic cell populations; effects on T cell subpopulations and macrophage populations in the gut; effects on plasma lymphocyte counts and proportions; and disruption of the mucus layer in the gut.

20. Overall, however, there is insufficient evidence of sufficient quality to conclude that food grade TiO2 (E171) is of concern with regards to immunotoxicity and inflammation.

Neurotoxicity

21. Overall, there is no new evidence on neurotoxicity to justify a change to the COT position on this endpoint as stated in its 2021 interim position paper. The findings of the studies on neurotoxicity were considered inconsistent by the COT. It was noted that the EOGRT study did not report any neurotoxic effects and that most of the other studies on this endpoint used titanium dioxide in the form of nanoparticles (TiO2 NPs).

22. The COT noted that in the EOGRT study the routine regulatory histopathology tests would have been less sensitive than the specific neuro-histopathology tests performed in some other studies. This qualification of the COT conclusion on neurotoxicity is more conservative than that of Health Canada who considered the EOGRT to be sufficiently sensitive and relevant to conclude on the lack of neurotoxicity potential of food grade TiO2 (E171).

23. The COT considered that the data from the relevant studies available indicated that TiO2 did not induce ACF, nor were there significant effects in studies that assessed inflammation and immunotoxicity, reproductive and developmental toxicity, and neurotoxicity. On balance, the Committee considered that the NOAEL of 1,000 mg/kg bw per day, the highest dose tested, from the EOGRT study, was robust.

Review of the genotoxicity of TiO2 by the COM

24. The COM reviewed a number of studies to assess the genotoxicity of TiO2. In addition to papers reviewed by EFSA, a literature search was conducted to find papers published on “genotoxicity” and “titanium dioxide”. Most papers identified used the nano-sized fraction of TiO2 and not the micro-sized form, nor the specific E171 form. Papers were assessed by experts and scored using a tiered approach to screen for both physico-chemical characteristics and ensure that only high-quality genotoxicity studies were included. The review therefore included papers on nano- and micro-sized TiO2 with particular attention given to the E171-form.

25. The COM stated that a definitive assessment of the safety of food grade TiO2 (E171) *per se* was difficult, when there were no high-quality OECD-compliant studies that adequately incorporate the study design considerations and characterisation of the nanoparticulate fraction present in E171. It was also noted that there is a lack of high-quality data sets that are OECD compliant on any form of the compound, and this led to conflicting data and some uncertainty in the risk assessment for TiO2. (COM, 2024b).

26. The COM’s opinion is that there is little evidence that TiO2 micro- or nano-sized particles are genotoxic in vitro or in vivo based on data from the few well conducted studies that are available. There is also a lack of replication of study outcomes using the same nanoparticle in different labs. (COM, 2024a).

27. Overall, therefore, the COM concluded that there was little evidence in the literature to suggest that there was a health concern related to genotoxicity induction by TiO2, particularly via the oral route and especially the micro sized TiO2 fraction (most studies in the literature used nano-sized material). Hence, any genotoxicity risk from dietary food grade TiO2 (E171) was considered to be low. (COM 2024b).

28. Following discussions of the COM report at their meeting in March 2024, the COT agreed with the conclusions of the COM.

Establishment of a Health-Based Guidance Value (HBGV)

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29. The Committee concluded that on the basis of the available evidence, 1,000 mg/kg bw/day was a robust Point of Departure (POD). This was based on the EOGRT study findings (Leuschner, 2020) as well as studies by Warheit et al., 2015b and Lee et al., 2019 that reported no effects up to the same dose. There was variability noted in the other studies, but nothing which would alter the proposed POD for food grade TiO2 (E171).

30. A standard uncertainty factor of 100 (10 for inter-species differences and 10 for inter-individual variability) was agreed by Members and applied to the POD which results in a HBGV of 10 mg/kg bw/day. There is likely to be additional conservatism in the application of this uncertainty factor to the NOAEL of E171 because 1,000 mg/kg bw per day was the highest dose of TiO2 tested and therefore the LOAEL (lowest observed adverse effect level) could actually be appreciably higher and, because there is no metabolism of TiO2 particles, the inter-/intra-species kinetic differences are likely to be lower than the defaults.

Exposure Assessment - (E171) Executive Summary

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31. Titanium dioxide (E171) can be found in a number of food categories, as well as in cosmetics and medicines. The exposures calculated and considered in this assessment are only for food and were for infants, toddlers, children, adolescents, adults, and the elderly using food consumption data from UK surveys. Maximum occurrence levels of titanium dioxide for specific food items, reported by EFSA (2021), were also used in the estimation of exposure.

32. The mean calculated total dietary exposures for TiO2 ranged from 3.3 to 11 mg/kg bw per day. The 95th percentile total dietary exposures for TiO2 ranged from 9.1 to 26 mg/kg bw per day. The 3 food groups that contribute the most to these exposures are: protein products; decorations, coatings and fillings; and sauces.

33. The exposure assessment took into account use levels in only sixteen food groups whereas E171 is approved in more categories (forty-eight). This may introduce underestimations for exposures. However, not all foods within the categories assessed will contain E171, which means exposure in those categories may be overestimated. In addition, the assessments are based on the assumption that all food in all categories assessed contain E171 at the maximum reported levels. This is unlikely and overall exposure is therefore more likely to be overestimated.

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34. Exposures for all population groups for the mean total diet are below or very close to the established HBGV of 10 mg/kg bw per day.

35. Exposures estimated for the 95th percentile total diet range from 9.1 to 26 mg/kg bw per day. The exposures for adults (18+) and the elderly are below the established HBGV and adverse health effects would not be expected. Although estimated exposures for infants, toddlers, children and adolescents are 1.3- to 2.6-fold higher than the HBGV, actual exposures are likely to be lower than those calculated. In addition, as noted in paragraph 30, the HBGV is likely to be conservative.

36. Therefore, exposures to food grade TiO2 (E171) from the diet are unlikely to present a risk to health for the UK population.

COT Overall Conclusion and References

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Overall Conclusion

37. The COT concludes that it is unlikely that there would be a risk to health from current UK dietary exposures of E171 TiO_2.

COT

August 2024

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