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.