Introduction - Statement on the safety of Titanium Dioxide (E171) as a Food Additive

In this guide

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Introduction

1. Titanium dioxide (TiO2) is an inorganic compound which exists in nature in different crystalline forms - the anatase and rutile being the two most common. Titanium dioxide is comprised of particles with a range of sizes. Food grade TiO2 (E171) is comprised of both nano- and micro-sized particles. The International Organization for Standardization (ISO) define nanoscale as a length range of approximately 1 – 100 nm. They also defined a nanomaterial as a material with any external dimension in the nanoscale (1 – 100 nm) or having internal structure or surface structure in the nanoscale (ISO, 2023). Microparticles are between 0.1 and ≥100 µm in size. EFSA have previously characterised E 171 and concluded that less than 50% of constituent particles in E171 have a minimum external dimension below 100 nm by number (EFSA, 2019).

2. Food grade titanium dioxide (TiO2) 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 in both anatase and rutile forms.

3. Food grade TiO2 (E171) 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 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. It has also been widely used in cosmetics and medicines (EFSA, 2016).

4. Where stated, titanium dioxide/TiO2 refers to both E171 and non-E171 forms and undefined specifications, E171 refers specifically to titanium dioxide which complies with the updated EFSA specification for titanium dioxide E171 as a food additive, which have been transferred over to assimilated Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council (Text with EEA relevance). (EC, 2012: Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council (Text with EEA relevance) (legislation.gov.uk))

5. In 1969, the FAO/WHO Joint Expert Committee on Food Additives (JECFA) allocated an acceptable daily intake (ADI) ‘not limited except for good manufacturing practice’. In 1975, the Scientific Committee on Food (SCF) did not establish an ADI for titanium dioxide, whereas in 1977, the SCF included titanium dioxide in the category ‘colours for which an ADI was not established but which could be used in food’.

6. Since 1975 TiO2 has been the subject of multiple safety evaluations which are detailed below.

Evaluations by EFSA prior to the 2021 Opinion

7. The EFSA Food Additives and Nutrient Sources (ANS) Panel (2016) did not establish an ADI, or other health-based guidance value (HBGV), during their review. This was due to the lack of an extended 90-day toxicity study, a multi-generation or an extended one generation reproductive toxicity (EOGRT) study with E171. These data were considered necessary due to possible adverse effects which were identified in the reproductive system from studies published in the peer-reviewed literature, using test substances that were non-food grade, or which contained inadequately characterised nanomaterial.

8. The EFSA 2016 review determined that E171 TiO2 consisted mainly of micro-sized TiO2 particles, with a nano-sized (< 100 nm) fraction which was less than 3.2% by mass. Uncertainties around the identity and characterisation of E171

were highlighted, noting that no limits for the particle size of E171 had been set. EFSA requested further data in the form of an EOGRT study, as no HBGV could be established due to the evidence gaps. These gaps included the lack of consistency and characterisation of the form of TiO2 in the test materials and in the test animal during absorption and metabolism, and considerations around the exposure to TiO2 test substances which contain higher levels of nanoparticles (NPs) that could change the toxicological profile. However, it is unclear by how much the toxicological profile could be changed, or at which point during the exposure. There was no comparison of results to food-grade TiO2; an ideal study had not been conducted.

9. The specifications of E171 TiO2 were reviewed again by EFSA in 2019. Based on the fraction of nanoparticles present in E171, it was determined that the food additive fell under the scope of the EFSA guidance on nanotechnology for “a material that is not engineered as nanomaterial but contains a fraction of particles, less than 50% in the number–size distribution, with one or more external dimensions in the size range 1–100 nm”. (EFSA, 2019).

Other Evaluations by Regulatory bodies prior to the EFSA 2021 Opinion

10. Following a report by the French Authorities in 2016 and a proposal for evaluation of titanium dioxide, the Committee for Risk Assessment (RAC) of the European Chemicals Agency (ECHA) concluded, in June 2017, that titanium dioxide met the criteria to be classified as a substance suspected of causing cancer (category 2) if inhaled. The main mechanism thought to explain the effects induced by titanium dioxide, in common with effects seen with other particulate substances, was inflammation and an indirect genotoxic effect through production of reactive oxygen species (ROS) arising from the biopersistence and insolubility of all forms of titanium dioxide particles. However, a direct interaction with DNA could not be excluded, since titanium dioxide had been found in the cell nucleus in various in vitro and in vivo studies. This was in line with the International Agency for Research on Cancer (IARC) evaluation which concluded that in relation to exposure via inhalation “titanium dioxide is possibly carcinogenic to humans (Group 2B) based on sufficient evidence in experimental animals and inadequate evidence from epidemiological studies.” (IARC 2010). However, the 2016 report by the French Authorities the Agency for Food, Environmental and Occupational Health and Safety (ANSES) concluded that there was no carcinogenic concern after oral or dermal administration.

11. In 2018, the Dutch Office for Risk Assessment and Research held a workshop on the “potential health effects of the food additive titanium dioxide (E171)”. The results were published in 2019, which overall stated the need for further studies to investigate the effects of titanium dioxide exposure, particularly for the endpoints of colon tumours and immunotoxicology based on the data gaps. Study limitations of the available database at the time were also highlighted. The need to better characterise the composition of E171 was noted. In 2020, a review was published (Bischoff et al., 2020) that summarised the outcomes of this workshop and additionally aimed to identify and evaluate recent toxicological studies on food-grade titanium dioxide and nano-sized titanium dioxide in ex-vivo, in-vitro, and in-vivo experiments along the gastrointestinal route, and to postulate an adverse outcome pathway (AOP) following ingestion. Adverse effects were identified including the generation of ROS, alterations of the gut microbiota, persistent inflammation, and other effects on the immune system. It was noted that the findings were inconsistent between the different species and independent research groups. With regards to the animal studies that reported positive effects on precancerous lesions/tumour formation, it was noted that those were mainly used as research models and a proper investigation of a dose-response relationship was not performed. Based on the available information, it was not possible to carry out a risk assessment. When considering the mode of action, it was postulated that it was closely related to the ability of titanium dioxide to induce ROS formation and promote inflammation. The potential key events were considered to be persistent inflammation and ROS generation (that can result in oxidative stress) as well as persistent epithelial cell injury which could potentially lead to DNA damage and exert a tumour-promoting effect of E171 which was seen in some of the studies. Finally, it was noted that it is generally assumed that the round and spherical crystal forms of TiO2 contribute to the induction of adverse effects to a lesser extent when ingested than nano-sized titanium dioxide particles which are suspected to induce more adverse effects than other particle sizes. However, a study by Proquin et al., (2017) was also mentioned that demonstrated that a mixture of nano- and micro-sized TiO2 particles, as present in E171, induced more adverse effects than the single fractions alone. The authors further expanded on possible interactions of E171 with its direct environment as well as other factors that could potentially affect agglomeration, for example and discussed how these might directly affect the properties of titanium dioxide. Bischoff et al. therefore, considered that “it is important to carefully examine and analyze the physicochemical characteristics of TiO2 particles in its vehicle, as well as in its surrounding matrix as their final milieu, to guarantee a profound assessment of potential adverse health effects of

E171 and to adequately compare different studies in the process of risk assessment.” (Bischoff et al.,2020).

12. In their most recent evaluation, the EU Scientific Committee on Consumer Safety (SCCS) assessed titanium dioxide used in cosmetic products that lead to exposure by inhalation. The SCCS considered both micro and nano-sized particles. With regards to mutagenicity and genotoxicity, the SCCS noted that in the 2010 evaluation, IARC concluded that most of the in vitro genotoxicity studies with titanium dioxide exposure were negative despite the high rate of false positives and that the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) Panel in 2016 considered that the positive genotoxicity results may have been due to experimental conditions associated with the induction of oxidative stress. The ANS Panel (2016) had concluded that orally ingested TiO2 particles (micro- and nanosized) are unlikely to represent a genotoxic hazard in vivo. The SCCS also noted that studies showing a positive association between exposure to the so-called group of Poorly Soluble Low Toxicity (PSLT) particles and genotoxicity are generally consistent with the mechanism that sub-toxic concentrations of PSLT particles can cause inflammation and oxidative stress, which may lead to mutations. Oxidative stress is considered the underlying mechanism of the proliferation and genotoxic responses to PSLT particles including titanium dioxide and thus there is a large body of evidence that titanium dioxide has no direct genotoxic potential. The SCCS was of the opinion that “The genotoxic effects of titanium dioxide most probably manifest through an indirect mechanism (oxidative stress), or secondary mechanisms (e.g. oxidative stress and inflammation caused by immune cells). The SCCS therefore considers it plausible that there is a practical threshold for this mode of action and therefore a risk assessment could be carried out for its use in cosmetic products.” They concluded that when used in cosmetic products, titanium dioxide does not pose a genotoxic risk (SCCS, 2020).

Opinion of the EFSA Panel on Food Additives and Flavourings (FAF), (2021)

13. In their 2021 Opinion, the EFSA FAF Panel had considered that some potential immunotoxicity and inflammation findings specifically with TiO2 food additive E171, as well as potential neurotoxicity with TiO2 nanoparticles may be indicative of adverse effects. They also concluded that there were indications of the induction of aberrant crypt foci (ACF) with E171 and that there were no studies which had been appropriately designed and conducted to investigate the

potential carcinogenicity of TiO2 nanoparticles. On the basis of the available evidence and the uncertainties, in particular a concern regarding genotoxicity that could not be resolved, the EFSA Panel concluded that E171 could no longer be considered as safe when used as a food additive. (EFSA, 2021).

COT and COM considerations of the 2021 EFSA Review

14. The COT and Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) conducted an initial review of the updated EFSA opinion on E171 (EFSA, 2021), following a request from the Food Standards Agency, and disagreed with EFSA's conclusion. It was considered that the conclusions were not robust, and it was decided that the UK should undertake its own evaluation. This would be undertaken by the COM for genotoxicity and the COT for inflammation, immunotoxicity, allergenicity, aberrant crypt foci (as a biomarker for colon cancer), reproductive and developmental toxicity and neurotoxicity.

15. The COT produced an interim position paper in 2022 (TiO2 COT Interim position paper (food.gov.uk) (COT, 2022)) outlining the views of the COM and the COT, on the EFSA Opinion. The available data have since been evaluated by both committees.

Evaluations by Authorities after the 2021 EFSA Opinion

16. Health Canada (2022) have published a document summarising the state of the science concerning the safety of TiO2 as a food additive. TiO2 is insoluble, poorly bioavailable and generally considered toxicologically inert via the oral route. However, Health Canada recognised that its safety has recently been questioned due to the presence of particles in the nanoscale range (<100 nm), (although not engineered as a nanomaterial). TiO2 particles in the nanoscale, as well as food-grade TiO2 containing nanoparticles, may produce toxic effects in various test systems when dispersed and stabilized in simple matrices such as water. However, it is not certain what the relevance is for human health, as TiO2 used in food preparations is of agglomerated particles that are not dispersed to the same degree. Health Canada noted that in vivo studies that administered TiO2 in the diet tended not to replicate findings from studies using dispersed TiO2

in simple matrices and also that the particles used in studies are often poorly described which makes it difficult to establish relationships between particle characteristics (e.g., size, agglomeration state, surface area, particle number, etc.) and toxicity. It is also difficult to determine the relevance to human exposure and the forms of TiO2 used in food.

17. Health Canada also noted that TiO2 is not metabolized to any significant degree and the majority is excreted unchanged in faeces. Studies in animals and human volunteers indicate that approximately 0.001%, may be systemically available via the oral route, and that where TiO2 particles have been found this was predominantly in Peyer's Patches, the liver and spleen. They further noted that in a study by Bettini et al. (2017) with TiO2 administered in drinking water at 10 mg/kg bw per-day for 100 days, large aberrant crypt foci (ACF) were observed in exposed animals. However, similar findings have not been found, even in other studies with concentrations orders of magnitude higher. In addition, there are three significant studies which assessed the effects of dietary exposure to TiO2 in food in rodents. A chronic rodent bioassay carried out by the United States National Cancer Institute (NCI, 1979) showed no evidence of intestinal tumours or other intestinal lesions, including inflammation, in rats or mice exposed to TiO2 incorporated into feed for two years. A study designed to replicate the findings of Bettini et al. (2017) (Blevins et al, 2019) found no evidence of ACF in the colon following 100 days exposure to food-grade TiO2 up to the highest concentration tested (236 – 300 mg/kg bw/day). In a recent extended one-generation reproductive toxicity study performed according to the Organisation for Economic Co-operation and Development (OECD) guideline No. 443 (Leuschner, 2020), no adverse effects were observed up to the highest dose tested (1,000 mg/kg bw per day) administered via the diet when rats were continuously exposed from pre-conception through to adulthood. Health Canada also concluded that the available evidence indicates that food-grade TiO2 is not genotoxic in vivo, although the number of studies available is limited and more research is recommended to confirm these findings.

18. Health Canada concluded that "In summary, the adverse effects associated with oral exposure to TiO2 are largely derived from non-standard studies that administered stable, homogenized suspensions of ultrasonically dispersed particles. While these intensive sample preparation steps are necessary and appropriate for particle characterization and hazard identification for nanoscale materials in general, in the opinion of Health Canada's Food Directorate they do not fully represent exposure to TiO2 as a constituent of food". Overall, Health Canada's Food Directorate did not identify any compelling health concerns for the

use of TiO2 as a food additive in the course of this review. While some uncertainties in the database were identified that would benefit from further research, the weight of available evidence suggested that these data gaps were not significant enough to warrant a more precautionary approach at this time. As is the case for food additives generally, Health Canada's Food Directorate will continue to monitor the emerging science concerning the safety of TiO2 used as a food additive and this conclusion may be revisited should new scientific information become available." Health Canada (2022).

19. The Food Standards Australia New Zealand (FSANZ) published a report (FSANZ, 2022) "Titanium Dioxide as a Food Additive" which had reviewed the key evidence relating to the safety of TiO2 as a food additive, following the assessment by EFSA in 2021. It was noted that most of the concerns around the safety of TiO2 as a food additive were based on studies with TiO2 in the nanoscale range, or in which food-grade TiO2 had been sonicated. These are not reflective of the human dietary exposure scenario. FSANZ noted that there is a lack of in vivo genotoxicity studies with food-grade TiO2 in the feed but there was no evidence that exposure through oral gavage or intraperitoneal administration was genotoxic in vivo. Their report supported the findings of Health Canada (2022) noting that ACF had been observed in a drinking water study (10 mg/kg bw per day) however this was not replicated in two studies using TiO2 administered via the diet at much higher concentrations (up to 267 or 1,000 mg/kg bw per day). This was also inconsistent with results from a 2-year bioassay conducted by the United States National Cancer Institute (NCI) which showed no evidence of toxicity or carcinogenicity at dietary concentrations of up to 50,000 ppm TiO2, (equivalent to doses of 2,250 to 8,350 mg/kg bw per day, depending on sex and species). It was also noted that there was no evidence of systemic toxicity, developmental or reproductive toxicity, developmental neurotoxicity or developmental immunotoxicity in the EOGRT undertaken by Leuschner (2020). Overall FSANZ concluded that "there is no evidence to suggest that dietary exposures to food-grade TiO2 are of concern for human health". (FSANZ, 2022).

20. More recently, JECFA (2024) have published the report of their risk assessment of titanium dioxide International Numbering System for Food Additives (INS) 171 (E171). It was noted that the absorption of TiO2 was very poor and that the bioavailability in humans was very low. Although there were uncertainties in the genotoxicity data, JECFA noted that INS 171 was not carcinogenic in adequately conducted 2-year studies at doses of up to 7,500 mg/kg bw per day for mice and 2,500 mg/kg bw per day for rats, the highest doses tested. JECFA also considered that there was no evidence for

reproductive or developmental toxicity effects in animals. There were no epidemiological studies that allowed conclusions to be drawn with respect to associations between dietary exposure to INS 171 and human health effects. JECFA concluded that “Considering the very low oral absorption of INS 171, and in the absence of any identifiable hazard associated with INS 171 in the diet, the Committee reaffirmed the ADI “not specified” established at the Thirteenth meeting.” (FAO/WHO, 2024).

Methodology of the COT review

21. This statement on the safety of TiO2 E171 as a food additive (considered at the request of the Food Standards Agency following the recent (2021) review of TiO2 E171 by EFSA) constitutes the views of the COT, on the safety of E171. This assessment has been conducted jointly by the COT and the COM. The COM statements are summarised and referenced in the genotoxicity section. The COM have assessed the available data on the genotoxicity of TiO2 in vitro and in vivo and the COM statements will be available on their website. The COT had previously considered and commented on the EFSA 2016 Opinion on TiO2, whilst the UK was part of the EU.

22. In 2023, the COT agreed that a small group of Members would be set up to undertake a more detailed critical evaluation on the additional endpoints (ACF, reproductive and developmental toxicology, neurotoxicity and immunotoxicity).

23. For the purposes of this assessment, the COT mainly considered the updated evidence provided in the 2021 EFSA opinion, with a focus on the EOGRT study commissioned after the 2016 EFSA review, together with several additional studies from 2015 to 2023. In this statement, references to ‘The Committee’ will be the COT unless otherwise stated.

24. The report by Health Canada, “State of the Science of Titanium Dioxide (TiO2) as a Food Additive” (2022) had previously been reviewed and commented on by COT Members prior to its publication. Therefore, some of the discussion and conclusions from this document have also been included in the endpoints discussed below. Conclusions and discussion from EFSA (2021), FSANZ (2022) and JECFA (2024) have also been included in the sections discussed below.

25. The EFSA updated opinion (2021) and the EFSA conclusions on the papers that were considered most relevant were reviewed by the COT with an additional literature search undertaken for the COT to consider relevant literature and opinions published between 2021 and 2023, including the Health Canada report

on Titanium Dioxide.

26. For the literature search, the database Lit-fetch was used to search the terms listed in Annex A between the dates 2021-01-01 to 2023-04-28. Lit-fetch searches used EBSCO, Scopus, Springer and Pubmed. For the first 2 search strings, the numbers in brackets denote (number of hits; number of relevant hits), the 3rd search string just denotes number of hits. An updated search was also carried out for the first 2 search strings for 2023-04-28 to 2024-03-01, but only for in vivo studies. The search terms can be found in Annex A.

27. Paper titles and abstracts were manually screened, and papers deemed not relevant were disregarded. The methodology used by the COM is described in detail in their statements (COM, 2024a; COM, 2024b).

28. The ADME and toxicity studies used in the assessment are summarised in the relevant sections. Summaries of the conclusions from the assessments by EFSA (2021), Health Canada (2022) FSANZ (2022) and JECFA (2024) are included at the end of each section. The COT review and conclusions for that endpoint are included at the end of the section. A summary table of all of the studies considered by the COT is available in Annex B.

29. The methodology for the exposure assessment is described in detail in the relevant section with additional information provided in Annex C.

30. Following the publication of the COT Interim Position Paper (COT, 2022), the COT have continued to review aspects of the safety of TiO2 as a food additive and have prepared the following additional papers:

- TOX/2023/16: Introduction - Review of EFSA Opinion | Committee on Toxicity (food.gov.uk),

- TOX/2023/32: Review of Titanium Dioxide: Discussion Paper for Additional Endpoints | Committee on Toxicity (food.gov.uk),

- TOX/2023/33 First draft statement on the safety of Titanium Dioxide (E171) as a Food Additive | Committee on Toxicity,

- TOX/2023/44 Exposure to Titanium Dioxide in the UK population | Committee on Toxicity (food.gov.uk),

- TOX/2023/56 Review of Titanium Dioxide – second draft statement,

- TOX/2024/02 Cover Paper and Tables - Third draft statement on the safety of Titanium Dioxide (E171) as a Food Additive | Committee on Toxicity,

- TOX/2024/14: Fourth draft statement on the safety of Titanium Dioxide (E171) as a Food Additive,

- TOX/2024/18: Fifth draft statement on the safety of Titanium Dioxide (E171) as a Food Additive- Introduction | Committee on Toxicity.