Fifth draft statement on the safety of Titanium Dioxide (E171) as a Food Additive- Exposure Assessment

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Exposure Assessment

Titanium dioxide (E171) can be found in a number of food categories. 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.

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

The exposure assessment takes 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 these categories contain E171 at the maximum reported levels. This is unlikely and overall exposure is more likely to be overestimated.

Risk Characterisation

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

Exposures calculated for the 95th percentile total diet range from 9.1 to 26 mg/kg bw/day. The exposures for adults (18 +) and the elderly are below the derived HBGV and adverse health effects would not be expected. Although exposures for infants, toddlers, children and adolescents are 1.3- to 2.6-fold higher than the HBGV the conservatism built into the calculated HBGV is likely to exceed that of the uncertainty factor of 100 and the calculated exposures are likely to be lower than calculated.

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

COT Overall Conclusion

In conclusion, based on the current available data and the derived HBGV, it is unlikely that there would be a risk to health from current dietary exposures of E171 TiO2.

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. TiO2 was an authorised Food Additive (E171) in the EU until 7th August 2022. It currently remains authorised in both anatase and rutile forms, in the UK, under Retained EU Regulation No. 1333/2008 and Retained EU Regulation No 231/2012. 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. 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).
2. 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.
3. TiO2 has been the subject of multiple safety evaluations; by the Scientific Committee on Food (SCF) in 1975 and 1977, and by the Joint FAO/WHO Expert Committee of Food Additives (JECFA) in 1969. In 1969, JECFA

allocated an acceptable daily intake (ADI) ‘not limited except for good manufacturing practice’. In 1975, the 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’.

Evaluations by EFSA prior to the 2021 Opinion

4. 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 in 2016. 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 literature studies using test substances that were non-food grade or which contained inadequately characterised nanomaterial.
5. 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 were 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 has not been conducted.
6. The specifications of E171 TiO2 were reviewed again by EFSA in 2019 (EFSA, 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”.

Other Evaluations by Regulatory bodies prior to the EFSA 2021

7. 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 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 possible carcinogenic to humans (Group 2B) based on sufficient evidence in experimental animals and inadequate evidence from epidemiological studies.” (IARC 2009). 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.

8. 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 of which were published in 2019, where overall the need for further studies to further investigate the effects of titanium dioxide exposure- particularly for the endpoints of colon tumours and immunotoxicology based on the data gaps and study limitations of the available database at the time was highlighted. The need to better characterise the composition of E171 was noted. In 2020, a review was published 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 lower extent when ingested and similarly that titanium dioxide nanoparticles 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, induce 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 could directly affect the properties of titanium dioxide. Therefore, they 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).

9. 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. 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 FAF Panel in 2016 considered that the positive genotoxicity results may have been due to experimental conditions associated with the induction of oxidative stress. The SCCS also noted that studies showing a positive association between the so-called group of Poorly Soluble Low Toxicity (PSLT) particles exposure 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).

2021 Opinion of EFSA (EFSA FAF Panel)

10. In their 2021 Opinion, the EFSA FAF Panel had considered that some immunotoxicity and inflammation findings specifically with TiO2 food additive E171, as well as 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 currently available evidence and the uncertainties, in particular a concern regarding genotoxicity which could not be resolved, the EFSA Panel concluded that E171 can no longer be considered as safe when used as a food additive. (EFSA, 2021).

COT and COM considerations of the 2021 EFSA Review

11. 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.

12. Note: The Genotoxicity data will be evaluated by the COM and a summary from their statement, once it has been published, will be included. [Placeholder for COM statement].

13. The COT produced an interim position paper in 2022 (TiO2 COT Interim position paper (food.gov.uk) (COT, 2022)) outlining their views on the EFSA

Opinion, and has evaluated the available data since. This draft statement constitutes the views of the COT, on the safety of E171.

Evaluations by Authorities after the 2021 EFSA Opinion

14. Health Canada (2022) have published a state of the science document for TiO2. The intention was to summarise 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, the 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. There is a lot of experimental toxicological literature which exists for TiO2, however, 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.

15. TiO2 is not metabolized to any significant degree and the majority are excreted unchanged in feces. Studies in animals and human volunteers indicate that approximately 0.001%, may be systemically available via the oral route. In studies, TiO2 particles have predominantly been found in Peyer’s Patches, the liver and spleen. In a study by Bettini et al. (2017) with TiO2 administered in drinking water at 10 mg/kg bw/day for 100 days, large aberrant crypt foci (ACF) were observed in exposed animals. However, similar findings have not been replicated, even at studies with concentrations orders of magnitude higher. There are three significant studies which assess the dietary exposure of TiO2 in food in rodent studies. 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) 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 OECD guideline No. 443 (LPT, 2020), no adverse effects were observed up to the highest dose tested (1000 mg/kg bw/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.

16. “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 suggests these data gaps are 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.”

17. Food Standards Australia New Zealand (FSANZ) published a report “Titanium Dioxide as a Food Additive”. This 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 safety of TiO2 as a food additive were based on studies with TiO2 in the nanoscale range, or in which food-grade TiO2 has 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 and there was no evidence that exposure through oral gavage or intraperitoneal gavage was genotoxic *in vivo*. Aberrant crypt foci had been observed in a drinking water study (10 mg/kg bw/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/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. 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 LPT. 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).

18. More recently, JECFA (2023) have published a summary of their risk assessment of titanium dioxide (E171). The summary noted that the absorption of TiO2 was poor and that the bioavailability in humans was very low. The Committee also considered that there was no evidence for carcinogenic, 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 E171 and human health effects. JECFA noted that the available data did not provide convincing evidence of genotoxicity when considering the limitations and some equivocal findings in the data on genotoxicity. It was recommended that there was a need for “more research to address the current uncertainty about the distribution of TiO2 particle sizes in food and to develop genotoxicity tests that are more appropriate for nanoparticles”. (FAO/WHO, 2023).

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