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TOX/2024/18

Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment.

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

1. Titanium dioxide (TiO₂) was an authorised Food Additive (E171) in the EU until 7th August 2022. It currently remains authorised 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.

2. Titanium dioxide has been the subject of multiple safety evaluations. In 2016, the EFSA ANS (Food Additives and Nutrient Sources) Panel evaluated the safety of E171 TiO₂ and identified several uncertainties in their evaluation included the unspecified identity and characterisation of E171 as it was not determined whether the test material was compliant with the specification of E171 requirements. 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. In 2019, the specifications of E171 titanium dioxide were reviewed by the EFSA FAF Panel (Food and Feed). A recommendation for re-assessment of the safety of titanium dioxide was proposed.

3. In the EFSA 2021 Opinion, the EFSA FAF Panel considered that some findings regarding immunotoxicity, inflammation and neurotoxicity with respect to TiO₂ nanoparticles may be indicative of adverse effects. On the basis of the currently available evidence and the uncertainties, in particular a concern regarding

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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.

4. In 2021 the COT published an interim position on titanium dioxide ([COT 2021](#)) capturing the outcomes of the discussions and outlining the next steps. Members were asked to evaluate the EFSA Opinion and comment on whether they agreed with EFSA's conclusions and further guidance on the next steps that should be taken; producing an opinion paper following a review of the new EFSA opinion and the extended one generation reproductive toxicity (EOGRT) study data by both the COT and COM (Committee on Mutagenicity).

5. This draft statement (Annex A) includes the COT conclusions on the following endpoints: ADME, Aberrant Crypt Foci as a marker for Carcinogenicity, Allergenicity, Reproductive and Developmental Toxicity, potential evidence of Immunotoxicity, Inflammation and Neurotoxicity and the derivation of a Health-Based Guidance Value, and a review and conclusions of genotoxicity endpoints by the COM. An Executive Summary has also been included and the conclusions have been updated.

Questions for the Committee

6. The Committee are asked to consider the following questions:
- i. Are Members content with the layout and structure of the statement?
 - ii. Are Members content with the layout and structure of the Executive Summary?
 - iii. Do Members agree with the risk characterisation and conclusions?
 - iv. Do Members have any other comments?

Secretariat

May 2024

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TOX/2024/xx Annex A

Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment.

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

[Executive Summary](#)

Introduction

Prior to August 7th 2022, titanium dioxide (TiO₂) was an authorised Food Additive (E171) in the EU. It currently remains authorised in the UK. It 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 has been the subject of multiple safety evaluations including three recent evaluations by the European Food Safety Authority (EFSA) in 2016, 2019 and 2021.

In their most recent Opinion (2021), EFSA considered that some findings regarding immunotoxicity, inflammation and neurotoxicity with respect to TiO₂ nanoparticles 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 when used as a food additive.

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

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Products and the Environment (COM), to assess the safety of TiO₂ as a food additive. This review is summarised below.

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, 2023).

Characterisation and ADME considerations

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 have all (100%) of their particles less than 100 nm in diameter and are colourless and would therefore be unsuitable for use as a pigment in food applications.

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

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)

Review of the Extended One Generation Reproductive Toxicity (EOGRT)

The COT reviewed the EOGRT provided to the Food Standards Agency (FSA) by the Titanium Dioxide Manufacturers Association (TDMA) (LPT, 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. The COT agreed that the EOGRT study was well conducted.

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Overall, there was no evidence of reproductive or developmental toxicity up to and including the highest dose tested (1000 mg/kg/day).

Review of toxicity for endpoints identified by the COT

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.

The Committee 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.

Aberrant Crypt Foci (ACF)

The Committee considered that although small numbers of ACF were observed in some animals exposed to TiO₂ alone, these could not necessarily be attributed to TiO₂, as ACF were also present in control groups without exposure to TiO₂ in other studies (e.g. the EOGRT). Additionally, none of the studies distinguished between hyperplastic or dysplastic ACF in any groups of control or treated animals. The Committee concluded that there was no conclusive evidence that TiO₂ induced ACF and no evidence to support progression to proliferative lesions in the colon.

Inflammation and immunotoxicity

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

Five studies using food grade TiO₂ 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) were considered by the COT. In several studies, differential cytokine and host defence gene expression was observed but was neither consistent across

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studies, nor ubiquitous in terms of pathway activation, making interpretation or formulation of conclusive statements challenging.

Other potential immunotoxic effects have been reported that are distinct from the induction or modulation of inflammation. These include, but are not limited to: induction of immune cell mediated inflammation in the gut, including in Peyer's patches, as well as in the spleen and via peripheral blood mononuclear cells; effects on broader host defence mechanisms, including antimicrobial peptides; effects in the gut microbiota; effects on dendritic cell populations in the gut; 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.

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

Reproductive and Developmental Toxicity

The COT considered the EOGRT report to be detailed and that the study was 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 of the testes and epididymides and a difference in weight of the right testes. However, the COT agreed with the authors' conclusions that these changes were spontaneous and not of toxicological relevance.

Overall, therefore, no adverse effects on reproductive and developmental toxicity were observed, up to the highest dose tested. The COT noted that the low absorption of TiO₂ may have contributed to the NOAEL (1,000 mg/kg bw per day) established in the EOGRT study.

Data from peer reviewed literature were inconclusive. The best overall quality additional studies were determined by the COT to be the studies by Warheit, Boatman and Brown, 2015, and & Lee et al., 2019. The COT concluded that there was no strong evidence that TiO₂ is reprotoxic and that the NOAELs in these additional studies are consistent with that from the EOGRT study.

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Neurotoxicity

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 effects and that most of the other studies on this endpoint used nanomaterials.

It was 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. It should be recognised that this qualified COT opinion 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.

Impact of Nanoparticles

The Committee concluded that there is uncertainty over the effect that TiO₂ nanoparticles have on toxicity. 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.

The COT considered that the data from the relevant studies available indicated that TiO₂ did not induce ACF, nor were there significant effects from 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 was robust.

Genotoxicity review of TiO₂ review by the COM

The COM reviewed a number of studies to assess the genotoxicity of TiO₂.

The COM stated that a definitive assessment of the safety of food grade E171 was difficult when there were no high-quality OECD-compliant studies that adequately

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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, and this led to conflicting data and uncertainty in the risk assessment for TiO₂. (COM, 2024b. Not yet published).

The COM opinion is that there is little evidence that TiO₂ micro-sized or nanoparticles are genotoxic in vitro or in vivo based on data from well conducted studies. There is also a lack of replication of study outcomes using the same nanoparticle in different labs. (COM, 2024a. Not yet published).

Overall, however, 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) (COM 2024b. Not yet published).

The COT agreed with the conclusions of the COM.

Derivation of a Health-Based Guidance Value (HBGV)

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 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).

A standard uncertainty factor of 100 (10 for inter species variability and 10 for 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 due to the low absorption of TiO₂ and because there is no metabolism of TiO₂ particles.

Exposure Assessment

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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

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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.

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Introduction

1. Titanium dioxide (TiO_2) is an inorganic compound which exists in nature in different crystalline forms - the anatase and rutile being the two most common. TiO_2 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/ TiO_2 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. TiO_2 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

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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

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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

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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

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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.

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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

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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.”

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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).

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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).

Methodology of the COT review

20. The EFSA updated opinion (2021) papers and the EFSA opinion 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.

21. A literature search was undertaken. The database Lit-fetch was used to search the following search terms between the dates 2021-01-01 to 2023-04-28. 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 B.

22. Paper titles and abstracts were manually sorted, and papers deemed not relevant were disregarded.

23. A summary table of all the studies considered by the COT is available in Annex D

This section will be updated.

Titanium Dioxide

General information

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24. Titanium dioxide (TiO₂) is an inorganic compound which exists in nature in different crystalline forms. The anatase and rutile being the two most important for its use as a food additive.

25. The Chemical Abstracts Service (CAS) Registry number for TiO₂ is 13463-67-7 and the European Inventory of Existing Commercial Chemical Substances (EINECS) number is 236-675-5. The Colour Index (C.I.) number is 77891.

26. TiO₂ was an authorised Food Additive (E171) in the EU in accordance with Annex II to Regulation (EC) No 1333/2008 in both anatase and rutile forms (Commission Regulation (EU) No 231/2012) and is currently authorised under GB Food Law (retained EU Regulation No 1333/2008 on food additives).

27. The uses of TiO₂ as a food additive include:

- As a colour to make food more visually appealing
- To give colour to food that would otherwise be colourless
- To restore the original appearance of food.

28. It is found in a wide variety of food items such as pastries, chocolate and sweets, sauces and chewing gum. It is also widely used in cosmetics and medicines (EFSA, 2016).

29. The current assessment of the safety of TiO₂ (E171) as a food additive is being considered at the request of the Food Standards Agency following the recent (2021) review of TiO₂ (E171) by EFSA. This assessment is being conducted jointly by the COT and the COM. The COM have assessed the available data on the genotoxicity of TiO₂ and the full COM statement will be available on their website. The COT previously considered and commented on the EFSA 2016 Opinion on TiO₂, whilst the UK was part of the EU.

30. For the purposes of this evaluation, 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. In this statement, references to 'The

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Committee' will be the COT unless otherwise stated. The COT considered updated evidence including the results of the EOGRT study reviewed by EFSA, and several additional studies from 2015 to 2020.

31. A 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.

32. Food Standards Australia New Zealand (FSANZ) have also published a report "Titanium Dioxide as a Food Additive". Some of the discussions and conclusions from this report have also been included in the sections discussed below.

Physicochemical Characterisation of food grade TiO₂

33. The characterisation of TiO₂ has been described in detail in EFSA (2016) and in the Health Canada State of the Science Report (2022). This section provides a summary of the TiO₂ characterisation to provide background information for the better understanding of this statement on the safety of TiO₂ as a food additive.

34. TiO₂ is a white powder used as a pigment in various industries and is valued for its high refractive index. 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 exists as an aggregate of smaller primary particles with a median particle size of 200 – 300 nm, which corresponds to roughly half the wavelength of visible light and provides optimal light scattering, thus producing the desired whitening effect (Winkler et al. 2018).

35. TiO₂ can also be in the form of engineered nano-TiO₂. One form of nano-TiO₂ that is generally sold for catalytic applications and is also widely used in toxicity studies is P25. Food-grade and P25 TiO₂ are engineered products, frequently synthesized from purified titanium precursors, and not milled from bulk scale minerals (Yang et al., 2014). Engineered nano-TiO₂ (e.g., P25, NM-101, NM-102, NM-103, NM-104 and NM-105) has 100% particles less than 100 nm in diameter, they are colourless and

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therefore would be unsuitable for use as a pigment/opacifier in food and pharmaceutical applications (Farrell and Magnuson, 2017).

36. Pure TiO₂ assembles in several crystal structures although only anatase, rutile or a mixture of the two are used in foods. For many applications, including cosmetics and some therapeutic products, surface coatings are applied to TiO₂ particles that alter their physicochemical properties. Therefore, toxicity and ADME studies using these materials may not be relevant to TiO₂ used as a food additive, which normally undergoes no surface treatment and is uncoated (EFSA 2016). Due to differences in the manufacturing processes food-grade TiO₂ differs in surface composition compared to other TiO₂ materials, such as TiO₂-NPs. The surface of food-grade TiO₂ particles is covered by superficial phosphate groups, while materials manufactured with alternative methods may have different surface functional groups (e.g., hydroxyl groups) (Dudefoi, et al., 2017a; Yang et al., 2014). The surface properties of TiO₂ materials have a significant impact on their behaviour in various environments, including biological media. Therefore, TiO₂ materials with surface properties that differ from food-grade TiO₂ may not be considered relevant models when studying the fate of dietary TiO₂ (Dudefoi et al. 2017a).

37. Suspended TiO₂ particles tend to aggregate/agglomerate to form larger clusters, although a majority of the individual particles may display a primary diameter < 100 nm. The term “aggregate” designates an assembly of particles held together by covalent or metallic bonds. Instead, “agglomerates” result from weak forces like van der Waals interactions, hydrogen bonding, electrostatic attractions or adhesion by surface tensions. Due to this aggregation and agglomeration it is important not to equate the nanoparticle fraction measured by number with the same value by mass (Winkler et al., 2018).

38. However, despite having a mean particle size in the desired range (200 - 300 nm), primary particles in food-grade TiO₂ form a broad size distribution that invariably contains particles below 100 nm, i.e. nanomaterial. A number of studies have measured particle size in food grade TiO₂ or E171 and found a range of 17 – 36% of

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TiO₂ NPs with a diameter less than 100 nm (Verleyesen et al. 2020 and 2021; Yang et al, 2014; Dufefoi et al. 2017; Weir et al., 2012). The percentage by number of particles with a diameter less than 30 nm is on the order of 1% or less (EFSA 2021a).

39. When calculating exposure to TiO₂ NPs the equivalent mass of NPs is more relevant than particle number. Several studies reviewed by Ropers et al estimated the mass (weight %) of nanoparticles present in E171 across a range from 0.31 to 12.5% (EFSA, 2016; Bachler et al., 2014; Rompleberg et al., 2016; and Dufefoi et al., 2017). This variation explains some differences in the estimated exposures to TiO₂ nanoparticles in the literature including between the study by Rompelberg et al. who used a value of 0.31% of NPs and the evaluation of EFSA who used a weight ratio of 3.2%. (Ropers et al., 2017).

40. Since E171 is a particulate material containing a fraction of nanoparticles, and toxicology studies mainly focus on the systemic absorption of this nanofraction after ingestion, EFSA noted the need for more data; for example, information related to the particle size distribution of the titanium dioxide, when used as a food additive. Moreover, it was noted that the EU specifications for E171 should also include full characterisation of the particle size distribution of the materials as well as indication on the percentage (in number and by mass) of the particles in the nanoscale together with the information on the analytical methods/techniques used for detection and quantification of the nanosized particles. In this respect, the European Commission called in early 2017 for data addressing those EFSA recommendations. (Verleyesen et al., 2020; Geiss et al., 2020).

Physicochemical Characterisation of nano grade TiO₂

41. The consensus within the risk assessment community on the need for standardised and validated dispersion methods to ensure reliable and reproducible results to help enable the advancement of risk assessment research is well known. Validated approaches for the preparation of TiO₂ nanoparticles solutions either

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monodispersed or protein stabilised dispersions in relevant biological media, are regularly used for acute *in vitro* or *in vivo* toxicity assessment. Dispersions in biological media, that remain stable for at least 48 h (acute testing timeframe) under typical incubation conditions, are considered useful. The initial objective of most studies is to determine whether monomodal nanoscale dispersions, in the tested media, can be achieved by mixing aqueous nanoparticle stock with a biological media without prior stabilisation with addition of FBS or BSA, or other dispersants more relevant to the test system. To achieve this mono-dispersion surface modifications of the NPs, during synthesis, is often undertaken. Most academic studies are based on assessment of dispersed particle solutions, to help answer specific questions, which may not fully represent specific products or properties of products on the market.

42. OECD WPMN 'Guidance Manual for Sponsors of the OECD Sponsorship Programme for the Testing of Manufacture Nanomaterials has collaborated with numerous international projects to develop an understanding of nanomaterials. The Joint Action, NANOGENOTOX, <http://www.nanogenotox.eu>, co-financed by the Executive Agency of the Directorate General for Health and Consumers of the European Commission and 11 EU member states was established to determine characterisation and testing of TiO₂, with a focus on reference materials produced by the Joint Research Centre laboratories. Examples of EU projects testing the materials from the Repository are MARINA (<http://www.marina-fp7.eu/>) and NANoREG (<http://www.nanoreg.eu/>). The NANOGENOTOX sample preparation protocol developed by CEA, INRS and NRCWE and the final dispersion protocol is published on the project's web page. To highlight the interventions required to achieve dispersion "2.56 mg/mL of material sterile-filtered 0.05 % w/v BSA-ultrapure water are sonicated (probe sonicator) for 16 minutes, placed in an ice bath, at 400 W and 10 % amplitude while controlling that the sonication probe does not touch the walls of the scintillation vial. Use of different sonication conditions (power and amplitude) require different sonication times. The energy input should be calibrated to be in the order of 3,136 MJ/m³." This highlights the upstream processes required prior to material assessment to investigate the possible relevance of the nano component in toxicology studies.

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43. The physico-chemical characterisation of the Titanium dioxide series from the JRC repository: NM-100, NM-101, NM-102, NM-103, NM-104 and NM-105 (NM-100 is included in the series as a bulk comparator). Each material is available as a 2,000 mg white powder kept in amber coloured vials under argon atmosphere. NM-103 and NM-104, have 2% of dimethicone as an external organic coating. The coatings of NM-103 and NM-104 may be unstable under in vitro test conditions. NM-105 is the purest form of the material with others showing trace background elements of C, O, K, Ca and Al.

Table 1. Properties and indicative content of TiO₂ for the JRC titanium dioxide series

NM code	Label name	Properties	Indicative content of TiO₂ (%Wt)
NM100	Titanium Dioxide	Bulk (non nano)	97.7
NM101	Titanium Dioxide	Anatase	98.1
NM102	Titanium Dioxide, Anatase	Anatase	99.6
NM103	Titanium Dioxide thermal, hydrophobic	Rutile	91.3
NM104	Titanium Dioxide thermal, hydrophilic	Rutile	92.7
NM105	Titanium Dioxide, Anatase-Rutile	Anatase-Rutile	99.8

44. TEM micrographs indicate that the TiO₂ NMs, detailed above, have a polydisperse particle size distribution; the average value of the primary particle size was estimated to be below 26 nm for NM-103, NM-104 and NM-105, below 10 nm for NM-102, and above 100 nm for NM-100; for NM-100 primary particle sizes ranging from 20 nm up to 300 nm were detected. The shape of the particles also varied with NM-103 and NM-104 given in Table 2.

Table 2. Particle characteristics of selected JRC titanium dioxide materials

Material	Sphericity	Shape Factor	General Morphology
NM-103	Low sphericity	Very angular to sub-angular	Angular, low sphericity
NM-104	Low sphericity	Angular to sub-rounded	Sub-angular, low sphericity

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45. It should be noted that the variation of these test material physicochemical characteristics and the nature of their preparation, required for study, should be considered, when attempting potential read-across activities.

Physicochemical Characterisation considerations for this assessment

46. Due to the differences in the characteristics of food grade TiO₂ and engineered nano-TiO₂ this assessment will focus on the data from studies using food grade TiO₂ or E171. Consideration has been given to studies that used nano-TiO₂ or food-grade TiO₂ which was de-agglomerated prior to testing. However, as these are likely to provide limited information for the evidence base for food grade TiO₂ or E171 when tested as consumed, these are usually summarised in the text but details of the studies can be found in the study summary tables in Annex D

47. The majority of studies tend to use percentage of NPs by particle number rather than mass. All of the information provided in a study will be used in the text and included in the study summary table.

Absorption, Distribution, Metabolism and Excretion (ADME)

48. The EU Scientific Committee on Consumer Safety (SCCS) produced an opinion on TiO₂ (nano form) coated with Cetyl Phosphate, Manganese Dioxide or Triethoxycaprylylsilane as UV filters in dermally applied cosmetics, confirming the safe use in cosmetics for products intended for application on skin (SCCS, 2017). Dermal absorption is not considered any further in this statement.

49. The COT reviewed a number of studies to assess the ADME of TiO₂, which were also reviewed by EFSA, Health Canada and FSANZ. These studies are described below and a brief overview of the review and conclusions of EFSA, Health Canada, and FSANZ are included. The COT review and conclusions are presented at the end of each section.

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Studies used to review the toxicokinetics and absorption of the E171 form of TiO₂

50. The toxicokinetics of E171 has been addressed in a few studies. The COT have assessed 9 studies in total in their review, 7 studies in mice and rats and 2 studies in humans. A summary of all the studies is provided in Annex B.

E171 studies in rats and mice

Talamini et al (2019)

51. Groups of NRF mice (n = 22/group) were treated with 5 mg/kg bw of E171 (99.3% pure anatase, 35% nanoparticles, 201.2 ± 8.5 nm) dispersed in water (no sonication or deagglomeration). The animals were treated for 3 days/week for 3 weeks receiving a total of 9 treatments in 21 days (average daily dose of 2 mg/kg bw) with E171 or water (control) which was slowly dripped with pipette in the mouths of the mice.

52. The animals were weighed at the beginning of the experiment and observed daily. On day 21, the animals were sacrificed and the lungs, liver, stomach, spleen, kidney, brain, testes and whole intestine were removed. The concentrations of titanium were determined in 4 animals.

53. No signs of general toxicity were observed. In the brain, kidney and testes, titanium levels were <0.03 µg/g, the limit of quantification (LOQ) of the analytical method for solid tissue samples. In lungs and spleen, the levels were low, with a not statistically significant, but slightly higher deposition in spleen of E171 treated animals compared to the controls. The authors reported that "Titanium concentration was one order of magnitude greater in the small intestine compared to the above tissues and distinctly higher in the stomach, large intestine and liver". The concentrations of titanium in treated animals were: 1.07 ± 0.38 µg Ti/g tissue in the large intestine and 0.94 ± 0.57 µg Ti/g tissue in the liver. These levels were 1.8 and 3.6 times higher compared to the controls, respectively.

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Comera et al (2020)

54. In the first round of experiments adult C57BL/6 mice (12–18 weeks) (4 animals/group) were treated with a single gavage dose of 40 mg/kg of E171 (> 95% anatase, 20–340 nm (transmission electron microscopy- TEM); 44.7% nanoparticles) suspended in water and sonicated or water only as a control. In the second round of experiments, 300 µg/mL of E171 was suspended in buffer and used to fill a closed mid- jejunal loop of 10cm, pre-treated with inhibitors of tight junctions, micropinocytosis, clathrin-mediated endocytosis or raft-dependent endocytosis.

55. Animals were sacrificed at 2-, 4-, 8- and 24-hours following treatment. Confocal microscopy and micro x-ray fluorescence imaging was used to analyse the existence of particles in both the first and second round of experiments, whilst ICP-MS was used to determine titanium concentration in blood and tissues (jejunum, ileum, colon). The jejunal or colonic intraluminal contents were recovered by gentle scraping.

56. In mice treated with a single dose of E171, the number of titanium dioxide reflective particles in the lumen of the small intestine was significantly increased, with analysis of the particles suggesting that no further agglomeration of titanium dioxide occurred during its transit through the intestinal tract, and that as it moved in the distal intestine, there was a decrease in its agglomeration state (as indicated by smaller particle size in the colonic versus the jejunal lumen).

57. An increase in the reflective particle content was observed in the jejunal and ileal villi, Peyer's Patches and colon crypts. The overall particle content in jejunal villi increased from 2 hours after gavage, peaked at 4 hours, and returned to basal values at 8 hours. A statistically significant increase ($p < 0.001$) 4 hours after E171 administration was observed in the titanium dioxide particle density in the jejunal mucosa (increased by 3.4-fold over the controls). A lower and non-significant trend of increased particle content was also observed at 4 hours in the ileum and colon, with the values decreasing close to control levels at the time 8 hours in all three intestinal

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sections. In the jejunum, the reflective TiO₂ particle spots displayed a mean diameter of 700 ± 59 nm (n = 70). From the content in the intestines and the weight of the mice tissues, the authors calculated that approximately 0.007% of the titanium administered was present in the entire intestine at the 4 hours timepoint. The authors concluded that titanium dioxide was absorbed predominantly in the ileum, partly in jejunum and that small amount absorbed in the colon. Based on the surface area information it was concluded that titanium dioxide is predominantly absorbed by the small intestinal villi and to a lesser extent through Peyer's patches.

Riedle et al., (2020)

58. C57BL/6 mice (6 males and 6 females per treatment group) were fed diets with food grade titanium dioxide (anatase, with a diameter of 119 nm) doses of 0, 6.25, 62.5 and 625 mg/kg of diet. These were equivalent to 0 and approximately equal to 1, 10 and 100 mg food grade TiO₂/kg bw per day.

59. The animals were sacrificed at 6, 12 and 18 weeks. Animals sacrificed at 18 weeks were also used to validate that the diet permitted uptake in the intestinal lumen. The basal regions of the Peyer's patches were surveyed, and reflectance confocal microscopy was used to determine the presence of titanium dioxide.

60. Reflectant foci, indicative of titanium dioxide presence, were found at the base of the Peyer's patches at all dose groups. SEM coupled to energy-dispersive X-ray (EDX) confirmed that the tissue contained subsurface particles rich in titanium. In the low and mid dose groups, weak signals were detected in the impacted cells at the base of the Peyer's patches, whereas higher signals were observed at the highest dose group.

Bettini et al., (2017)

61. The COT noted that there was evidence of significant clearance through organs, including the liver, in the Bettini *et al.* (2017) study which examined the fate of TiO₂ along the gut-liver axis in adult male rats. Firstly, rats (10 per group) were dosed

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daily by gavage with food-grade TiO₂ (E171) or TiO₂ NM-105 (Paragraphs 58 – 59) (10 mg/kg bw/day) for 7 days (the control group was given water). These animals were used for tissue imaging, flow cytometry and cytokine assays and to carry out tissue inflammation and gut permeability measurements.

62. It was found that TiO₂ particles re-agglomerated *in vivo* during transit through the gut, but not after dosing with a subsequent bolus. Following absorption, light-diffracting TiO₂ particles were found along the small intestine, in the colonic (large intestine) mucosa and liver of rats dosed orally with E171 for 7 days, but not in the controls. Using NanoSIMS analyses of subcellular TiO₂ distribution in the immune cells of Peyer's patches after 7 days of oral exposure, Ti was detected in the gut lumen and in the colon mucosa which corresponded to the residual dose of E171. TiO₂ was also found in the liver, with the highest density found close to the portal vein sinus. There was no significant change in epithelial paracellular permeability to ⁵¹Cr-EDTA observed in the E171 group when compared with control animals. This indicates that oral exposure to E171 did not affect gut permeability *in vivo*.

Farrell and Magnuson (2017)

63. A GLP-compliant study of food-grade TiO₂ (anatase) was carried out in rats and performed in accordance with the OECD TG 417 to assess the absorption, distribution and excretion routes in rats after oral exposure to TiO₂. The TiO₂ form of interest from this study was the anatase form (D50 = 133 – 146 nm) and was incorporated into the feed at a dose equivalent to 30 mg/kg bw/day of male and female SD rats (n = 10 per sex per group). This is based on 200 ppm and assumes daily consumption of 30 g diet per day by a 200 g rat. Rats received either TiO₂ or a control diet (no TiO₂ added, background concentration 7 – 9 ppm) for 7 days. Following the 7 days treatment, the TiO₂ was withdrawn from diet and 3 rats per sex were sacrificed at 1, 24 and 72 hours. After the TiO₂ was removed from the diet, the animals were housed individually and those allocated to the 72 hours group were housed in metabolic cages and all excreta including the cage washings were kept. The Ti content of excreta, whole blood, liver, kidneys and muscle was measured using inductively coupled plasma – atomic emission spectrometry (ICP-AES).

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64. TEM micrographs indicated that the anatase TiO₂ exists primarily as aggregated particles up to approximately 1 µm in diameter and approximately 36% of the discrete but aggregated particles are <100 nm in diameter. However, it is not anticipated that these will de-aggregate during the manufacture of pharmaceutical or food products or in the GI tract. Dynamic light scattering technique was also used to assess the aggregation of the anatase TiO₂. The median particle size (d₅₀) was determined as 336 nm. There was no measurable quantity of TiO₂ that had a particle size <100 nm.

65. No adverse effects were found during clinical observations related to the consumption of TiO₂. Liver, kidney and muscle samples were analysed for Ti and 65% were below the LOD. In tissues, Ti levels were below the LOD (0.1 to 0.2 mg/kg wet weight) in most rats at most time points; of a total of 270 tissue samples (90 each from liver, kidney and muscle), 176 (65%) were below the LOD. Sporadic observations of levels above the LOD were in the range of 0.1 to 0.3 mg/kg wet weight, and occurred at similar levels and frequency in TiO₂-exposed rats as those in the control group.

66. The majority of Ti was excreted in feces with concentrations in urine below the limit of quantification (LOQ; < 0.04 mg/L; equivalent to < 2% daily dose/L) for all samples except the 0 to 24 h urine sample for one rat (0.05 mg/L); collection of urine and feces began after withdrawal of the test diet and therefore no mass balance recovery was estimated. Whole blood Ti concentrations were also below the limit of detection (LOD) in both treatment and control groups. The authors conclude that food-grade TiO₂ administered in diet is not appreciably absorbed and distributed in mammalian tissues, and there is no evidence of accumulation in liver, kidney and muscle following repeated oral exposure for 7 d.

LPT (2020)

67. “A second GLP-compliant, multi-site toxicokinetics study of 5 different grades of TiO₂ was carried out in accordance with OECD 417 test guidelines and a summary report of this unpublished study was submitted to Health Canada by industry (EBRC 2022). In this study, male and female CD rats (n not stated, although the test guideline stipulates a minimum of four animals per sex per dose group) received

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either a vehicle control or a single dose of 1,000 mg/kg bw of TiO₂ administered by oral gavage and the total Ti content of whole blood was measured for 96 h post-dosing. The relative oral bioavailability of the various grades of TiO₂ was compared to a soluble Ti reference substance (Titanium(IV) bis(ammonium lactato) dihydroxide solution – 50 wt. % in H₂O) that was administered orally (100 mg/kg bw) or intravenously (10 mg/kg bw). The test articles included a food-grade form of TiO₂ identified as E171-E which had a median particle diameter (SD) of 99.9 ± 2.0 nm and contained approximately 50-51% of constituent particles in the nanoscale (LNE 2020). The other four particles were identified as G6-3 (a rutile TiO₂-NP coated with alumina and hydrophobic organic, D50 = 9.2 ± 2.0 nm), G2-5 (uncoated anatase TiO₂-NP, D50 = 5.5 ± 2.0 nm), G3-1 (uncoated pigmentary rutile TiO₂, D50 = 146.9 ± 5.9 nm) and G4-19 (pigmentary rutile TiO₂ coated with alumina and polyol, D50 = 177.5 ± 3.9 nm). Details of the vehicle and the dispersion protocol were not provided. Blood was collected at 0, 0.5, 1, 2, 4, 8, 12, 24, 48, 72 and 96 h post-dosing and Ti concentrations measured by ICP-MS/MS following microwave-assisted acid digestion of whole-blood samples using H₂SO₄. This method accounts for the bioavailability of particulate TiO₂ as well as any dissolved Ti. Blood Ti concentrations of vehicle-control treated rats were highly variable with several males considered statistical outliers and excluded from data processing. The mean blood Ti concentrations of male and female rats were below 0.2 µg Ti/g blood following oral administration of all test articles. Administration of the soluble Ti reference resulted in blood Ti concentrations up to 90 µg/g blood and 0.9 µg/g blood following i.v. and oral dosing, respectively. The highest blood Ti concentrations following oral dosing were observed in the group that received the food-grade TiO₂ test item E171-E. The resulting areas under the curve (AUC) were plotted for rats exposed to the reference substance orally or by i.v. and compared to the AUC of rats that received E171-E by oral administration. The maximum relative oral bioavailability of E171-E was determined to be 0.0013% (rounded to one significant figure for the purpose of this report). The measured blood Ti levels of the other four forms of TiO₂ were below the LOD after background correction (LOD not stated). The authors concluded that oral bioavailability of all TiO₂ grades tested was close to the LOD of the analytical system. They also stated that most reagents used in the process contain low but measurable background concentrations of Ti, which makes analysis of low levels challenging. In

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addition, the authors reported that the background level of blood Ti in controls rats was highly variable, especially in males, which is consistent with time zero levels measured during dietary studies.” (Health Canada, 2022).

E171 studies in humans

Pele et al., (2015)

68. The test material used was E171, anatase, d50=250nm (EFSA, 2021).

69. Eight healthy volunteers (self-reported) with normal intestinal permeability were given a permeability solution. At 7am, following an overnight fast baseline, urine samples were collected. After consumption of the solution, urine samples were collected for 5 hours.

70. Baseline blood samples were also taken at 9 am. Following that, the subjects received two tablets containing 50 mg of E171 (total dose 100 mg). Blood samples were collected at: 30 minutes, 1-, 1.5-, 2-, 3-, 6-, 8- and 10-hours post E171 ingestion. Of the 8 volunteers, only 7 completed the study as blood could not be withdrawn from the cannula of 1 subject.

71. Dark field microscopy was used to identify titanium dioxide in the blood. Random areas were visualised and the estimation of particles within each field was based on four reflective grades: 0 (<5 particles/field); 1 (5-10 particles/field); 2 (10-20 particles/field); and 3 (>20 particles/field). This analysis was only performed in 5/7 subjects due to blood clotting in two subjects. ICP-MS was used to quantify titanium in the blood for 0 - 10 hours, except in two subjects for which samples could not be collected at 8 hours (2 subjects) and 10 hours (1 subject).

72. Based on the results of the dark field microscopy, it was determined that some of the ingested titanium dioxide was absorbed directly into the blood. A significant increase in positive signals was observed from 2 hours onwards and both dark field microscopy and ICP-MS demonstrated a peak in absorption at 6 hours, reaching up

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to 11 ng/mL and decreasing to around 5 ng/mL by 10 hours post exposure. Only the titanium levels from 6 hours post exposure onwards were significantly different than the baseline. A positive correlation between reflective grades and total titanium levels was observed.

73. The authors hypothesised that two routes of uptake in the gut were involved: one proximal (in the duodenum/jejunum) and one distal (Peyer's patches in ileum). This was because at two hours the uptake was visible in the dark field microscopy and the levels peaked at 6 hours as determined by ICP-MS (i.e., early absorption and late peak).

Guillard et al., (2020)

74. The test material used was: titanium dioxide particles with a mean particle size of 104.9 ± 44.9 nm and a particle size distribution ranging from 20 to 440 nm, with 55% of NPs by number.

75. Human placentae were collected at term from normal pregnancies. Transplacental passage of titanium dioxide was determined using an ex vivo placental transfusion model. The samples were analysed using ICP-MS and scanning transmission electron microscopy (STEM) coupled to EDX spectroscopy for content analysis of titanium and analysis of titanium dioxide particle deposition, respectively.

76. All placental samples (n=22) contained titanium with the total content ranging from 0.01 to 0.48 mg/kg of tissue. STEM-EDX confirmed the presence of titanium and oxygen in the particle deposits seen by TEM, as well as aluminium, silicone, iron, zinc and tin trace elements. Most of the analysed titanium dioxide particles were below 100nm. Size particle analysis of all particles indicated that 50% were below 100nm in diameter.

77. Meconium samples were also collected from nappies. In 50% of the meconium samples (total of 18 samples), titanium was detected (0.02-1.5 mg/kg). TEM-EDX analysis confirmed the presence of titanium and oxygen elements in the particle

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deposits, alongside silicone, aluminium, iron and zinc. Analysis of all particles indicated a diameter of 5-194nm, with 26/33 (80%) in the nano range.

78. In the transplacental passage experiment, of the 7 ex vivo isolated perfused placentae, round shaped or small particle aggregates of titanium dioxide were observed. Titanium dioxide particles were recovered in the syncytiotrophoblast microvilli and had translocated in deeper areas of the placental chorionic mesenchyme surrounding foetal vessels. The particles had a diameter of below 250 nm, with 17 of them in the nano range.

79. The authors concluded that the results indicated the passage of titanium dioxide particles across the human placenta with potential local accumulation during pregnancy, depending on the individual. The findings of the perfused placenta experiment indicated, according to the authors, that the human placental barrier is unable to completely prevent the passage of titanium dioxide from dietary sources and protect the fetus.

80. Based on both experiments (results of perfused placenta study and the titanium levels in the placenta and meconium), the authors noted that there was a need to assess the risk of titanium dioxide nanoparticle exposure in pregnant women and warranted specific attention for oral exposure to the nanosized fraction of the E171 food additive.

Studies used to review the toxicokinetics and absorption of the nanoparticle form of TiO₂

Studies in rats and mice

81. Three studies investigated the kinetics of TiO₂ nanoparticles using intravenous injection (Geraets et al., 2014; Disdier et al., 2015; and Kreyling et al., 2017a), where just under 100% of the administered dose was biodistributed to the organs. However, in their gavage study Kreyling estimated the absorption from the GI tract at 0.6% of the administered dose. Therefore Kreyling et al. (2017b) concluded that the kinetics

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patterns of the i.v. route were very different to those of the oral route and the “i.v. injection appears not to be an adequate surrogate for assessing the biodistribution and potential health effects occurring after oral exposure to TiO₂ nanoparticles”. The focus of this assessment is TiO₂ as a food additive and the route of administration is oral, therefore, these studies using i.v. administration will not be considered further but the study details are included in the Study Summaries in Annex C.

82. Of the remainder of the studies that assessed TiO₂-NP treatment, none were through the diet. Seven studies dosed the animals using gavage (Kreyling et al., 2017b; Geraets et al., 2014; Ammendolia et al., 2017; Chen et al., 2020; Warheit, Boatman and Brown, 2015; Tassinari et al., 2014; and Bettini et al., 2017), the study by Hendrickson et al., 2016 used intragastric administration and the study by Hendrickson et al., 2020 used an isolated intestinal loop technique.

83. The studies that used nanoparticles (not classed as food-grade or E171 by the authors) constituted a range of sizes. The majority of the studies measured individual nanoparticles (engineered nanoparticles such as NM-101 at 5-10 nm up to about 60 nm in one dimension) and the size of agglomerates/aggregates (ranged from about 80 nm up to approximately 2 µm) predominantly using transmission electron microscopy (TEM), scanning electron microscopy (SEM) and dynamic light scattering (DLS) techniques. A few studies also assessed the percentage (by number) of the particles. For example, in Ammendolia et al. (2017) the average diameter of NPs in MilliQ water was 70 – 1,200 nm with a peak at 60 – 90 nm. There was an abundance of 11% at 76 nm and only 13% had dimensions less than 100 nm. In the study by Tassinari et al (2014) TEM showed individual NPs of 20 – 60 nm and analysis using SEM showed 87% of particles were 30 – 900 nm and 48% of those were 100 – 300 nm with an average diameter of size distribution of 284 ± 43 nm. Thirteen percent of particles had dimensions <100 nm whereas the size distribution was dominated by agglomerates up to 1.6 µm in diameter.

84. The study by Kreyling et al (2017b) which looked at the distribution of [⁴⁸V]TiO₂NP in rats after 1 hour and up to 7 days following gavage estimated only approximately 0.6% of the dose was absorbed across the intestinal barrier in the first

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hour after gavage. This fraction was reduced to about 0.05% after 7 days. The rest of the material is excreted in the feces. Other studies agreed that the absorption of TiO₂ across the gut epithelium was very low following dosing via gavage (oral route).

85. Kreyling et al (2017b) and others (Geraets et al., 2014; Ammendolia et al., 2017; Chen et al., 2020; Warheit, Boatman and Brown, 2015; Tassinari et al., 2014; and Bettini et al., 2017) also looked at deposition of TiO₂ NPs in various tissues. The tissues in which measurable levels were detected across all studies include small intestine, liver, spleen, kidneys, lungs, heart and brain. However, the studies used different nanomaterials, different dosing duration and evaluation timepoints, and different methods of analysis.

studies in humans

Heringa et al., (2018)

86. Titanium was measured using high resolution ICP-MS in liver and spleen from 15 deceased human subjects (nine women and six men) who had donated their bodies for research and educational purposes. The LOD of the method was 10 ng/g tissue.

87. TiO₂ particles were detected in 7/15 liver and 13/15 spleen samples. The number-based TiO₂ particle size distributions in liver and spleen were comparable and had a size range of 85–550nm and 85–720nm, respectively. In the tissues, 24% of the TiO₂ particles in the number-based size distribution was < 100 nm, but this fraction may be underestimated considering that the smallest titanium dioxide particle that could be detected with the method used was 85 nm.

88. The particle mass concentration in liver ranged from 0.01 to 0.3 mg titanium/kg tissue. In the spleen, the concentration ranged from 0.01 to 0.4 mg titanium/kg tissue. The average concentration in samples where titanium could be determined was 40 ng/g in the liver and 80 ng/g in the spleen.

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89. Small tissue grains of liver and spleen from two subjects were analysed using SEM-EDX to visualize the titanium dioxide particles. The observed particles were composed of titanium and oxygen and were present as an aggregate or agglomerate, consisting of smaller primary particles of 75–150 nm. Presence of titanium was also confirmed semi-quantitatively by EDX analysis in dry-ashed liver and spleen samples.

Peters et al (2020)

90. Post-mortem human liver, spleen, kidney, jejunum and ileum samples were analyzed from 15 human subjects, 7 male and 8 female, who died at the age of 64–98 years. From these persons, written informed consent was obtained during life that allowed the use of their entire bodies for educational and research purposes.

91. The total titanium concentration in the organs ranged from 0.01 to 2.0 mg titanium/kg tissue with an average value of 0.17 mg titanium/kg tissue and a standard deviation of 0.33 mg/kg. The authors considered that this was an indication of large differences between subjects and organs. The highest concentrations were detected in the jejunum and ileum (average of 0.34 and 0.43 mg titanium/kg respectively), followed by the kidney, spleen and liver (0.08, 0.06 and 0.03 mg titanium/kg respectively).

92. The particle sizes were measured by spICP-MS and ranged between 50 and 500 nm in the different tissues (50 nm was the lower size detection limit). The titanium dioxide particle concentrations were considered by the authors to represent about 80% of the total titanium concentration.

EFSA review and conclusions on ADME of TiO₂

93. “On the basis of the data available, the EFSA ANS Panel concluded that the absorption and oral bioavailability of titanium dioxide was low, independent of size. EFSA noted that the toxicokinetics of E 171 was assessed in three mouse studies and two human studies. The studies by Comera et al., (2020) and Talamini et al.,

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(2019) allowed the derivation of estimates of internal exposure of 0.01 and 0.1% of the external dose, respectively.” (EFSA, 2021).

94. “However, these estimates are based on Ti concentrations measured in a limited number of organs. Although it is uncertain to what extent TiO₂ distributes to other organs, the Panel’s estimates have always included the Ti amount in the liver, which accounted for about 12.5% of the Ti amount in the body (Kreyling et al., 2017b). The underestimation in body burden and absorption is therefore unlikely to be more than 5-fold.” (EFSA, 2021).

95. “The Panel noted that in mice, TiO₂ can be taken up from the small intestine by the paracellular pathway and by endocytosis. Furthermore, in two of the studies (Comera et al., 2020; Riedle et al., 2020), uptake of TiO₂ particles was demonstrated into M-cells of the Peyer’s patches, whereby the quantitative contribution to the systemic exposure seems to be low. In humans, the Panel considered that after oral administration of 100 mg E 171, Ti concentration in blood increased ca. 5- to 10-fold from 6 to 10 h post-dosing (Pele et al., 2015), demonstrating some oral systemic availability. TiO₂ particles were found in human placenta in low concentrations (Guillard et al., 2020), indicating that TiO₂ is systemically available after ingestion and also can distribute to the placenta. In an ex vivo human placenta model, particles were transferred and the size distribution of the particles was similar to the E 171 present in the perfusate. The Panel noted that the extent of transfer across placental membranes was small. The Panel noted that materials other than E 171, mainly TiO₂ NPs, were investigated in rats and humans. In rats, two intravenous studies (Disdier et al., 2015; Kreyling et al., 2017a) demonstrated long half-lives and, hence the potential for accumulation. Together with data from an intravenous study (Geraets et al. (2014) already addressed in the EFSA opinion on the re-evaluation of E 171 (EFSA ANS Panel, 2016), half-lives of 83 days (for liver) and of 450 days (for whole body) were estimated and accumulation factors between 135 and 450. Based on these data, the steady state would be reached between 1.5 and 5 years. Out of five oral rat studies, one provided an estimate for oral systemic availability of 0.0002% based on a limited number of organs (Hendrickson et al., 2016) and another study provided an estimate of 0.6% (Kreyling

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et al., 2017b). The Panel noted that in a study employing the model of isolated loop technique, the authors could provide data indicating the presence of TiO₂ NPs either as single particles or as smaller and larger agglomerates in intestine, liver and spleen (Hendrickson et al., 2020). The other studies did not give data suitable to quantify absorption and/or accumulation. The Panel considered that in two studies analysing tissues from deceased subjects, deposition of Ti-containing nanoparticles was observed in liver, spleen and kidney as well as in intestine. After quantification of the Ti amount in the organs and comparison with the estimated mean daily intake of E 171, the Panel concluded that the oral systemic availability of TiO₂ NP ingested from a number of sources, including dietary exposure to E 171, would be low (less than 1% by mass).” (EFSA, 2021).

96. “In summary, the Panel considered that E 171 has a low oral systemic availability, probably not greater than 0.5%. It may pass the placenta and may be transferred to the fetus. Furthermore, the Panel considered that rat studies with TiO₂ NPs, consisting of nanoparticles with primary particle sizes between 7 and 90 nm, showed long half-lives (roughly 200–450 days), a potential for accumulation (accumulation factor of 290 to 450) and long time to reach steady state (3–5 years) (Geraets et al., 2014; Disdier et al., 2015). The oral systemic availability of these materials was low (most probably < 1%) but higher than for E 171. In tissues from deceased subjects, TiO₂ particles were identified in liver and spleen, the low Ti amount of the investigated organs indicating low oral systemic availability of TiO₂ ingested from a number of sources, including dietary exposure to E 171.” (EFSA, 2021).

Health Canada review and conclusions

97. Engineered TiO₂-NPs are often used as surrogates in toxicity tests to represent the fraction of particles in the nanoscale in food-grade TiO₂. However, unlike food-grade TiO₂, these particles have a distribution wholly in the nanoscale, generally have a substantial fraction of particles.

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98. Evidence of very low and size-dependent oral absorption of TiO₂ particles in rodents and humans that may occur primarily via the GALT, with the absorbed material mainly being retained in the intestines, liver, spleen, and kidneys (Bettini et al. 2017; Coméra et al. 2020; Farrell and Magnuson 2017; Heringa et al. 2018; Hummel et al. 2014; Peters et al. 2020; Riedle et al. 2020; Talamini et al. 2019; EBRC 2022). Only one GLP- and OECD guideline-compliant toxicokinetics study with food-grade TiO₂ was identified in the literature (Farrell and Magnuson 2017). In this study, repeated exposure to food-grade TiO₂ in the diet at concentrations of 200 ppm (equivalent to 30 mg/kg bw/d) for 7 days resulted in no appreciable absorption or distribution to tissues or organs and no evidence of accumulation in the liver, kidney, or muscle of male or female rats. A second unpublished multi-site toxicokinetics study conducted according to OECD and GLP guidelines was submitted to Health Canada by industry (EBRC 2022). In this study, the maximum relative bioavailability of 5 different TiO₂ grades was approximately 0.001% following a single oral dose of 1000 mg/kg bw in CD rats.

99. Taken together, evidence from rodent and human studies indicates very low and size-dependent oral absorption of TiO₂ particles may occur primarily via the GALT, with the absorbed material mainly being retained in the intestines, liver and spleen (Winkler et al. 2018; Heringa et al. 2018).

100. It should be noted that the majority of studies that investigated the toxicokinetics of TiO₂-NPs in rodents administered particles by oral gavage. Gavage studies conducted with insoluble particles have several issues that may complicate interpretation of the results, including disruption of the gastric mucus layer, which may enhance systemic bioavailability of the administered dose. In addition, concentrated bolus doses used in gavage may produce artifactual changes in particle size distribution which would not be reflective of how humans are exposed through the diet; for example, higher bolus doses of TiO₂ may lead to greater agglomeration of particles and paradoxically lower exposure to primary particles. The lack of exposure to a food matrix prior to ingestion may also potentially affect toxicokinetic properties.

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FSANZ review and conclusions

101. In a previous TiO₂ review, FSANZ had noted that in humans “the presence of inorganic microparticles containing TiO₂ and other sub-micron particles in basal ‘pigmented cells’ of Peyer’s patches is a normal occurrence throughout Western populations. These particle-containing pigmented cells are mainly mature macrophages that appear to be of low metabolic and immunological activity, with no evidence for differential phenotype or activation” (FSANZ, 2022).

102. Studies submitted to FSANZ with food grade TiO₂ indicated that the relative oral bioavailability in rats is ≤0.0013%. They noted that the majority of the material that is absorbed is retained in the Peyer’s patches of the intestine. There is some distribution to the liver, spleen and kidneys. Data from human cadavers shows relatively low bioavailability and with the age of the subjects the TiO₂ would be expected to have reached steady state. FSANZ concluded that, considering the animal and human study data, the absorption of TiO₂ following oral exposure is very limited. (FSANZ, 2022).

COT review and conclusions

103. It should be noted that various chemical forms of TiO₂ were used in the different studies and can be used as the food additive. Therefore, the COT considered that the size and shape of TiO₂ particles in test materials can affect absorption and particle agglomeration. The modes of delivery varied, with some studies administering TiO₂ in water instead of via complex food groups (Bettini *et al.*, 2017; Karimpour *et al.*, 2018; Khorsandi *et al.*, 2016; and Lee *et al.*, 2019). The COT raised concerns that TiO₂ may not fully dissolve in water which could affect absorption, however the effect remains unclear. The EOGRT study did not provide details of the form of the material in the organs or which was taken up by the cells, which increases uncertainty of toxicity.

104. The COT further noted concerns around absorption, especially relating to nanoparticles/nanofractions as these were variable in the test substances and hence

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varied in absorption in the studies described above. The COT could not separate the impact of the TiO₂ form and matrix of administration to conclude on the observed variability in absorption.

105. The COT noted that TiO₂ particles can cross the placenta. However, because absorption is low, this would be a very small number. It was unclear whether this was by passive diffusion or active uptake and, what form the TiO₂ was in by the time it got to these barriers. Once intracellular, materials can change in response to different pH and be transferred elsewhere.

106. Several studies stated the method by which the TiO₂ was dosed. The EOGRT and Blevins *et al.* studies were dietary, Bettini *et al.* was in drinking water (via gavage). In some studies where the TiO₂ was prepared in solution, included added stabilisers to force deagglomeration, while some studies had long sonication steps which can cause structural changes to TiO₂.

107. The COT opinion with regard to absorption, was that there was no reason to believe that titanium dioxide particles behaved differently to other particles in the gastrointestinal tract. It was also observed that the percentage of absorption was reported to be higher in the EFSA 2021 Opinion, based on the same dataset considered previously. Members were advised that newer studies used in the previous evaluation were re-considered (indications that titanium dioxide could cross the placenta). The duration of the animal studies was not sufficient to evaluate at which levels steady state would be reached and therefore it was considered that absorption had previously been underestimated. Finally, the EOGRT study provided indirect evidence for systemic exposure following administration of titanium dioxide.

108. The COT noted that the Heringa *et al.* (2018) study would have assessed lifetime accumulation of TiO₂. The study does not comment on the sources of the TiO₂ but is likely to be an overestimation of the quantities that can be accumulated over a lifetime from food.

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109. Overall, the COT concluded that the form of TiO₂ may affect the likelihood and quantity of the TiO₂ material crossing barriers into organs. Additionally, absorption might be affected due to the presence of nanosized particles. The wide variance of test materials used in each study between nano, micro and a mixture in sizes of TiO₂ was noted. Due to the large variance in test materials (form and size) as well as the potential impact of the matrix of administration to the absorption of TiO₂, the Committee could not ascribe a percentage for the absorption of TiO₂. However, the Committee considered that absorption is low, based on the available evidence.

Review of toxicity for endpoints identified by the COT

110. The following endpoints were reviewed initially by the COT and then in more detail by a sub-group of COT Members: Aberrant crypt foci (as a potential marker for carcinogenicity), genotoxicity, inflammation and immunotoxicity, allergenicity, reproductive and developmental toxicity and neurotoxicity. The sub-group's reviews are discussed in detail in each of these sections for the endpoints.

111. Following a call for data by EFSA to address several data gaps for TiO₂, an extended one generation reproductive toxicity (EOGRT) study was conducted (LPT, 2020). Because the data from this study covered aberrant crypt foci, reproductive and developmental toxicity, immunotoxicity and neurotoxicity, the study methodology is summarised in the following paragraphs and the results discussed in the relevant endpoint sections.

Summary of the EOGRT study (LPT, 2020)

Methodology

112. The Extended One Generation Reproductive Toxicity (EOGRT) study was commissioned by interested business operators to address the data gaps identified in the EFSA 2016 Opinion. The protocol was later amended to include a satellite group per dose at the F0 generation to accommodate the investigation of additional parameters related to the occurrence and titanium dioxide-related induction of aberrant crypt foci (ACF) in the colon (preneoplastic lesions that had been reported

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by Bettini et al. (2017)). The study was carried out according to OECD TG 443 and good laboratory practice (GLP) compliance.

113. The test material: Titanium dioxide E171-E, Particle size (ECD); (number measurement, primary particle size) x10 = 0.070 µm x50 = 0.110 µm x90 = 0.180 µm via the diet. The doses used were: Group 1: 0 mg/kg b.w./day; Group 2: 100 mg/kg b.w./day; Group 3: 300 mg/kg b.w./day; 4: 1000 mg/kg b.w./day. Twenty male and 20 female rats from each dose group were evaluated. The concentration of the test item in the diet was adjusted based on the mean group food consumption per sex. The concentration was adjusted weekly using the food consumption values from the previous week.

114. The test item was administered at doses of 0, 100, 300 and 1,000 mg/kg bw/day to several groups of males and females from 10 weeks prior to mating, during mating and until weaning of the F1 and F2 generations. The F1 generation was dosed in the same way as the F0 generation after weaning. Until weaning, the exposure of the F1 Pups to the test item was indirectly through the breast milk, however the pups additionally received the test item directly when commencing feeding by themselves during the last week of the lactation period. The duration of dosing depended on the requested endpoints for the different cohorts of the F1 generation. Cohort 1B animals were maintained on treatment beyond PND 90 and bred to obtain an F2 generation. Detailed examination of key developmental endpoints, such as offspring viability, neonatal health, developmental status at birth, and physical and functional development until adulthood, was performed to identify specific target organs in the offspring. Possible endocrine disruptor effects of the test item were also examined.

115. Table 3 describes the parameters considered in the EOGRT study with the corresponding generation, cohort and number of animals assessed for each.

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Table 3. Parameters considered in the EOGRT study

Generation	F0	F0 satellite	F1 pups	F1 1A	F1 1B	F1 2A	F1 2B	F1 3	F2
Number of animals /sex per group	20	30		20	20	10	10	10	
Mortality	X		X	X	X	X		X	
Clinical signs	X			X	X	X		X	
Body weight	X		X	X	X	X		X	X
Food consumption	X			X	X	X		X	
Water consumption	X				X				
Haematology	X			X					
Clinical biochemistry	X			X					
Lymphocyte typing (spleen)				X					
Urinalysis	X			X					
Sexual hormone levels	X			X	X	X	X		
Thyroid hormone levels	X		X	X					
Sexual maturation				X	X	X		X	
Oestrous cycle data				X					
Sperm parameters	X			X					

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Necropsy	X		X	X	X	X		X	X
Histopathology	X			X		X	X		
Reproductive parameters	X		X		X				
Pre-postnatal development			X		X				X
Functional neurotoxicity observations						X			
Neurohistopathology						X	X		
Lymphocyte typing (spleen) after KLH immunisation				X				X	
Anti-KLG IgM levels after KLH immunisation								X	
Aberrant crypt foci (ACF) scoring		X							

EOGRT: extended one-generation reproductive toxicity; KLH: keyhole limpet haemocyanin; IgM: immunoglobulin M.

This table is taken from EFSA (2021).

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Results

Evaluation of Sexual Function and Fertility

Male fertility

116. No statistically significant or dose-related effects on sperm motility, total spermatids/gram testis, percentage of abnormal spermatozoa and male mating index were observed in the F0 generation. The slight decrease in the number of successful matings at doses of 300 and 1,000 mg/kg bw per day appears unrelated to the male partners, as all males that failed to impregnate their females showed normal sperm motility and sperm counts. Only one of the high-dose males was found to have a lower testicular spermatid content (50% of the group mean), a finding that was also associated with a slightly lower testis weight (85% of the group mean). The number of abnormal sperm was low in all dose groups and remained below 2% in the few males in which abnormal sperm were found.

Female fertility

117. No effects on mean oestrus cycle duration were noted in F0 and F1 (cohort 1B) parental generations and all F0 females in the control, 100, 300 and 1,000 mg/kg bw per day groups mated. In the F1 generation 2 and 3 animals from the mid- and the high-dose groups, respectively, were erroneously removed from the study, before mating had been unequivocally confirmed. All other females mated, except one F1 female in the 100 mg/kg bw per day group. With few exceptions, mating occurred at the first oestrus after the females were housed with males. No effects of treatment were observed. The pregnancy rate was slightly lower in the F0 generation at 300 and 1,000 mg/kg bw per day (100, 96, 92 and 92%). This finding was not confirmed in the F1 generation (100, 95, 94 and 100%).

118. No effects were noted on pregnancy duration, number of implantation sites and post-implantation loss. Although they occurred in the mid- and high-dose groups, three single total litter losses, either from total resorption of all embryos or from death of the litter during or shortly before birth, were not considered to be due to treatment.

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This is because the two F0 dams had unusually small litters of two pups each, which were stillborn, and the F1 dam showed total resorptions of eight implants at necropsy after failing to litter. Live litter sizes and litter weights were comparable to control values in all dose groups in the F0 and the F1 generation.

Developmental Toxicity

119. Pre- and postnatal lethality and structural abnormalities: No treatment-related pre- or postnatal loss was observed in the F0 and F1 generations. The average litter size at birth in all dose groups was comparable or higher than in the control group and the sex ratio was unaffected. No external or internal abnormalities were detected in F1 and F2 pups at termination.

120. Growth and sexual development: No treatment-related effects were observed in birth weights and growth of the pups. There were no indications for any androgenic and/or oestrogenic effects on the male and female anogenital distance (AGD) and the retention of nipples in males.

121. The mean age at vaginal opening was comparable between control and treated groups. The statistically significant lower body weight on the day of vaginal opening in cohort 1A at 300 mg/kg bw per day was not considered to be biologically relevant due to the slightly higher litter sizes in all treated groups. A divergence from the required method was examination of balanopreputial gland cleavage instead of examining balanopreputial separation which does not comply with the OECD TG 443 and therefore cannot be considered a measure of puberty in males.

122. Neurofunctional screening: Male and female F1 cohort 2A offspring were tested for auditory startle response between PND 23 and 25, and for a functional observation battery including grip strength evaluation and for quantitative locomotor activity between PND 58 and 64. No differences in the response to an auditory startle stimulus were observed between the control and all the tested doses. Compared to controls, an increase in hindlimb splay was observed in females, reaching statistical significance at 100 and 1,000 mg/kg bw per day. A statistically significant increase in

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mean forelimb grip strength was noted at 300 mg/kg bw per day in both males and females.

Developmental Immunotoxicity

123. Effects on developmental immunotoxicity were determined in the F1 cohort 3 animals through an examination of their ability to raise an antibody response to a foreign antigen. Animals were sensitised and the primary IgM antibody response to the sensitising antigen, in this case to keyhole limpet haemocyanin (KLH) antigen, was measured. The ability of the test compound to modulate serum anti-KLH antibody titre was taken as indicative of a developmental immunotoxic effect. A KLH-immunised control group also exposed to a known immunosuppressant (i.e., cyclophosphamide (CY)), resulting in at least 50% inhibition in serum IgM anti-KLH titre, was considered crucial for the verification of assay performance.

124. These data can be considered in combination with additional data related to potential immunotoxic effects. In the F1 cohort 1A animals, the following may also contribute to the general assessment for immunotoxicity: weight and histopathology of the spleen, thymus and lymph nodes, as well as bone marrow histopathology, total and differential peripheral WBC count and splenic lymphocyte subpopulation distribution and the T-cell-dependent anti-KLH response (KLH assay). Determination of serum anti KLH-IgM antibodies was performed in F1 cohort 3 (10/sex per group, PND 53–61) using an enzyme-linked immunosorbent assay (ELISA).

125. The animals were sacrificed 5 days after intravenous bolus injection (tail vein) of KLH, blood was withdrawn and the level of anti-KLH IgM was measured in serum. In addition, satellite animals of F1 (10/sex, PND 55) were immunised with KLH and treated with CY (single administration of 40 mg/kg bw by gavage on the same day of KLH treatment) to provide a positive control (for an inhibition of immune response).

126. A slight, but statistically significant decrease in the antigen specific IgM level was measured at the highest dose tested (1,000 mg/kg bw per day) in males only (–9%) and without an apparent dose response. In addition, the Panel noted that

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treatment with CY was not performed at the same time as the rest of F1 cohort 3, without a separate control for the CY response, conducted at the same time.

127. The sensitivity of the test was not demonstrated due to invalid CY positive control results. It was noted that the assay conditions may have not been optimal resulting in an apparent low antibody response to KLH when compared to literature (Gore et al., 2004).

128. It was considered that all tested animals in the study had a weak immunogenic response to KLH that was insufficient to identify a T-cell-dependent immunotoxic effect of E171 therefore no conclusion can be drawn on the effect of E171 on the developing immune system.

129. Assessment of pathology, haematology and splenic lymphocyte subpopulations at necropsy, pathology of lymphoid organs, haematology and lymphocyte subpopulations in the spleen were investigated. The following lymphocyte subpopulations were determined via flow cytometry analysis (FACS): T cells, T helper cells, T suppressor/cytotoxic cells, NK cells and B cells.

130. For the splenic lymphocyte subpopulation analysis, no statistically significant differences were observed in the percentage of T cells, T helper cells, T suppressor/cytotoxic cells, NK cells and B cells of any of the treated groups compared to control in both sexes. The study authors concluded that no test substance-related effect was observed on the proportion of the examined lymphocyte subtypes.

131. Compared to the animals of F1 cohort 1A, F1 cohort, 3 animals showed a shift in the lymphocyte subpopulation that indicated activation of the immune system by injection of KLH and concluded that increased B-cell proliferation may have led to the production of antigen-specific antibodies. In the F1 cohort 3 animals, no differences in the relative size of the lymphocyte subpopulations were observed between the control group and the E171-treated groups, after immunisation of the animals with KLH.

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132. The proposed reason was that the B-cell shift in F1 cohort 3 was caused by KLH immunisation, supported by the fact that there was no such shift found for the positive control animals that were sensitised to KLH and treated with CY. It was also considered that KLH induced an immune reaction, and that this response was influenced by CY as expected; KLH would increase the percentage of splenic B cells and decrease the percentage of T cells.

133. Some findings regarding immunotoxicity and inflammation with E171 as well as neurotoxicity with TiO₂ Nanoparticles may be indicative of adverse effects including indications of an induction of ACF with E171.

Developmental Neurotoxicity

134. Male and female offspring were tested for auditory startle response between PND 23 and 25, including grip strength evaluation and for quantitative locomotor activity between PND 58 and 64. No differences in the response to an auditory startle stimulus were observed and an increase in hindlimb splay was observed in females, reaching statistical significance at 100 and 1,000 mg/kg bw per day. A statistically significant increase in mean forelimb grip strength was noted at 300 mg/kg bw per day in both males and females.

135. Grip strength and hindlimb splay belong to the same domain of neurological function, however, the increase in hindlimb splay and increase in mean forelimb grip strength are opposed in this case - increases in hindlimb splay indicate muscular weakness but an increase in mean forelimb grip strength may indicate myotonia. No dose response was observed for any of these endpoints or for the two functional measurements, indicating that the likelihood of an association with test substance is low. No other changes were observed including in histopathological findings in brain or in peripheral nerve tissue.

Aberrant Crypt Foci

136. A satellite group of the EOGRT study used doses up to 1,000 mg/kg bw per day and up to this dose did not induce ACF in the colon.

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Aberrant Crypt Foci Examination in Satellite F0 Animals (EOGRT Study).

137. Evaluation of ACF in the colon of a satellite group of F0 animals (10/sex per group) treated with 0, 100, 300 and 1,000 mg E171/kg bw per day and terminated after weaning was undertaken. The colon was excised, opened longitudinally and the contents removed by rinsing with a 0.9% NaCl solution. Thereafter, the tissue was divided in parts of a suitable size for fixation by immersion in 5% buffered formalin. A blind examination of these samples stained with 0.5% (w/v) methylene blue in water was performed under a stereomicroscope at 50x magnification for presence of ACF. The Panel noted that the design of the study did not include a positive control group (e.g., treatment with a known gastrointestinal tract tumour initiator such as dimethyl hydrazine (DMH) for the development of ACF.

138. The definition of ACF used was 'foci containing more than 2 aberrant crypts (ABCs), taken from Shwter et al. (2016). No ACF were found in the colons of the control and the treated groups. A mildly increased morphological variability (increased size and intensity of the staining of a small portion) of the crypts in the two caudal parts of colon was observed in seven animals (See tables 4 & 5 below). These changes were assessed as inconsistent with the appearance and definition of ACF discussed above. Incidence of these single crypts observed in the mid and high doses was not significantly different from the control. The EFSA Panel agreed with this conclusion.

Table 4: Aberrant Crypt Foci Presence in Satellite F0 Animals.

Aberrant Crypt Foci Present

Dosage Group	Control	Low-Dose	Mid-Dose	High-Dose
Females	1/10	0/10	1/10	2/10
Males	1/10	0/10	1/10	1/10

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139. An additional submission of data included photomicrographs of mildly increased variability in crypt morphology from all seven animals. A re-examination was extended to an additional randomly selected nine control animals (4 males and 5 females) and eight high-dose group animals (3 males and 5 females). A mild increased variability in crypt morphology was observed in eight of the nine controls and six of the eight high-dose animals (see Table x).

Table 5: Aberrant Crypt Foci Presence in the Re-Examination of Satellite F0 Animals.
Aberrant Crypt Foci Present

Dosage Group	Control	High-Dose
Females	4/5	3/3
Males	4/4	3/5

EFSA review and conclusions

140. The EOGRT study with E171 did not indicate adverse effects up to a dose of 1,000 mg/kg bw per day. Also, no effects were seen in studies retrieved from the literature with TiO₂ NP < 30 nm up to the highest dose tested of 100 mg/kg bw per day.

141. EFSA Opinion of the EOGRT Study: The Panel considered that there was no systematic bias in group testing order and that this was therefore not a plausible explanation for the observed group differences. Grip strength and hindlimb splay belong to the same domain of neurological function, i.e. motor function and/or sensory–motor coordination. However, the effects observed (i.e. increase in hindlimbs play and increase in mean forelimb grip strength) seem to point in opposite directions when it comes to muscle strength. In particular, an increase in hindlimb splay can be interpreted as muscular weakness whereas an increase in mean forelimb grip strength could be indicative of myotonia. The Panel noted that the effects observed were not correlated to any other changes (e.g. alterations in muscle tone, righting reflex, gait, wire manoeuvre, posture). No dose response was observed

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for any of these endpoints or for the two functional measurements, indicating that the likelihood of an association with test substance is low. No other changes in the functional observation battery measurements or locomotor activity were noted. There were no notable histopathological findings in brain or in peripheral sciatic nerve. Based on all the above considerations, the Panel considered that the effects on grip strength and hindlimb splay were not treatment-related. However, the Panel noted that quantitative information on peripheral nerves was not available. Overall, the Panel considered that E171 at these doses had no adverse effects on neurofunctional endpoints in F1 cohort 2A offspring. EFSA conclusions on developmental toxicity results of the EOGRT study: No effects of E171 on pre- and postnatal development were observed. Data on the attainment of puberty in males (i.e. an appropriate assessment of the timing of the balanopreputial separation) were missing. The Panel did not consider this to be critical in this case.

142. The conclusion was that the immune response was affected by CY but was not adversely affected by the TiO₂ test substance. The Panel did not agree with the conclusion of the study authors that a shift to B cells by KLH was substantiated. The Panel considered that it is incorrect to compare the groups of F1 cohort 1A and of F1 cohort 3 because the groups of animals of F1 cohort 3 had a different age than that of the animals in F1 cohort 1A at the time of sacrifice (PND 87–96 vs. PND 53–61, respectively). In addition, the FACS analyses on the splenic cell suspensions were not all performed in the same round of analysis but were performed separately, while it is known that this may have influenced staining and subsequent quantification. The authors suggested that even if the positive CY control did not perform as expected, the data still indicate there is no effect of E171 on sensitisation to KLH.

143. It is worth noting that the EFSA Panel did not agree with this conclusion and overall considered that the data did not allow to conclude on developmental immunotoxicity with respect to E171.

COT review and conclusions

144. The Committee agreed that the EOGRT (Extended One Generation

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Reproductive Toxicology) study was very detailed and well conducted. There was no evidence of reproductive or developmental toxicity. An extensive range of endpoints had been considered in the study and it was noted that there was some small evidence of focal effects on the testes and sperm abnormalities in the sperm production in one treated rat, however, these changes were not statistically different to the control group.

145. Overall, it was agreed that there was no evidence that titanium dioxide caused reproductive or developmental toxicity at the doses tested.

Aberrant Crypt Foci (ACF) as a potential biomarker for carcinogenicity

146. ACF are a potential biomarker for colorectal cancer. Genetic changes in the malignant transformation process of colorectal mucosa lead to either inactivation or activation of specific target genes. A number of biomarkers associated with genetic changes have been identified for early detection of colorectal cancer including identification of ACF as an early pre-invasive lesion and its relationship to the development of cancer (Cheng and Lai, 2003). The number of ACF were found to be significantly higher in patients with colon cancer compared to those without, but have been found in healthy patients (Health Canada, 2022). However, most ACF do not appear to develop into neoplasms and there is evidence to suggest that dysplastic changes in ACF are a better predictor of cancer (Clapper et al 2020). Most preclinical studies do not distinguish between hyperplastic and dysplastic changes in ACF.

Studies using the E171 form of TiO₂ (in mice)

147. With regards to the effects of TiO₂, ACF were evaluated in the studies by Bettini *et al.*, 2017, Blevins *et al.*, 2019 and the EOGRT study (TDMA, 2020). These are described below. Additional studies have also been evaluated to assess evidence for proliferative changes in the colon.

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EOGRT study (LPT, 2020)

148. Details of this study are given in paragraphs 113 – 115 and 136 – 139, but the overall conclusion based on data from this study was that oral exposure to E171 at doses up to 1,000 mg/kg bw per day did not induce ACF in the colon. In addition, no evidence of morphological changes in the colon were identified during routine microscopic histopathological examination of tissues from this study.

Blevins et al., (2019)

149. The Blevins *et al.* (2019) study used test material E171, anatase, 110 – 115 nm (SEM), 36% of the particles had a particle size <100 nm. Internal exposure was not examined. Six-week-old male Wistar Han IGS rats were exposed to E171 in a standard diet at 4 concentrations between 0 - 5,000 mg/kg diet and a control in two studies each of 7 days (n = 5/group) (equal to 1.8, 4.8, 31.4 and 374 mg/kg bw per day) and one study of 100 days (n = 15/group) and (equal to 1.3, 3.5, 22.4 and 267 mg/kg bw per day). Prior to exposure to E171, animals in groups 1 - 4 were treated with one intraperitoneal injection of a sterile dose of 180 mg/kg bw dimethylhydrazine dihydrochloride (DMH) (an inducer of GI tract tumours) in 1.5% EDTA-0.9% NaCl, pH 6.5. Animals in groups 5 - 8 were treated with a single sterile dose of 1.5% EDTA-0.9% NaCl, pH 6.5 without DMH.

150. In the 100-day study no treatment related histopathological changes were found in the duodenum, jejunum, ileum, spleen, liver, lung and testes in animals exposed only to E171. Rats that were initiated with DMH only and those which received E171 in the diet after the initiation displayed several histopathological abnormalities. There were two invasive adenocarcinomas in the large intestine in one animal in the 1.3 mg E171/kg bw per day + DMH group, and single adenomas in the large intestines in one animal in the 3.5 mg E171/kg bw per day + DMH group and in one animal in the 22.4 mg E171/kg bw per day + DMH group.

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151. There were no other histopathological changes in the large intestines of the other animals treated with DMH. One rat in the 1.3 mg E171/kg bw per day + DMH group and one rat in the 22.4 mg E171/kg bw per day + DMH group had subpleural lymphocytes in the lung, but without any evidence of acute inflammatory changes or hyperplasia.

152. The 100-day study also showed that there was, as expected, a significant increase in ACF/cm² and ABC/cm² in animals pre-treated with 180 mg/kg bw DMH compared to the non-pre-treated groups. E171 doses administered after DMH did not result in statistically significant increases in ACF or ABC. Of note was that ACF and ABC were also seen at low levels in control animals. Overall, no effects of E171 on histopathologic evaluations of ACF were noted although the authors did include a caveat that much of the epithelial surface of the colon samples (proximal, middle and distal) were obscured when observed by light microscopy limiting examination of the entire surface of the colon samples. The Committee noted that Blevins *et al.* (2019) pointed out the limitations of their study, however, authors of other studies did not report on any limitations in their studies.

Bettini et al., (2017)

153. In the Bettini study, in addition to the 7-day studies described in paragraphs 58 and 59, a second group of rats (n = 11 to 12 per group) were treated (or not) with 1,2-dimethylhydrazine (DMH) to induce colon carcinogenesis and were exposed daily to E171 at 200 µg or 10 mg/kg bw/day through drinking water for 100 days. Control animals (n = 12) received water only. Rats were used for flow cytometry and cytokine assays and for gut inflammation and ACF assessments.

154. DMH and TiO₂ treatment at 10 mg/kg bw/day for 100 days significantly increased the total number of aberrant crypts and large ACF per colon when compared with both control animals and the 200 µg/kg bw/day group. However no overall significant difference between the number of ACF per colon between groups of rodents was observed. The authors did not explicitly give their definition

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of an ACF, however, the authors defined a 'large ACF' as consisting of more than three aberrant crypts per ACF.

155. Spontaneous development of ACF was also observed in 4 rats (from a total of 11) in the E171 group without a colon carcinogenesis initiator. Three or fewer ABCs were observed per ACF in three of these 4 positive samples and one which contained 12 ABCs.

156. Growth-promoting effects on colonic preneoplastic lesions were investigated for TiO₂ differentially affecting the viability of normal or preneoplastic cells. This was investigated using the comparative cytotoxicity of food-grade TiO₂ particles on non-mutated and pre neoplastic cells (Apc^{+/+} and Apc Min/+). Results showed that 24-hour exposure to TiO₂ was more cytotoxic to Apc^{+/+} than Apc Min/+ cells.

Additional studies examining evidence of histopathological proliferation in the colon

157. No microscopic evidence of proliferative changes in the intestine have been seen in other chronic toxicity studies with TiO₂ including Warheit, Brown and Donner, Agaki et al and the NCI Unitane ® carcinogenicity 1979 study. The Agaki study was not included in the EFSA review as it was published after the EFSA Opinion, in 2023. The EFSA review also did not include the NCI 1979 study because, although the EFSA ANS Panel did conclude that the study indicated that TiO₂ was not carcinogenic in rats and mice, the Panel scored the study of low reliability as they considered that the analytical information on the test item was insufficient. However, the COT notes that the Titanium Dioxide Manufacturers Association (TDMA) has more recently completed work that shows that the test item used in the NCI 1979 study is comparable to the specifications of E171 today, and so the study should be considered reliable.

Studies using E171 or equivalent form of TiO₂

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Han (2020)

158. The 90-day oral repeated dose toxicity study of E171 was carried out in male and female SD rats in a study conducted according to OECD TG 408 and to GLP. Five-week-old male and female Sprague-Dawley rats (50 male and 50 female). Ten rats per sex and per dosage group were randomly assigned to one of four groups (0, 10, 100, or 1,000 mg/kg dosage of E171) administered daily by oral gavage for 13 consecutive weeks (90 days). The control group was provided with the equivalent volume of water. AGS cells (70 - 80 % of confluence) were incubated with E171 (0, 10, 20, 40 µg/mL) for 24 h. Colon sections and AGS cells (human gut epithelial cell line) were fixed, sectioned and images were produced for analysis using a TEM.

159. There were no dose-related changes in the endpoints. Effects observed included E171 deeply penetrating cells lining the stomach tissues at the maximum dosage (1,000 mg/kg), and significantly lower blood IgM (male and female) and GM-CSF (female) levels in the E171-treated animals than in control animals. Colonic superoxide dismutase (SOD)-1 protein levels decreased in males and females and SOD-2 protein levels decreased in females with increasing Titanium accumulation.

160. E171 (40 µg/mL) accumulated in the perinuclear region following exposure of AGS cells for 24 hours. The E171 treatment affected expression of ER stress-related proteins but did not induce cell death up to 40 µg/mL (the maximum dosage in this study).

161. The authors concluded a NOAEL for E171 of less than 1,000 mg/kg for both male and female rats based on the 90-day study and proposed that further chronic toxicity studies be conducted due to the potential for E171 to reduce a host's immune defence function by decreasing antioxidant capacity.

NCI (1979)

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162. The Unitane ® 0-220 study (NCI, 1979), a compound which was previously manufactured in the US for use as food-grade TiO₂, was considered by the sub-group due to recent work by the TDMA which demonstrated that the test item tested in this study was comparable to the updated specification of E171. EFSA scored this study low on reliability and discounted the study from their evaluation as they considered that the analytical data on the test item was not sufficient. A 13-week study of male and female B6C3F1 mice and Fischer 344 rats (n = 10 per sex per group) were fed doses of Unitane ® 0-220 (TiO₂, anatase, 98% purity). A further cohort (n = 50 per sex per group) were fed Unitane ® 0-220 via the diet for 103 weeks at concentrations equivalent to 0, 3,250 or 6,500 mg/kg bw/day and 0, 4,175 or 8,350 mg TiO₂/kg bw/day for male and female mice (respectively) and doses of 0, 1,125 or 2,250 mg/kg bw/day and 0, 1,450 or 2,900 mg/kg bw/day for male and female rats (respectively) and then observed for 1 additional week. Fifty untreated rats of each sex and 50 untreated mice of each sex were used as controls. All remaining rodents were euthanised at 104 weeks. No evidence was observed (in either species) of lesions in the gastrointestinal tract e.g., proliferative non-neoplastic or neoplastic findings. It was also noted following examination of the pathology data that nematodes were recorded in the intestine which introduces an additional potential for GI inflammation which could potentially induce inflammatory and proliferative changes, however none were observed in the study.

Warheit, Brown and Donner (2015)

163. Warheit, Brown and Donner (2015) summarised 3 OECD-type studies of TiO₂ in rats of varying particle sizes and surface coatings. Study 1 was a subchronic 90-day study (OECD TG 408) in which adult male and female rats were fed rutile, surface-coated pigment-grade TiO₂ test particles (d₅₀ ¼ 145 nm, 21% nanoparticles) by oral gavage for 90 days with a NOAEL of 1000 mg/kg bw/day. Study 2 was a 28-day repeated-dose oral toxicity study (OECD TG 407) in adult male rats fed two rutile-type, uncoated, pigment-grade TiO₂ test particles (d₅₀ ¼ 173nm) by oral gavage at a dose of 24,000 mg/kg bw/day with no test item-related effect. Study 3 was an acute oral toxicity study (OECD TG 425) with

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female rats were fed surface-treated rutile/anatase nanoscale TiO₂ particles (d₅₀ ¼ 73 nm) up to 5,000 mg/kg. The oral LD₅₀ for the test substance was >5,000 mg/kg bw. Collectively, no test item-related adverse effects were found including during microscopic examination of colon.

Studies using the nanoparticle form of TiO₂

Akagi et al., (2023)

164. In the Akagi *et al.*, 2023 study, TiO₂ NPs (6 nm) were examined in male and female rats by repeated oral administration of 10, 100, and 1,000 mg/kg bw/day for 28 days (5 individuals per sex per dosage group) and of 100, 300, and 1,000 mg/kg bw/day for 90 days (10 individuals per sex per group). The authors reported that no mortality was observed in either study length with no treatment-related effects observed in body weight, urinalysis, hematology, serum biochemistry or organ weight. Histopathological examination of the 28-day study specimens found TiO₂ particle deposition in the gastro-intestinal lumen, nasal cavity, epithelium and stromal tissue as well as in the 90-day study in Peyer's patches in the ileum, cervical lymph nodes, mediastinal lymph nodes, bronchus-associated lymphoid tissue, and trachea. Despite this, no inflammation or tissue damage were observed. Little evidence was found of TiO₂ absorption or accumulation was found in the liver, kidneys, and spleen. Immunohistochemical analysis showed no extension in colonic crypts of the proliferative cell zone or preneoplastic cytoplasmic/nuclear translocation of β -catenin up to 1000 mg/kg bw/day in males or females. With regard to genotoxic endpoints, no observed increase in micro-nucleated or γ -H2AX positive hepatocytes was found. No effects were observed after repeated oral administration of TiO₂ (6 nm particle size) up to 1,000 mg/kg bw/day.

EFSA review and conclusions

165. The EFSA Panel (2021) noted a considerable variability in the results, which may mask possible effects. The Panel considered that the effect of E171 in

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producing ACF reported by Bettini et al. (2017) was not replicated in later investigations (EOGRT study and Blevins et al., 2019). One source of uncertainty was that it was noted that there were methodological limitations in Blevins et al. A further source of uncertainty is being unclear to what extent animals were exposed to TiO₂ Nanoparticles in both the EOGRT study and Blevins et al. The Panel concluded that E171 may induce ACF in male rats at a dose of 10 mg/kg bw per day when the test substance is pre-dispersed and stabilised in a liquid medium preventing agglomeration of nanoparticles prior to administration by gavage.

Health Canada review and conclusions

166. As mentioned in paragraph 14, the Health Canada State of the Science report on titanium dioxide had previously been reviewed by the Committee and its conclusions endorsed. The Committee sub-group agreed that the report was comprehensive and included further discussions with pathologists from the Blevins *et al.* study to evaluate the colon findings.

167. Papers included in the Health Canada report which discussed effects on the gut microbiota were reviewed by the sub-group. These include Lamas, Martins Breyner and Houdeau 2020.

168. Papers included in the Health Canada report which discussed carcinogenicity and chronic toxicity endpoints were reviewed by the sub-group. These include Bettini et al. 2017, Blevins et al. 2019, EOGRT study (TDMA, 2020), and Han et al. 2020. The Health Canada report concluded that while there were some uncertainties identified which may require further studies, they did not identify consistent evidence of preneoplastic lesions in the colons of rodents exposed to food-grade TiO₂ via the oral route. A single non-guideline study in which rats were exposed to food-grade TiO₂ dispersed in drinking water at doses of ~10 mg/kg bw/d for 100 days (Bettini et al., 2017) resulted in formation of ACF, but these results were not replicated in subsequent dietary studies when higher doses were administered, up to ~236 - 300 mg/kg bw/day for 100 days in a non-guideline study (Blevins et al., 2019) and up to

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1000 mg/kg bw/day for ~18 - 19 weeks in a GLP- and OECD guideline-compliant EOGRT study (TDMA, 2020). An OECD guideline-compliant study that administered food-grade TiO₂ dispersed in water to rats via oral gavage at doses up to 1,000 mg/kg bw/day for 90 days showed no histopathological changes in the gastrointestinal tract (GIT) (or any other tissues) (Han et al., 2020). The report concluded that there was no evidence of, and therefore that there was a low level of concern for, carcinogenicity, chronic toxicity, or other non-neoplastic lesions of the GIT. A weight of evidence approach did not identify data gaps of such significance to require a precautionary approach currently.

169. In the final summary the authors concluded that "Health Canada Food Directorate's position is that there is no conclusive scientific evidence that the food additive TiO₂ is a concern for human health".

FSANZ review and conclusions

170. FSANZ noted that there were no chronic toxicity or carcinogenicity studies available in the literature which used oral administration of food-grade TiO₂. The NCI 1979 Unitane study was previously considered suitable for assessing the carcinogenicity of food-grade TiO₂ however FSANZ noted that the EFSA 2021 opinion on TiO₂ raised questions regarding the suitability of use of Unitane 0-220 for this purpose.

171. FSANZ noted EFSA's concerns from the 2021 opinion around the risk of induction for ACF in the colon identified in Bettini et al. (2017). FSANZ' previous assessment of the Bettini et al. (2017) study concluded that this study had limited relevance in humans due to design limitations including the use of sonicated test item delivered via drinking water and its applicability to the use of TiO₂ as a food additive. This concern was raised by ANSES in 2017 in which it was concluded that the Bettini et al. (2017) study results could not be used for risk assessment without a confirmatory study which included dietary administration of the test item.

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It was also noted that in their 2018 opinion on TiO₂, EFSA also agreed that these data “were not sufficient to raise a concern on the potential initiation or promotion properties of TiO₂ on colon carcinogenesis”.

172. FSANZ noted that while Bettini et al. (2017) noted evidence suggesting increased incidence of ACF related to oral exposure to TiO₂, two additional studies using sonicated food-grade TiO₂ administered to rats via by gavage for up to 90 days (Blevins et al. 2019 and the unpublished EORGT Study (LPT 2020)) found no evidence of treatment-related histopathological changes in the gastrointestinal tract up to doses of 1000 mg/kg bw/day. In addition, FSANZ also noted that the NCI 2-year bioassay results “found no evidence of carcinogenicity of a test item comparable to food-grade TiO₂ in the colon or other tissues in rats and mice at dietary concentrations up to 50,000 ppm, equivalent to doses of 2250 – 2900 and 6500 – 8350 mg/kg bw/day, respectively”, a finding which was also inconsistent with the Bettini et al. (2017) results.

173. FSANZ concluded that dietary administration of the food-grade test item were considered most relevant to human exposure to TiO₂ in foods and beverages and that the different modes of exposure may explain the difference in results between these studies. Given the increased weighting given to these studies, FSANZ stated that “dietary exposure to food-grade TiO₂ is unlikely to induce pre-neoplastic or neoplastic lesions in the colon or other tissues”.

COT review and conclusions

174. The Committee questioned whether studies in which TiO₂ was administered by methods other than through the diet represented consumption of food. In one study that assessed ACF (Bettini *et al.*, 2017) the test item was administered via water whereas the EOGRT study and Blevins *et al.*, 2019 were dietary. Studies with TiO₂ prepared in solution had bovine serum albumin (BSA) added to provide protein with sonication for up to 1 hour which can affect the material. This may not

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be representative of the exposure in humans via use in food, due to the sonication affecting the structure of TiO₂.

175. The results from the Bettini study where ACF were identified in rats pre-treated with DMH and in a small subset of animals with TiO₂ alone, exposed to food-grade TiO₂ dispersed in drinking water at doses of ~10 mg/kg bw/d for 100 days, were not replicated in the Blevins or EOGRT dietary studies. In addition, there was no evidence of further proliferative changes in the colon mucosa in these studies or other studies where histopathology of the colon was performed. The committee concluded that there was no conclusive evidence that TiO₂ induced ACF and no evidence to support progression to proliferative lesions in the colon.

176. The Committee considered that although small numbers of ACF were observed (Bettini *et al.*, 2017) in some animals exposed to TiO₂ alone, these could not necessarily be attributed to TiO₂, as ACF were also present in control groups without exposure to TiO₂ in other studies (Blevins et al, EOGRT). Additionally, none of the studies distinguished between hyperplastic or dysplastic ACF in any groups of control or treated animals. The Committee also concluded that there was very little evidence that the effects of TiO₂ were systemic at the doses tested but also that TiO₂ measurements were not accurate in the analysis of TiO₂ in the tissues. One explanation for why the NOAEL is 1,000 mg/kg bw/day, the highest dose tested, is because the absorption is low.

Genotoxicity

177. An overview of the genotoxicity studies evaluated is included in the COM statement and supporting documents published on their website. A link to COM narratives which detail the studies that they have assessed will be included once published.

178. Add in brief overview of COM methodology – RAG ratings to understand the COM conclusions. To be included once COM documents have been finalised.

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EFSA review and conclusions

179. EFSA considered that several in vitro studies demonstrated that TiO₂ NPs can induce gene mutations in cultured mammalian cells. Five in vivo studies were considered, one indicated the induction of large DNA deletions, however the remaining four studies, that could identify point mutations and small deletions, gave consistently negative results. They concluded that the available experimental data do not confirm the potential of TiO₂ NPs (< 30 nm) to induce gene mutations in vivo.

180. Taking into account the available evidence, EFSA considered that - on balance - TiO₂ NPs have the potential to induce micronuclei (MN)/chromosomal aberrations (CA). They noted “that a significant portion of the studies was performed using TiO₂ NPs < 30 nm, however some positive results were observed with TiO₂ particles > 30 nm and no clear dependence of the particle size on positive effects in MN/CA assay was observed” (EFSA, 2021).

181. EFSA concluded that based on the results of the in vitro and in vivo comet assays, TiO₂ particles have the potential to induce DNA damage. As noted for MN and CA effects, “a significant portion of the studies were performed using TiO₂ NPs < 30 nm, however some positive results were also observed with TiO₂ particles > 30 nm and no clear dependence of the particle size on positive effects in Comet assay was observed” (EFSA, 2021).

182. EFSA also concluded that there is evidence, from both in vitro and in vivo studies, for interaction(s) of TiO₂ NPs with DNA. However, due to the techniques employed, it was not possible to determine whether these interactions involved covalent or non-covalent binding.

Health Canada review and conclusions

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183. Health Canada's Food Directorate notes that, based on the currently available studies including the EOGRT study (LPT 2020), food grade TiO₂ is not genotoxic in-vivo. However, they recognise that the studies available on this endpoint are limited and additional OECD guideline-compliant studies are recommended to confirm this. They conclude that any adverse effects found related to oral exposure were based on the findings from non-standard studies in which the form of TiO₂ administered homogenized suspensions of particles with ultrasonic dispersion and that these do not fully represent exposure to food grade TiO₂.

FSANZ review and conclusions

184. No in-vivo genotoxicity studies were found that used food-grade TiO₂ with dietary administration were found. FSANZ identified four in-vivo genotoxicity studies, two studies using orally-administered food-grade TiO₂ (Bettini et al., 2017 and Jensen et al., 2019) and two studies using intraperitoneal administration of Unitane (0-220) (Shelby et al, 1993 and Shelby and Witt, 1995). These latter two studies assessing genotoxicity by the NCI/NTP were identified by the US National Toxicology Program and were assumed to use Unitane 0-220 as the test item was not described (Shelby et al, 1993 and Shelby and Witt, 1995)

185. FSANZ noted that DNA damage was not observed either in the two comet assays (food-grade TiO₂, oral gavage) or the micronucleus and chromosomal aberration studies (Unitane 0-220, intraperitoneal injection). In-vitro studies (GLP- and OECD test guideline-compliant, food-grade TiO₂) found no evidence of mutagenicity in mammalian cells nor clastogenicity or aneugenicity or cellular uptake (micronucleus assay using human peripheral blood lymphocytes). There was some observation of uptake and internalisation by A549 cells however no particles were detected in the nucleus. FSANZ noted that "the absence of confirmed cellular uptake in the in vitro genotoxicity studies may limit confidence in the negative results, although alternatively the absence of direct exposure of the nucleus to food-grade TiO₂ in these studies may indicate a low intrinsic hazard from a direct genotoxicity perspective (OECD 2014)".

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COM review and conclusions

186. The COM have provided conclusions on the in vitro and the in vivo studies separately and are presented below.

COM Opinion of the in vitro genotoxicity studies reviewed

187. “After reviewing the in vitro genotoxicity studies performed to date on TiO₂, we note the following points:

- i. There were four in vitro studies of the highest quality (labelled “green” here) that used TiO₂ nanoparticles of different sizes and forms in the micronucleus assay. Only one study tested micro-sized (anatase)TiO₂ that was more representative of E171 (Demir et al. 2015) which was negative in the micronucleus assay. All four “green” studies that used anatase TiO₂ nanoparticles reported negative results for the MN endpoint. Of the two green studies that used rutile TiO₂ nanoparticles, one was negative and the other was weakly positive for MN induction in a non-standard cell line but only at the two lowest doses used (1 and 5 mg/ml) (Di Bucchianico et al 2017). Two green studies used TiO₂ nanoparticles of mixed anatase/rutile form and both were negative for MN induction.
- ii. There were two green studies that both used anatase/rutile TiO₂ nanoparticles in either the hprt gene mutation assay or CA assay. The TiO₂ nanoparticles were negative in the hprt assay. In the CA assay, the TiO₂ nanoparticles were positive, but the CA frequency decreased with increasing TiO₂ concentration, and despite the significant induction of CA, this study was negative with the micronucleus assay.
- iii. There were eight amber studies (i.e., ones that contained some suboptimal aspects) that used TiO₂ nanoparticles of different sizes and forms in the micronucleus assay. Four studies used anatase TiO₂ nanoparticles and three of these were negative for micronuclei induction. The one positive study reported a dose-dependent increase in micronuclei induction in lymphocytes from healthy

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individuals. All three studies that used nanoparticles of mixed anatase/rutile TiO₂ were negative for micronuclei induction. Two studies that used anatase/brookite TiO₂ nanoparticles reported positive results for micronuclei induction.

- iv. The one amber study on hprt mutations was positive at low anatase TiO₂ nanoparticle doses but not at higher doses (Vital et al. 2022).
- v. Some “green” studies included other assays (e.g. Comet assay) to provide mechanistic information but results were inconsistent, showing either no increase (Demir et al., 2015), or an increase in oxidative DNA damage (Di Bucchianico et al., 2017) but only at the highest dose (Unal et al., 2021). Andreoli et al., 2018 and Stoccoro et al., 2017 showed ROS involvement.

188. Overall, the COM opinion is that there is little evidence that TiO₂ micro-sized or nanoparticles are genotoxic in vitro based on data from well conducted studies. The limited number of positive studies all report no dose-response effects, with significant effects being observed at the lowest doses used, although it is acknowledged this may be due to differences in dispersion and agglomeration at low and high doses. There is also a lack of replication of study outcomes using the same nanoparticle in different labs.

189. Currently a definitive assessment of the safety of food grade E171 is difficult when there are no high-quality OECD-compliant studies that adequately incorporate the study design considerations and characterisation of the nanoparticulate fraction present in E171. With the exception of one study, the studies identified in this report are not representative of E171, where the fraction of nanoparticulate is <50% and according to the recent "Guidance on the implementation of the Commission Recommendation 2022/C 229/01 on the definition of nanomaterial" (<https://data.europa.eu/doi/10.2760/143118>), E171 would not fall under the definition of a NM, hence we need GLP studies with E171 that also include robust physicochemical characterisation and nano-specific adaptations to the TG protocol to definitively assess the hazard. Nanoparticles of TiO₂ are considered worst-case scenario for E171, as E171 is anticipated to be less reactive.

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190. We also note that there is a dearth of high-quality datasets available with well documented nanomaterial characteristics where the relevant OECD test guidelines (using suitably adapted protocol designs for the testing of nanomaterials) have been followed.” (COM, 2024a)

COM Opinion of the in vivo genotoxicity studies reviewed

191. “After reviewing the *in vivo* genotoxicity studies performed to date (up to 2023) on TiO₂, we note the following points:

- i. The highest quality *in vivo* studies labelled here as “green” (n=2), both show negative results for the micronucleus endpoint (Donner et al., 2016; Sadiq et al., 2012). There were no “green” studies for other endpoints.
- ii. Only Donner et al., (2016) used pigment grade TiO₂ (including micro-sized anatase that was most similar to E171) and therefore was most relevant to the concern for human health in this case. This study showed no micronucleus induction.
- iii. The Donner et al., (2016) paper also used a physiologically relevant oral route, which is most appropriate for the assessment of dietary exposure of food grade TiO₂. The authors acknowledge that absorption from the GI tract is low, meaning poor bone marrow exposure. This is important for risk assessment purposes where the oral bioavailability of E171 in humans is very low (≤0.0013% - refer to COT opinion/)
- iv. The Sadiq et al., (2012) study, that used an i.v. route (a route that is most likely to achieve bone marrow exposure), also showed a negative micronucleus response and confirmed bone marrow exposure to titanium.
- v. The studies labelled as “amber” (i.e., contained some suboptimal aspects) showed a mixture of positive (4/9) and negative (5/9) results for the genotoxicity endpoints studied.
- vi. The positive studies included chromosomal and DNA damage endpoints and were all associated with cytotoxicity and/or indirect mechanisms of

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genotoxicity, such as oxidative damage and inflammation. There was no evidence of gene mutations, however no definitive conclusion can be made due to the deficiencies in the study designs and limited number of available studies.

vii. The route of administration of nano-sized TiO₂ in these “amber” studies was often not via the most relevant oral route (only 2/9 studies) when considering the use of E171 as a food grade material. The less relevant endotracheal route was employed in 3/9 studies and the i.v. route and i.p. route were employed in 3/9 and 1/9 studies, respectively. Often the dosing regimens employed in these studies were suboptimal and did not follow the recommendations of the OECD test guidelines, which also makes interpretation difficult.

viii. All these “amber” studies used a nano-sized TiO₂ material which is less relevant to the E171 material.

192. Overall, we conclude that there is little evidence in the literature to suggest that there is a health concern related to genotoxicity induction by TiO₂, particularly via the oral route and especially the micro sized TiO₂ fraction (most studies used the nano-sized material).

193. Currently a definitive assessment of the safety of food grade E171 is difficult when there are no high-quality OECD-compliant studies that adequately incorporate the study design considerations and characterisation of the nanoparticulate fraction present in E171. We also note that there is a dearth of high-quality data sets that are OECD compliant and this has led to a lot of conflicting data and uncertainty in the risk assessment for TiO₂.” (COM, 2024b).

COT review and conclusions

194. The COT agree with the COM conclusions.

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Inflammation and Immunotoxicity

Studies using E171 or equivalent form of TiO₂

LPT (2020)

195. The immunotoxicology results of the EOGRT (LPT, 2020) are described in the EOGRT summary section (paragraphs 123 – 133).

Riedle et al., (2020)

196. Riedle *et al.*, (2020) dosed C57BL/6 mice with food grade (E171) TiO₂ at doses of 0, 6.25, 62.5, or 625 mg TiO₂/kg of diet (equivalent exposures of 0 and ≈1, 10, and 100 mg TiO₂/kg body weight per day for 6, 12 or 18 weeks, at which point the GI tracts were harvested and Peyer's patches assessed. This study showed that these diets deliver the TiO₂ particles to the basal cells of intestinal lymphoid follicles. It also showed that up to 18 weeks of exposure, food intake, weight gain, and Peyer's patch immune cell profiles do not differ between TiO₂-fed groups or controls.

Blevins et al., (2019)

197. To assess gut immunopathology in the Blevins *et al.*, (2019) study as described in paragraphs 149 - 152, the number of CD103+ dendritic cells (DC) in the periphery and in Peyer's patches was quantified. CD103+ DC routinely make up less than 1% of the total leukocyte population in peripheral blood and spleen, and these percentages were not affected by the E171-containing diet following exposure to TiO₂ for seven or one hundred days. However, the frequency of CD103+ DC increased modestly in the peripheral blood and spleen of animals in the 100-day study compared to the 7-day study. Pre-treatment with DMH had no effect on CD103+ DC content, with or without E171 consumption. When combining these results, there was no change in the percentage of CD103+ DC in peripheral blood, spleen or Peyer's patches due to acute or chronic dietary E171 consumption.

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Talamini et al., (2019)

198. Talamini et al., (2019) carried out a study with repeated oral administration to mice of an E171 suspension by an oral drip. Mice were divided into 2 groups of 22 animals/group and dosed with water (vehicle) or E171 suspension (no sonication or deagglomeration) at 5 mg/kg bw over 3 weeks dosed for 3 days per week (receiving an average daily dose of ~ 2 mg/kg bw/day). This study focussed on the GI tract as it had previously been suggested that this was one of the main targets of E171-induced biological effects. Deposition of TiO₂ was found in internal organs including the intestine and liver and in the digestive tract as discussed in paragraph 50. Neither overt structural and morphological histological alterations or significant recruitment of monocytes/macrophages were observed in the stomach and whole intestine of E171 fed animals. Liver effects included necro-inflammatory foci with tissue macrophages recruitment. Additional effects were seen in the stomach and intestine including increased superoxide production, compared to controls. There was no difference in the circulating levels of IL-1 β and TNF- α concentrations in the intestine with E171 dosing. However, concentrations of IL-6 and SDF-1 were increased 2.2- and 3.1-fold, respectively. Gene expression analysis of pro-inflammatory cytokines showed a significant upregulation of IL-1 β in the stomach (65%) and gut (74%) and a non-significant increase in the liver, TNF- α levels were not modified in the stomach and liver but were reduced (60%) in the whole intestine, where a decrease in ICAM-1 was also observed. Expression levels of COX-2 were not changed in any of the tissues, ICAM-1 showed no change in the stomach and SDF-1 in the liver. Tissue expression levels of anti-inflammatory cytokine IL-10 was only significantly modified in the liver with a 40% reduction. (Talamini *et al.*, 2019).

Pinget et al., (2020)

199. Pinget *et al.*, (2020) investigated the impact of food grade TiO₂ (E171) dosed via drinking water on gut microbiota of mice. Five- to six-week-old male C57BL/6JAusb mice were allowed access to feed and water ad libitum. The drinking water contained 0 or 2, 10, or 50 mg/kg bw/day sonicated TiO₂ E171 for

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3 weeks. TiO₂ had a low impact on the microbial composition in the small intestine/colon. However, TiO₂ exposure *in vivo* altered the release of bacterial metabolites and *in vitro* encouraged biofilm formation and altered the spatial distribution of commensal bacteria. Reduced expression of the colonic mucin 2 gene was found for 10 and 50 mg/kg bw/day, which the authors suggested has a detrimental impact on the mucus layer. However, no change was observed in the expression of *Tjp1* which indicated that there was no impact of TiO₂ on gut permeability. Another major mechanism of bacterial exclusion is through the release of antimicrobial peptides. Defb3 (encoding for beta-defensin-3) was elevated at 10 and 50 mg/kg bw/day. However expressions of other antimicrobial peptides such as granzyme B, cathelin-related antimicrobial peptide (CRAMP), regenerating islet-derived protein 3 gamma (REG3 gamma) and p-lysozyme (PLYz) were unchanged. Colonic neutrophil and dendritic cell populations were unchanged; however, the numbers of macrophages, CD8+ T cells and Th17 cells were increased by TiO₂ at 10 and 50 mg/kg bw/day. The levels of IL-6, TNF- α , IFN- γ , IL-17A and IL-10 were upregulated in the colon of TiO₂ treated mice. A significant decrease in crypt length was also observed in the colon. Conversely, neither regulatory T cells (Treg) nor TGF-beta were affected by TiO₂ treatment.

Bettini et al., (2017)

200. Bettini et al. dosed rats with E171 TiO₂, TiO₂ NPs (NM-105) or water via gavage for 7 days (paragraph 58 - 59) or through their drinking water for 100 days (paragraphs 153 - 156). In a third series of experiments, untreated rats (n= 4) were used for ex vivo cytotoxicity and proliferative assays on isolated immune cells.

201. After 7 days of oral exposure, both NM-105 and E171 induced a significant increase in the dendritic cell frequency in Peyer's Patches without affecting the spleen at the systemic level. These early effects on the dendritic cells in the Peyer's Patches were found to be transient, as they were not detected in rats exposed for 100 days through drinking water. NM-105 nanomaterial had no effect on Treg cells in the Peyer's patches after 7 days of oral exposure. However, E171 led to a significant decrease in this cell subset. This effect was still observed in Peyer's patches after 100 days of exposure. The authors noted that decreased

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levels of Tregs appeared along with a decrease in CD4+ CD25+ T helper (Th) cells, which the authors suggest indicates a failure of Th cell expansion. No changes were detected in the content of basal cytokines TNF- α , IFN- γ , and IL-17 in the mucosa of the small and large intestine compared to control rats, after 7 days of TiO₂ dosing.

Han et al., (2020)

202. “The 90-day oral repeated dose toxicity study of E171 was also investigated in male and female SD rats in a study conducted according to OECD TG 408 (Han et al. 2020). Animals (10 per sex per group) received E171 by oral gavage at doses of 0, 10, 100 or 1000 mg/kg bw/d for 90 d. Particles were dispersed in distilled water by at least 10 minutes of sonication and dose formulations were prepared at least once per week, with homogeneity determined by sampling from the top, middle and bottom of all preparations (Dr. Seokjoo Yoon, pers. comm. 09 September 2021). No mortality or effects on body weight, clinical chemistry, urinalysis, organ weights or gross or histopathological endpoints were observed. Gene profile analysis indicated that changes in immune-response associated microRNAs were associated with E171 exposure. A slight but statistically significant decrease in relative lymphocyte count (~8%) was observed in high and low dose males and alterations in granulocyte-macrophage colony-stimulating factor (GM-CSF) in females and plasma IgM (both sexes) were observed at the highest dose tested, but given the lack of a doseresponse and the natural variability in these parameters, it is unclear if these changes can be considered adverse; notably, the EFSA FAF Panel (2021a) considered the highest dose tested in this study to be the NOAEL.” (HC, 2020)

Urrutia-Ortega et al., (2016) (Study summary to be deleted)

203. A study by Urrutia-Ortega et al. (2016) had also been reviewed by the EFSA ANS Panel in 2016, the EFSA FAF Panel in 2021 and Health Canada (2022) and due to the nature of the study, a carcinogen-induced colitis-associated cancer (CAC) model, it was deemed not suitable for use in the risk assessment of TiO₂

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as a food additive. The COT agreed with these conclusions and this study will therefore not be included in this assessment.

Mortensen et al., (2021)

204. “The effects of oral exposure to food-grade TiO₂ on inflammation and immunity in the GIT of male and female rat pups was investigated by Mortensen et al. (2021). Lactating Sprague Dawley rats with standardized litters of five male and five female pups at PND 2-3 were acclimated for 4-5 days prior to dosing. Between PND 7-10, pups received a daily dose of either 10 mg/kg bw/d of E171 (purchased from Pronto Foods Co., Chicago, IL, USA) dispersed in water or an equivalent volume of water by oral gavage (three litters per dose group for a total of 15 male and 15 female pups per group). Particles were dispersed by ultrasonication in deionized water and DLS was used to measure the hydrodynamic diameter every 2- 5 minutes until the change in diameter was less than 5% (the critical delivered energy was determined to be 1690 J/ml); dosing solutions were prepared fresh each day. One male and one female from each group was sacrificed 4 h after the fourth and last dose on PND 10 and the duodenum, jejunum, ileum, and colon were harvested for histopathology. The remaining pups were sacrificed on PND 21 and the liver and brain were collected. The study also included a three-phase in vitro digestion model to investigate the stability of E171 during simulated digestion. DLS measurements of E171 showed increased hydrodynamic diameter and polydispersity index during simulated digestion, which was confirmed by SEM. Enhanced darkfield microscopy with hyperspectral imaging (EDM-HSI) was used to evaluate intestinal uptake of particles. The majority of particles were present in the lumen, although some particles were also detected in the gastric mucus and a smaller number still in the underlying epithelial tissue; the percentage of E171 in all areas of intestinal tissues was higher in female pups than in male pups. There was no increase in liver Ti concentration based on ICP-MS. Histopathological analyses included an evaluation of changes in the number of intraepithelial lymphocytes (IEL) and granulocytes in the duodenum and colon. Following E171 administration, a

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significant increase in the number of IEL was observed in the duodenum but not the colon in both sexes, while the number of granulocytes increased in both the duodenum and the colon. No sign of active inflammation was observed. The authors concluded that oral exposure to E171 well dispersed in water leads to the recruitment of immune cells in young rats, with the strongest effect observed in the small intestine, although whether this early life exposure is associated with long-term effects on intestinal homeostasis is unknown.” (HC, 2022).

Warheit, Brown and Donner, (2015)

205. Warheit, Brown and Donner (2015) reported the outcomes of two repeated-dose studies of TiO₂ in rats as described in paragraph 165. In the 90-day study, TiO₂ particles were detected within the digestive tract, draining lymph tissues and the nose, although there was no evidence of an adverse tissue response. In the 28-day study TiO₂ particles were observed in intestinal lymphoid tissue. However, no treatment-related adverse effects on any endpoints were observed.

Duan et al., (2021)

206. “Duan et al. (2021) exposed male ICR mice (n = 6 per group) to two different forms of TiO₂ via the diet for 1, 3 or 6 months. The particles are described as “food-grade” and were incorporated into a commercial pelleted diet at a concentration of 1% by mass; control animals received the basal diet (Ti content not reported). Based on JECFA conversion factors (JECFA 2000), a concentration of 1% w/w in food is equivalent to a dose of 1500 mg/kg bw/d in mice. The first particle type was a nano-sized anatase form with an average primary particle diameter of 38.3 ± 9.3 nm. The second particle type was a micro-sized rutile form with an average primary particle diameter of 128.0 ± 33.4 nm and both particle types were reported to be nearly spherical in shape based on TEM imaging. After receiving control or TiO₂-mixed feed for one, three or six months, animals were euthanized and blood and organs (liver, spleen, and kidney) were collected for histological and elemental analyses. Ti content in blood was higher in animals fed a TiO₂-containing diet for 6 months than those fed for 1

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or 3 months, although the Ti content of organs was not significantly different from controls. The authors evaluated the influence of TiO₂ exposure on the homeostasis of 22 trace elements in blood, liver, kidney and spleen (17 of which were above the LOD). The results are reported as ratios of these elements relative to controls and a number of minor alterations were observed that are of unclear toxicological significance. Moreover, the number of statistical comparisons was large (4 tissues x 17 elements x 3 dosing conditions x 3 time points = 612 comparisons) and the authors do not appear to have controlled for the familywise error rate (i.e., Bonferroni correction or similar); with an alpha level for significance of 0.05, the approximately 18 statistically significant observations reported does not exceed that which would be expected based on chance alone. Histopathological examination revealed that after six months of feeding, mice exposed to the micro-sized particles showed occasional protein casts in kidney canaliculus but no significant effects in liver or spleen. Mice exposed to the nanoform showed some liver pathology, described as punctate cell necrosis and infiltration of mononuclear lymphocytes, as well as macrophages engulfing particle aggregates in the spleen, the latter of which could be viewed as an adaptive response. It should be noted that the equivalent dose on a mass basis would result in far greater exposure on a particle number basis to those animals receiving the nanoform. As only one dose was tested, no indications of a dose-response could be gleaned from this study.” (HC, 2022).

Studies using the nanoparticle form of TiO₂

Huang et al., (2017)

207. Huang *et al.*, (2017) treated Raw264.7 and J774a.1 cell lines and primary mouse macrophages with TiO₂ NPs with primary diameters of 10 nm (NP10) or 50 nm (NP50) at concentrations of 0.1, 1, or 10 µg/mL and incubated for 48 h. C57BL/6 wild type and C57BL/6 TLR4-knockout (TLR4^{-/-}) were fed standard laboratory diet or diet containing 0.1% TiO₂ NP10 or NP50 for 1 month. Experiments for macrophage chemotaxis, phagocytosis and bactericidal activity, nitric oxide production and mouse cytokine analysis were carried out in both the

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macrophage cell lines and macrophages from the mice. A lipopolysaccharide (LPS) septic shock model and cecal ligation and puncture procedure was performed on the mice.

208. After a month of dietary exposure to TiO₂ NPs in mice no changes in bodyweight were observed. NP10 and NP50 TiO₂ led to an elevation in the levels of pro-inflammatory IL-1 β , IL-6, IL-12a, TNF- α , and nitric oxide synthase-2 (Nos2). Conversely, anti-inflammatory factors IL-4 and IL-10 were reduced in groups exposed to TiO₂ NPs. These mice showed an M1-like pro-inflammatory activation state. Mice exposed to NP10 or NP50 showed a reduction in TG4-induced macrophages compared to the control group. The data also suggest that the phagocytic activity of the macrophages exposed to TiO₂ NPs was inhibited. These findings indicate that exposure to TiO₂ NPs could disrupt immune homeostasis and aggravate the inflammatory responses to an external stimulus such as LPS. After a month of dietary exposure to low doses of TiO₂ NPs and following LPS challenge, an aggravated septic shock occurred which caused increased levels of inflammatory cytokines in serum and reduced overall survival. The authors stated that “These results demonstrate that TiO₂ NPs induce an abnormal state of macrophages characterized by excessive inflammation and suppressed innate immune function in a TLR4-dependent manner, which may suggest a potential health risk, particularly for those with additional complications, such as bacterial infections”. Huang et al., (2017).

209. For exposure *in vitro* to TiO₂ NPs (10 and 50 nm), the expressions of pro-inflammatory genes in macrophages were increased, and the expressions of anti-inflammatory genes were decreased. In addition, for macrophages exposed to TiO₂ NPs *in vitro* and *in vivo*, their chemotactic, phagocytic, and bactericidal activities were lower. In mice, after a month of dietary exposure to low doses of TiO₂ NPs, an aggravated septic shock occurred in response to lipopolysaccharide challenge, leading to elevated levels of inflammatory cytokines in serum and reduced overall survival. Moreover, TLR4-deficient mice and primary macrophages, or TLR4-independent stimuli, showed less response to TiO₂ NPs.

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Kampfer et al., (2021)

210. “Kampfer and colleagues (2021) also exposed male and female mice C57BL6/J to TiO₂-NPs via the diet in order to evaluate effects on intestinal tissue. The particles were P25 (a mixed phase catalytic material consisting of spherical particles ~85% anatase/15% rutile, average primary diameter 21 nm) and were incorporated into pelleted diet at either 0.2% or 1% TiO₂ by mass, corresponding to an estimated dose of 400 or 2000 mg/kg bw/d. Mice (n = 10 per sex per group) were exposed to a control diet (basal Ti content not stated) or one of the two TiO₂-enriched diets for 28 days. Following exposure, intestinal tissue was harvested and analyzed for DNA damage via the alkaline comet assay in freshly isolated colonocytes as well as for markers of inflammation and gene expression analysis. No increase in DNA damage was observed in animals exposed to TiO₂-NPs, there was no enhancement in proinflammatory cytokines and none of the investigated genes related to DNA repair, oxidative stress or inflammation were differentially expressed. The authors concluded there is an absence of major local adverse effects in the intestines of mice following repeated exposure to TiO₂-NPs via the diet. They note that incorporating TiO₂-NPs in the feed allows for a highly realistic manner of exposure that mimics human exposure via the diet, although the results may not be consistent with studies that employ bolus dosing by gavage.” (HC, 2022).

Gao et al., (2020)

211. Gao and colleagues (2020) exposed male Sprague Dawley rats (n = 10 per group) to two types of uncoated anatase TiO₂, a nano-sized form or a micro-sized form, intended to represent the two fractions of food-grade TiO₂. The nano-sized particles had a mean primary particle diameter of 24 ± 5 nm, whereas the micro-sized particles averaged 120 ± 30 nm in diameter. The particles were dispersed in water by ultrasonication and administered via oral gavage at doses of 2, 10, or 50 mg/kg bw/d for 30 consecutive days; control rats were treated with an equal volume of ultrapure water. Following exposure for 30 days, rats were fasted overnight and anesthetized in order to examine the influence of TiO₂ particles on

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nutrient absorption and metabolism through an in situ intestinal loop experiment. Blood samples were collected at baseline as well as at 1 and 2 h following the introduction of a mixed nutrient solution to the closed intestinal loop. Following the in situ closed-loop experiment, animals were euthanized for histopathological examination of the small intestine and the ultrastructure of the small intestine mucosa was further analyzed by TEM. Sparse and short microvilli and inflammation of the small intestine was observed in the 50 mg/kg bw/d groups exposed to either nano- or micro-sized particles, although the effects were more pronounced in the nano-sized group. In the intestinal absorption experiment, six amino acids (Thr, Met, Val, His, Lys, and Trp), three metal elements (Mg, Mn, Zn), and glucose were injected into the in situ intestinal loop. Some inhibition of added histidine absorption was observed, particularly in the groups exposed to TiO₂-NPs, although there was no clear indication of a dose-response relationship. There was no influence on the remaining amino acids, glucose levels or metal elements. Ti levels were elevated in blood after exposure to TiO₂-NPs but not microscale particles.

Akagi et al., (2023)

212. The COT sub-group also reviewed a paper by Akagi *et al.* (2023) (methods described in paragraph 164) which was not included in the review by Health Canada. In a 28-day subacute oral toxicity study the authors stated that “hematological analysis... showed that a significant decrease in white blood cell count (WBC) was observed in the 10 mg/kg bw/day group of males” (Akagi *et al.*, 2023).

213. In the 90-day subchronic oral toxicity study also reported by these authors, it was stated that “Hematological analysis showed significant changes in leukocyte fractions [increase in neutrophils, monocytes, and basophils, and a decrease in lymphocytes in the male 1000 mg/ kg bw/day group. However, no differences were observed in the WBC count and absolute numbers of each leukocyte type. A significant decrease in mean corpuscular hemoglobin and an increase in the absolute number of eosinophils were observed in the female 1000 mg/kg bw/day

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group. A significant decrease in the fraction and absolute number of basophils was observed”.

214. However, in the discussion it was noted “The significant decrease in MCH observed in the 1000 mg/kg bw/day group of females in both the 28-and 90-day studies was considered of little toxicological significance because the change was minor, and there were no changes in the other erythrocyte markers suggestive of anemia in red blood cell count (RBC), hemoglobin (HGB), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC)” (Akagi *et al.*, 2023).

215. It was also noted “In the 90-day study the significant changes in leukocyte fractions in the 1000 mg/kg bw/day group of males were of little toxicological significance because no significant differences in total leukocyte counts and absolute numbers of each leukocyte type were observed. The significant increase in the absolute number of eosinophils observed in the 1000 mg/kg bw/day group of females was considered to have no toxicological significance, as it was a minor change and no fractional changes were observed.” (Akagi *et al.*, 2023).

With respect to induction of inflammation, in the discussion it was noted that “Notably, no reactive changes such as inflammatory reactions or tissue injury were observed at the site of deposition, which is consistent with previous findings, suggesting that there were no adverse effects on the organs.” (Akagi *et al.*, 2023).

EFSA (2021) review and conclusions

216. With regards to other endpoints the EFSA Panel discussed findings observing immunotoxicity and inflammation with E171 as well as neurotoxicity with TiO₂ NPs may be indicative of adverse effects.

217. The Panel noted that there were methodological shortcomings in the design of the immunotoxicology part of the EOGRT study. Therefore, the Panel could not conclude on immunotoxicity.

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Health Canada review and conclusions

218. The summary provided by Health Canada in their report on the State of the Science of Titanium Dioxide as a Food Additive states, “The evidence suggests that TiO₂ particles when well dispersed in simple matrices can produce inflammation and immunological perturbations and may alter gut microbiota and metabolism in ways that may potentially be adverse. In studies using food-grade TiO₂ specifically, some effects on inflammation and immune dysregulation were observed when the substance was administered in water in stable dispersions; however, these findings could not be replicated when TiO₂ was administered via the dietary route. Therefore, it is concluded that concerns that food-grade TiO₂ may produce inflammation or immunotoxicity appear to a great extent contingent on the oral dosing paradigm. Liu *et al.*, (2020) have pointed out that specific absorption of biomolecules (i.e., the particle corona) can also alter the immunological identity of particles and may either enhance or diminish their immunogenicity. Studies in humans have consistently demonstrated accumulation of pigment including TiO₂ in macrophages in the base of Peyer’s patches of the terminal ileum but not elsewhere in the GIT. However, no association between the presence of particles and immune activation or pathological state has been observed. Further information is required in order to investigate the potential mitigating effects of the food matrix on local toxicity in the GIT to determine whether studies involving stably dispersed food-grade TiO₂ in simple matrices are relevant to the hazard characterization of this substance when used as a food additive.”

219. In their overall summary the Health Canada conclusions on inflammation and immunotoxicity stated “No consistent evidence of inflammation or immunotoxicity in the GIT of rodents exposed to food-grade TiO₂ via the oral route. While a few non-guideline studies suggest food-grade TiO₂ when administered in water may produce inflammation or immune dysregulation in male mice and rats at doses up to 50 mg/kg bw/d (e.g. Pinget et al., 2020; Bettini et al. 2017, Talamini et al. 2019), these findings were not observed when food-grade TiO₂ was administered in the diet in a non-guideline study in male rats at doses up to ~236-300 mg/kg

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bw/d (Blevins et al. 2019), in male and female mice at doses up to 100 mg/kg bw/d for 18 weeks (Riedle et al. 2020), in a GLP- and OECD-guideline-compliant EOGRT study in male and female rats at doses up to 1000 mg/kg bw/d (LPT 2020 as cited in EFSA 2021a), or a two-year chronic bioassay with a form of TiO₂ highly comparable to the form of TiO₂ added to food at concentrations up to 5% w/w in male and female mice and rats (NCI 1979). In addition, no treatment-related histopathological abnormalities were observed in the spleen, thymus, lymph nodes and bone marrow and no abnormal hematological findings were reported for any immune-related parameters in the EOGRT (LPT 2020 as cited in EFSA 2021a) or chronic bioassay (NCI 1979). Similarly, no treatment-related changes in hematology or gross or histopathological abnormalities in lymphoid organs were observed in rats following the gavage administration of food-grade TiO₂ dispersed in water at doses up to 1000 mg/kg for 90 days in another OECD guideline-compliant study (Han et al. 2020).”

FSANZ review and conclusions

220. FSANZ identified three studies which fulfilled the criteria for assessing immunotoxicity (two used food-grade TiO₂ administered by the diet and one sonicated food-grade TiO₂ by gavage, at doses up to 1000 mg/kg bw/day for 90 days (Blevins et al., 2019 and Riedle et al., 2020, and the EOGRT study (LPT 2020). These studies found no adverse developmental immunotoxic effects in rodents (mice or rats).

COT review and conclusions

221. The COT sub-group noted that in reflection of the dietary dosing paradigm, only the three studies (Riedle et al., 2020; Blevins et al., 2019; and LPT, 2020) use E171 TiO₂. These studies showed that no adverse effects on inflammation or immunotoxicity were observed when using an E171 dietary dosing approach.

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222. Five studies using food grade TiO₂ in water (Talamini et al., 2019; Pinget et al., 2020; Bettini et al., 2017; Han et al., 2020; and Mortensen et al., 2021) were considered by the COT. These exposures of mice and rats to food grade TiO₂ in water has yielded mixed findings. In several studies, differential cytokine and host defence gene expression was observed but was neither consistent across studies, nor ubiquitous in terms of pathway activation (for example, one antimicrobial defence gene *Defb3* was increased, but others including cathelin-related antimicrobial peptide (CRAMP) and lysozyme were not). Non-dose dependent increases in expression of IL-10, TNF and IL-6 were observed by Pinget et al, but in Talamini et al., a significant increase in proinflammatory cytokine IL-1 β mRNA transcripts were reported in stomach (~65%) and whole intestine tissues (~75%), but not liver tissues. TNF- α levels were only significantly different (a reduction of 60%) in the intestine. A statistically significant reduction in liver anti-inflammatory cytokine IL-10 mRNA transcript expression was observed (~40%). It was also noted that Bettini et al did not observe any changes in the content of basal cytokines TNF- α , IFN- γ , and IL-17 in the mucosa of the small and large intestine compared to control rats, after 7 days of TiO₂ dosing. It is suggested therefore that tissue specificity and differential cytokine expression may play a role.

223. It is important to note that cytokine mRNA or protein measurement can take place in a variety of tissues or cell types that are not consistent across studies and thus considering these as an additional variable will likely be of value in the context of immunotoxicity.

224. The available data suggest that food grade E171 and TiO₂-NPs, delivered either by dietary exposure, or in water, or dispersant, could have the potential to induce inflammogenic responses. This is likely species, tissue- and cell-dependent, and it is suggested that ROS or oxidative stress may be key in the initiation of inflammation. The oral dosing paradigm, is also a key factor. However, the sub-group highlighted an inconsistency in the findings of the studies in this area, making interpretation or formulation of conclusive statements challenging.

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225. Various immune toxicity targets have been identified in the context of TiO₂ immunotoxicity, that are distinct from the induction or modulation of inflammation. These include, but are not limited to: induction of immune cell mediated inflammation in the gut, including in Peyer's patches, as well as in the spleen and via peripheral blood mononuclear cells; effects on broader host defence mechanisms, including antimicrobial peptides; effects in the gut microbiota; effects on dendritic cell populations in the gut; 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.

226. Broadly, there are a number of studies, in which immune cell activation, alteration, change in number or suppression of normal function has been observed, although this is highly inconsistent between studies, and appears to be affected by the nature of exposure and the form of TiO₂ used. The quality of data available and the use of appropriate controls in some studies therefore impacts consensus interpretation of the data and the toxicological significance of some of the findings.

227. Overall the weight of evidence for the food grade TiO₂ is not sufficient or of sufficient quality to arrive at a consensus that food grade TiO₂ is of concern with regards to immunotoxicity and inflammation.

Reproductive and Developmental Toxicity

Studies using E171 or equivalent form of TiO₂

LPT., (2020)

228. The Extended One Generation Reproductive Toxicity (EOGRT) study is described in paragraphs 113 - 122. The reproductive and developmental effects from this study are described within those paragraphs.

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Reproductive and developmental studies using the nanoparticle form of TiO₂

Lee et al., (2019)

229. Mated female Sprague–Dawley rats (12 females per group) were treated with TiO₂ NPs (21 nm) daily by gavage at dose levels of 0, 100, 300 and 1,000 mg/kg bw per day from GDs 6 to 19. The physicochemical characterization of TiO₂ nanoparticles, included analyses of primary shape, primary size, purity and hydrodynamic size. The majority of the TiO₂ nanoparticles had spherical and anatase crystal shapes with a purity of 100%. The mean primary size of the TiO₂ NPs was 17.8 ± 5.46 nm. The hydrodynamic size of the TiO₂ NPs was 341.5 nm, which indicated that TiO₂ NPs were prone to aggregation.

230. In the Lee *et al.*, (2019) study, no statistically significant differences were noted in general clinical signs, bodyweight, organ weights (absolute and relative to body weight), macroscopic findings. No significant differences for caesarean section parameters and fetal external and visceral examinations. The COT considered that no adverse maternal and developmental effects were reported with TiO₂ NPs (21 nm) up to 1,000 mg/kg bw per day, the highest dose tested.

Warheit, Boatman and Brown, (2015)

231. Warheit, Boatman and Brown (2015) undertook an oral prenatal developmental toxicity study in rats using five different TiO₂ materials, TiO₂ nanoparticles or TiO₂ containing a fraction of nanoparticles up to a dose of 1,000 mg TiO₂ NPs/kg bw. See paragraphs 97-99.

232. Gross necropsy included gross examination of the dam, counting of the number of corpora lutea, implantation sites, resorptions, live and dead foetuses, fetal sex and weight. Foetal pathological external, visceral and skeletal examinations were performed in order to identify any abnormalities. At 1,000 mg

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uf-1/kg per day, mean fetal sex ratio and the means for male and female fetuses per litter were statistically significantly different from the control group means. The mean number of male fetuses was 7.2 compared with 5.5 male fetuses for the concurrent control group; the test facility historical control group data ranged at that time from 5.2 to 7.4. The mean number of female fetuses was 4.8 compared with 6.7 for the concurrent control group; the test facility historical control group data ranged at that time from 5.8 to 8.3. Mean fetal sex ratio of the 1,000 mguf-1/kg bw per day group was 60% (males/females) compared with a sex ratio of 46% in the concurrent control group; the test facility historical control group data ranged at that time from 43% to 53%.

233. Apart from some incidental changes in body weight and feed intake, no other changes were observed in the dams or the fetuses in these studies. The authors concluded that there were no significant toxicological or developmental effects in females or fetuses at any of the dose levels or compounds tested and considered the NOAEL for each compound to be 1,000 mg/kg bw per day, the highest dose tested.

EFSA review and conclusions

234. Male Fertility: The Panel noted that the epididymal sperm parameters were not evaluated but that this deviation has no effect on the final conclusion of the study. There were no effects on any of the sperm endpoints in the cohort 1A.

235. Female Fertility: The EFSA Panel concluded that there were no indications of effects on general toxicity, thyroid or sex hormone levels, reproductive function and fertility in either male or female rats, no effects on pre- and postnatal development or on neurofunctional endpoints in F1 offspring.

236. Developmental Toxicity: To check whether the significant differences in grip strength and hindlimb splay could be due to systematic bias in group testing order, the testing order was checked. The Panel considered that there was no systematic bias in group testing order and that this was therefore not a plausible explanation for

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the observed group differences. Grip strength and hindlimb splay belong to the same domain of neurological function, i.e., motor function and/or sensory–motor coordination. However, the effects observed (i.e., increase in hindlimbs play and increase in mean forelimb grip strength) seem to point in opposite directions when it comes to muscle strength. In particular, an increase in hindlimb splay can be interpreted as muscular weakness whereas an increase in mean forelimb grip strength could be indicative of myotonia.

237. The Panel noted that the effects observed were not correlated to any other changes (e.g., alterations in muscle tone, righting reflex, gait, wire manoeuvre, posture). No dose response was observed for any of these endpoints or for the two functional measurements, indicating that the likelihood of an association with test substance is low. No other changes in the functional observation battery measurements or locomotor activity were noted.

238. Furthermore, there were no notable histopathological findings in brain or in peripheral nerve (sciatic). Based on all the above considerations, the Panel considered that the effects on grip strength and hindlimb splay were not treatment related. However, the Panel noted that quantitative information on peripheral nerves was not available. Overall, the Panel considered that E171 had no adverse effects on neurofunctional endpoints in F1 cohort 2A offspring at the doses used.

239. EFSA conclusions on developmental toxicity results of the EOGRT study: No effects of E171 on pre- and postnatal development were observed. Data on the attainment of puberty in males (i.e., an appropriate assessment of the timing of the balanopreputial separation) were missing. The Panel did not consider this to be critical in this case.

240. To check whether the significant differences in grip strength and hindlimb splay could be due to systematic bias in group testing order, the testing order was checked. The Panel considered that there was no systematic bias in group testing order and that this was therefore not a plausible explanation for the observed group differences. Grip strength and hindlimb splay belong to the same domain of neurological function, i.e., motor function and/or sensory–motor coordination.

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However, the effects observed (i.e., increase in hindlimb splay and increase in mean forelimb grip strength) seem to point in opposite directions when it comes to muscle strength. In particular, an increase in hindlimb splay can be interpreted as muscular weakness whereas an increase in mean forelimb grip strength could be indicative of myotonia.

241. The Panel noted that the effects observed were not correlated to any other changes (e.g., alterations in muscle tone, righting reflex, gait, wire manoeuvre, posture). No dose response was observed for any of these endpoints or for the two functional measurements, indicating that the likelihood of an association with test substance is low. No other changes in the functional observation battery measurements or locomotor activity were noted.

242. Furthermore, there were no notable histopathological findings in brain or in peripheral nerve (sciatic). Based on all the above considerations, the Panel considered that the effects on grip strength and hindlimb splay were not treatment related. However, the Panel noted that quantitative information on peripheral nerves was not available. Overall, the Panel considered that E171 had no adverse effects on neurofunctional endpoints in F1 cohort 2A offspring at the doses used.

243. EFSA conclusions on developmental toxicity results of the EOGRT study: No effects of E171 on pre- and postnatal development were observed. Data on the attainment of puberty in males (i.e., an appropriate assessment of the timing of the balanopreputial separation) were missing. The Panel did not consider this to be critical in this case.

244. EFSA concluded that there were no indications of effects on general toxicity, thyroid or sex hormone levels, reproductive function and fertility in either male or female rats, no effects on pre- and postnatal development or on neurofunctional endpoints in F1 offspring.

245. With regards to other endpoints EFSA concluded that no effects on reproductive and developmental toxicity were observed up to a dose of 1,000

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mg/kg bw per day, the highest dose tested, of a commercially-available E171, were observed in the EOGRT study. No other reliable studies were found in the literature addressing these effects with E171.

246. EFSA Opinion: The Panel agreed with both the author and Food Additives & Nutrient Sources Added to Food Panel conclusions (2016 ANS Panel - Overall, the Panel noted that prenatal developmental studies with three pigment-grade (pg-1, pg-2 and pg-3) and three ultrafine (uf-1, uf-2 and uf-3)/nanoscale (anatase and/or rutile) TiO₂ particulates performed according to the OECD guidelines (TG 414) did not give concern for maternal or developmental toxicity up to the highest dose tested (1,000 mg/kg bw per day)).

Health Canada review and conclusions

247. Health Canada noted that the EORGT study (LPT 2020) was designed to investigate the induction of ACF using a commercially-available food grade E171 at doses from 0-1000 mg/kg bw/day and that no effects were observed up to the highest dose tested. They concluded on the basis of the EORGT study (LPT 2020) that a NOAEL of 1000 mg/kg bw/d could be established for reproductive and developmental effects and that the EGORT study (LPT 2020) was assessed as the most reliable currently-available study that addresses potential reproductive and developmental effects of E171.

FSANZ review and conclusions

248. FSANZ noted the 2016 EFSA request for an additional developmental and reproductive toxicity study in the form of the subsequent EORGT study. FSANZ stated that the EORGT study (LPT 2020) using food-grade TiO₂ showed no evidence of developmental or reproductive toxicity with an upper test dosage and NOAEL of 1000 mg/kg bw/day.

249. FSANZ noted an additional study (food-grade TiO₂ by oral gavage and in the diet) which described germ cell sloughing in mouse testes (Rodríguez-Escamilla et

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al., 2019) however these changes were not observed in the follow-up study. Combined with the lack of histopathological changes observed in the testes in three additional studies up to 1,000 mg/kg bw/day (EOGRT (LPT, 2020), Han et al. 2021 and Blevins et al. 2019)

COT review and conclusions

250. The COT considered the EOGRT report to be detailed. No toxicological effects on the reproductive & developmental system were observed. A range of endpoints, including body weight, haematology, urinalysis, sex hormone levels, thyroid hormone levels, circadian rhythm and sperm parameters, which were detailed looking at both the F0 and F1 generations.

251. It was noted that there was a suggestion of focal effects on the testes and epididymides in 1 male in the highest dose group, which was considered to be spontaneous and not test item-related, and abnormalities in sperm. Data from the EOGRT study showed test item-related differences for the examined absolute and relative testis weights between the control and the treatment groups (low = 100 mg, intermediate = 300 mg or high = 1,000 mg TiO₂ E171/kg bw per day). These slight differences included a small but statistically significantly reduced absolute right testes weight at the intermediate and the high dose level. These were only noted for the right testes, not the left testes and it was concluded that changes were spontaneous and not of toxicological relevance. The COT agreed with the authors' conclusions. Overall, no adverse effects on reproductive and developmental toxicity were observed, up to the highest dose tested.

252. The COT considered that the EOGRT study was carried out according to the relevant scientific guidelines with no obvious deficiencies. Based on the evidence reviewed, no reproductive & developmental toxicity was observed. Data from peer reviewed literature were inconclusive.

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253. The best overall quality additional studies were determined by the COT to be the studies by Warheit *et al.*, 2015, and & Lee *et al.*, 2019. The COT considered the 1,000 mg/kg bw per day NOAEL established in the EOGRT study may result from low absorption. Other studies indicate NOAELs consistent with that from the EOGRT study. The COT noted variations in method endpoints between several of the studies and as a consequence the physicochemical differences between the test items were unclear.

254. The COT concluded that there was no strong evidence that TiO₂ was reprotoxic.

255. No evidence of reproductive or developmental toxicity or gross or histopathological abnormalities were found in male or female reproductive organs in the EOGRT in rats following dietary exposure to food-grade TiO₂ at doses up to 1,000 mg/kg bw/day. No other reliable studies with food-grade TiO₂ were identified in the literature. In the final summary the authors concluded that "Health Canada Food Directorate's position is that there is no conclusive scientific evidence that the food additive TiO₂ is a concern for human health" and the sub-group agrees with these conclusions.

Neurotoxicity

Studies using E171 or equivalent form of TiO₂

EOGRT Study (LPT, 2020)

256. The EOGRT study is described in detail in the EOGRT summary section (paragraphs 134 - 135).

Studies using the nanoparticle form of TiO₂

Sofranko et al., (2021)

257. Sofranko et al., 2021 investigated the effects of silver and TiO₂ NPs on behaviour and neuropathology in male and female C57BL/6J mice. Mice were fed

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pellets with 10 mg/g TiO₂, 2 mg/g polyvinylpyrrolidone-coated Ag or control pellets for 28 days with and without a 14-day post-exposure recovery. Inflammation, oxidative stress, and blood-brain barrier integrity were not significantly affected in male or female mice. No consistent significant treatment-related effects on anxiety and cognition were observed for either test material. The authors concluded that there were no substantial neuropathological changes following subacute exposure to foodborne TiO₂ in mice.

Canli et al., (2020)

258. Female albino rats were given sublethal doses (0.5, 5.0 and 50 mg/kg bw per day) of sonicated TiO₂ NPs for 14 days via oral gavage in 200 µL water (n = 6 per group). Control animals (n = 6) received 200 µL water only. Following dosing, total protein, the activities of ATPases and acetylcholinesterase A(ChE), the levels of TBARS and the different forms of GSH were measured in tissues from the liver, kidney, brain and intestine.

259. The anatase TiO₂ NPs were about 21 nm, >30 m²/g surface area, >99% purity, and a density of 4.26 g/cm³. None of the rats died within the 14-day treatment period. TEM images showed that TiO₂ NPs seemed to accumulate in the brain, kidney and liver tissues in a dose-dependent manner. There was no TiO₂ NP deposition in the same tissues from control animals. Brain AChE activity decreased at all TiO₂ NP doses compared to controls, however, brain ATPase activities were generally stimulated. ATPase activities in the intestine and kidney did not change significantly. Levels of the different forms of GSH did not change significantly. There were no significant changes in TBARS levels, except for a decrease at the highest TiO₂ NP dose.

Grissa et al., (2016)

260. The effects of oral intake of TiO₂ NPs (5–10 nm) at 0, 50, 100, and 200 mg/kg bw for 60 days on the brain of Wistar rats was investigated. The coefficient of the brain, AChE activities, level of IL-6, and the expression of glial fibrillary acidic protein (GFAP) were assessed to quantify the brain damage. The results showed that high-

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dose TiO₂ NPs could induce a downregulated level of AChE activity and an increase in plasma IL-6 compared to the control group. There was a dose-dependent decrease between doses and an associated increase in cerebral IL-6 as a response to a local inflammation in the brain. Elevated levels of immunoreactivity to GFAP were observed in the rat cerebral cortex. The authors concluded that oral intake of TiO₂ NPs can induce neuroinflammation and could be neurotoxic and hazardous to health. (Grissa *et al.*, 2016 (Abstract only)).

EFSA review and Conclusions

261. The EFSA conclusions on the neurotoxicity of the EOGRT study considered that the effects on grip strength and hindlimb splay were not treatment-related but that quantitative information on peripheral nerves was not available. Overall, the Panel considered that E171 had no adverse effects on neurofunctional endpoints at the doses used.

262. No neurotoxicity studies performed with E171 were identified from the published literature that were considered sufficiently reliable. Some papers were identified noting effects of TiO₂ NP.

Health Canada review and Conclusions

263. Health Canada (2022) summarised the evidence for neurotoxicity of TiO₂ as follows: “The concerns pertaining to potential neurotoxicity associated with TiO₂-NPs in E171 appear to be based predominantly on studies which used test articles that did not correspond to food-grade TiO₂ and/or dosing paradigms that are considered to be of limited relevance to human dietary exposure. In an EOGRT study where developmental neurotoxicity was investigated in rats exposed to E171 at doses up to 1,000 mg/kg bw/d via the diet, no adverse effects on neurodevelopmental or neurofunctional endpoints were observed. Endpoints examined included auditory startle response as well as a functional battery that included grip strength and locomotor activity. No treatment-related changes were observed in any of these endpoints and there were no notable histopathological findings in the brain or

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peripheral nerves. Similarly, in the only available study in which TiO₂-NPs were administered via the diet (Sofranko *et al.*, 2021), no neurotoxicity was observed.”

264. Health Canada’s more detailed analysis of neurotoxicity potential focussed on some of the studies used by EFSA and this is extracted at the end of their document. However, Health Canada did not include the study by Canli *et al.* (2020) which found reduced brain cholinesterase activity and increased brain Na/K-ATPase activity in female rats dosed for 14 days with TiO₂ nanoparticles, presumably because of the limited relevance to human dietary exposure. Nevertheless, EFSA had concluded that these adverse effects, which were seen at the lowest of three doses tested (0.5 mg/kg bw/day), were “the most sensitive endpoint”, even though this was not consistent with the findings of Grissa *et al.* (2016) which reported reduced brain cholinesterase activity at 100 but not 50 mg/kg bw/day in male rats dosed for 60 days with TiO₂ nanoparticles.

265. Health Canada also noted the study by Sofranko *et al.* (2021), published after the EFSA Opinion, which concluded that sub-acute (28 day) exposure to TiO₂-P25 did not cause neurotoxicity (although P25 is referred to as foodborne, this is not considered as a food additive). Other studies which have been published since the 2021 EFSA Opinion are of very limited relevance as they were conducted *in vitro* using rat cortical cells (Gerber *et al.*, 2022) or involved i.v. injection of sonicated TiO₂ nanoparticles (Ciu *et al.*, 2021; Naima *et al.*, 2021).

FSANZ review and Conclusions

266. FSANZ only identified one study with neurotoxicological endpoints using food-grade TiO₂ administered via the diet, the EORGT Study (LPT 2020). FSANZ concluded that no adverse neurofunctional effects were observed (in auditory startle response and a functional observation battery).

COT review and Conclusions

267. 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, namely

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“The findings of the studies on neurotoxicity were considered inconsistent by the COT. It was noted that the EOGRT study did not report any effects and that most of the other studies on this endpoint were of nanomaterials. In the EFSA evaluation, the issue of the test material in the EOGRT not being dispersed was taken into consideration with regards to the conclusions on this endpoint, as they considered that had it been dispersed and stabilised in the nano form some effects could possibly have been observed. The COT, as previously, questioned the relevance of such dispersion to the real-world use of TiO₂. Members noted that the histopathology tests performed for the EOGRT study were standard and were not sensitive enough in comparison to other studies on this endpoint that performed specific neuro-histopathology testing.” COT, 2022.

268. It should be recognised that this qualified COT opinion 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.

Derivation of a Health-Based Guidance Value (HBGV)

269. The Committee concluded that on the basis of the available evidence, 1,000 mg/kg bw/day was a reasonably robust Point of Departure (POD). This was based on the EOGRT study findings 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 undermine the value of 1,000 mg/kg bw/day to be used as the POD. It was also noted by the COT that this is the highest dose of E171 or equivalent TiO₂ tested in a study of this quality.

270. A standard uncertainty factor of 100 (10 for inter species variability and 10 for 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 due to the low absorption of TiO₂ and because there is no metabolism of TiO₂ particles.

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Exposure Assessment

271. The exposures to TiO₂ from medication, personal healthcare products and through dermal, inhalation and intravenous routes are not considered in this assessment. The exposures considered are only those from food.

272. Titanium dioxide can be found in a number of food categories including bakery products, soups, broths, sauces, salads, savoury based sandwich spreads and processed nuts. It is also used in confectionary, chewing gum, food supplements and cake icing (EFSA, 2016).

Occurrence data in food

273. Only food as a source of TiO₂ is considered in this exposure assessment. Food consumption data from the National Diet and Nutrition Survey (NDNS) (Bates, 2014; 2016; Roberts 2018; Bates, 2020) and the Diet and Nutrition Survey of Infant and Young Children (DNSIYC) (Department of Health, 2013) were used to estimate exposure to titanium dioxide. Maximum occurrence levels of titanium dioxide for specific food items, reported by EFSA (2021), were also used in the estimation of exposure (Table 6). Food categories were created by the FSA Exposure Assessment Team (EAT) using data from NDNS and DNSIYC to reflect those created by EFSA for the Food Additive Intake Model and as presented in Annex II of Regulation (EC) No 1333/2008, part D. Foods in NDNS and DNSIYC were matched to food categories associated with the regulation on food additives to enable an assessment of exposure based on maximum levels reported by industry for titanium dioxide and those reported in the scientific literature. Assessments were carried out in Crème which is the software used by the FSA EAT to conduct exposure assessments.

274. The occurrence data used were those reported in EFSA, 2021 which were obtained from industry as reported by the Dutch National Institute for Public Health and the Environment (RIVM) along with levels reported in analytical studies. These levels are presented in Table 1 for sixteen food categories, although titanium dioxide is approved in many other food categories (forty-eight in total) (Table 1, Annex E). For the exposure assessment, only use levels for these sixteen food categories were

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taken into account, as no data were available for the other categories and it was not possible to use the maximum permitted levels (MPLs) for TiO₂ as they were established at quantum satis, rather than a specific value being ascribed. The assessment was based on maximum use levels reported to provide conservative scenarios of exposure for the population groups considered (Table 7).

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Table 6: Occurrence levels of titanium dioxide (E171) used in the exposure assessment scenarios (mg/kg or mg/L as appropriate)

EFSA Food category number	Food category name	Concentration levels used in the exposure assessment (Maximum reported)	MPL (mg/L or mg/kg as appropriate)
01.4	Flavoured fermented milk products including heat-treated products	48	QS
01.8	Dairy analogues, including beverage whiteners	125	QS
03	Edible ices	857	QS
05.2	Other confectionery including breath-refreshening microsweets	4,500	QS
05.3	Chewing gum	16,000	QS
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	20,000	QS
07.2	Fine bakery wares	318	QS
12.5	Soups and broths	193	QS
12.6	Sauces	4,000	QS
12.7	Salads and savoury-based sandwich spreads	3,000	QS
12.9	Protein products, excluding products covered in category 1.8	5,000	QS
14.1.4	Flavoured drinks	70	QS
15.2	Processed nuts	7,000	QS
16	Desserts, excluding products covered in categories 1, 3 and 4	200	QS
17.1	Food supplements supplied in a solid form, excluding food supplements for infants and young children	26,950	QS
17.2	Food supplements supplied in a liquid form, excluding food supplements for infants and young children	26,950	QS

QS – *quantum satis* (no maximum numerical level is specified, and substances will be used in accordance with good manufacturing practice, at a level not higher than is

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necessary to achieve the intended purpose and provided the consumer is not misled).

Exposure Estimation

275. Exposure assessments were carried out for the following population groups: Infants, toddlers, other children, adolescents, adults and the elderly. There are two toddler groups. One group represents ages 1 - 1.5 years and the data used were from DNSIYC as this survey covers infants and young children aged 4 - 18 months (1.5 years). The other toddler group covers ages 1.5 - 3+ years and data were obtained from the NDNS, as this survey covers all age groups from 1.5 years. The mean and 95th percentile estimates are presented for each population group and food category in mg/kg bw/day in Table 2.

276. The reported data are consumer-based, meaning that only subgroups of the population that consumed these categories of food were considered. The mean and 95th percentile total exposures are derived from the combined exposure for each individual in the respective age group. The mean and 95th percentile total exposures have not been derived by adding up the mean and 95th percentile values respectively, for the food categories, as not all consumers will be exposed in each category. Total exposures were calculated to estimate exposures from the total diet in Table 8. These were calculated from a distribution of individual total exposure of any combination of food categories, rather than by summation of the respective mean/95th percentile consumption values for each of the food categories.

277. The mean calculated total exposures for TiO₂ from foods 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, except 4.2.4; and Sauces.

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Table 7: Estimated mean and 95th percentile (P95) exposures (mg/kg bw/day)^a to titanium dioxide E171 from its use as a food additive based on the maximum reported use level. The reported data are consumer-based.

	Infants (4 – 11 months)	Toddlers (1 - 1.5 years)	Toddler s (1.5 - 3 years)	Children (4 - 10 years)	Adolescents (11 - 18 years)	Adults (19 - 64 years)	Elderly (≥65 years)
Food group	Mean (P95^b)	Mean (P95^b)	Mean (P95^b)	Mean (P95^b)	Mean (P95^b)	Mean (P95^b)	Mean (P95^b)
1.4- Flavoured fermented milk products including heat-treated products	0.24 (0.6)	0.2 (0.49)	0.16 (0.4)	0.097 (0.23)	0.05 (0.12)	0.044 (0.11)	0.049 (0.11)
1.8. Dairy analogues and whitener	0.91 (1.9)	1.6 (5.6)	1.9 (5.7)	0.63 (2.3)	0.21 (0.78)	0.15 (0.47)	0.18 (0.75)
12.5. Soups and broths	0.45 (1.5)	0.53 (1.7)	0.55 (1.3)	0.37 (0.94)	0.23 (0.56)	0.21 (0.54)	0.24 (0.64)
12.6. Sauces	4 (14)	4 (12)	3.5 (11)	3.3 (9.2)	2.2 (6.1)	1.7 (5)	1.4 (3.8)
12.7. Salads and savoury based sandwich spreads	2.2 (9.4)	3 (12)	2.9 (9.2)	1.9 (4.6)	0.92 (2.7)	0.95 (2.7)	0.94 (2.9)
12.9. Protein products	14 (55)	27 (190)	38 (160)	11 (49)	5.3 (18)	5.3 (18)	7.6 (30)
14.1.4. Flavoured drinks	0.15 (0.53)	0.35 (1.1)	0.47 (1.4)	0.44 (1.2)	0.4 (1)	0.24 (0.73)	0.12 (0.32)
15.2. Processed nuts	1.3 (4.6)	2.1 (8.1)	2.8 (9.9)	2 (7.2)	1 (3.6)	1.2 (3.9)	1.2 (3.4)
16. Desserts excluding products covered in categories 1, 3 and 4	1.2 (3.2)	1 (2.6)	0.64 (1.8)	0.33 (0.85)	0.14 (0.38)	0.11 (0.28)	0.15 (0.4)
17.1. Food supplements supplied in a solid form, excluding for	1.4 (3.3)	1.7 (2.7)	1.9 (4.2)	1.1 (2.6)	0.82 (1.3)	0.95 (2.1)	0.85 (2.2)

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infants and young children							
17.2. Food supplements in a liquid form, excluding for infants and young children	3.9 (16)	3.2 (12)	3.3 (11)	1.4 (4)	3.2 (9)	1.7 (5.6)	0.91 (2.3)
3. Edible ices	0.88 (2)	1.1 (3)	1.3 (3.3)	1 (2.5)	0.55 (1.5)	0.32 (0.74)	0.35 (0.84)
5.2. Confectionery and sweets	1.2 (2.9)	2.7 (6.9)	2.6 (7)	2.4 (7.6)	1.5 (4.7)	0.79 (2.9)	0.54 (1.8)
5.3. Chewing gum	0 (0)	1.5 (1.5)	0.73 (1.6)	0.6 (1.7)	0.57 (2.2)	0.41 (1.2)	0.63 (0.82)
5.4. Decorations, coatings and fillings, except 4.2.4	6 (16)	7.2 (19)	6.8 (17)	6.6 (18)	3.8 (10)	2.3 (6)	2.3 (6.3)
7.2. Fine bakery wares	0.3 (0.9)	0.5 (1.4)	0.6 (1.5)	0.55 (1.3)	0.3 (0.76)	0.21 (0.55)	0.23 (0.57)

*Estimates are rounded to 2 significant figures.

**Calculated from a distribution of individual total exposure of any combination of food categories rather than by summation of the respective mean/95th percentile consumption values for each of the food categories.

^a Exposure per bodyweight was calculated for each individual before calculating the mean and P97.5 exposure. The mean and 95th percentile estimates are presented for each population group and food category in mg/kg bw/day in Table 2. The estimates are based on individual bodyweights and not the average for the population group. However, for context, the average bodyweights of the population groups are 9.11 kg for infants; 10.9 kg for 12-18 months old; 14.6 kg for 1.5 -3 years; 27.1 kg for 4-10 years; 58.9kg for 11-18 years; 78.6 kg for 19-64 years; and 70.6kg for the elderly.

^b 95th percentile exposures have been reported for the UK to aid the comparison with data reported by EFSA.

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Table 8: Estimated mean and 95th percentile (P95) total exposures (mg/kg bw/day)^a to titanium dioxide E171 from its use as a food additive based on the maximum reported use level.

	Infants (4 – 11 months)	Toddlers (1 - 1.5 years)	Toddlers (1.5 - 3 years)	Children (4 - 10 years)	Adolescents (11 - 18 years)	Adults (19 - 64 years)	Elderly (≥65 years)
	Mean (P95^b)	Mean (P95^b)	Mean (P95^b)	Mean (P95^b)	Mean (P95^b)	Mean (P95^b)	Mean (P95^b)
Total exposure**	3.9 (14)	6.9 (19)	11 (26)	9.5 (24)	5 (13)	3.7 (10)	3.3 (9.1)

^a Exposure per bodyweight was calculated for each individual before calculating the mean and P97.5 exposure. The mean and 95th percentile estimates are presented for each population group and food category in mg/kg bw/day in Table 2. The estimates are based on individual bodyweights and not the average for the population group. However, for context, the average bodyweights of the population groups are 9.11 kg for infants; 10.9 kg for 12-18 months old; 14.6 kg for 1.5 -3 years; 27.1 kg for 4-10 years; 58.9kg for 11-18 years; 78.6 kg for 19-64 years; and 70. 6kg for the elderly.

^b 95th percentile exposures have been reported for the UK to aid the comparison with data reported by EFSA.

** The total exposure includes all of the following food groups:

- 1.4- Flavoured fermented milk products including heat-treated products
- 1.8. Dairy analogues and whitener
- 12.5. Soups and broths
- 12.6. Sauces
- 12.7. Salads and savoury based sandwich spreads
- 12.9. Protein products
- 14.1.4. Flavoured drinks
- 15.2. Processed nuts
- 16. Desserts excluding products covered in categories 1, 3 and 4
- 17.1. Food supplements supplied in a solid form, excluding for infants and young children
- 17.2. Food supplements in a liquid form, excluding for infants and young children
- 3. Edible ices
- 5.2. Confectionery and sweets
- 5.3. Chewing gum
- 5.4. Decorations, coatings and fillings, except 4.2.4
- 7.2. Fine bakery wares

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Assumptions and uncertainties

Evidence base

278. To be updated

Exposure assessment

279. 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. It is unlikely that all foods in each category assessed will contain E171 and at the maximum reported levels. This assumption may overestimate exposure.

280. There are differences between the granularity of food groups used by EFSA for the purposes of their exposure assessment, and those used here for the UK population. This could introduce uncertainties about the comparability of the data.

281. The European Union (EU) banned the use of titanium dioxide as a food additive in 2022. This followed EFSA's update on the safety of the food additive and taking into account uncertainties around genotoxicity concerns (Commission Regulation (EU) 2022/63). Titanium dioxide has not been banned in the UK. However, the EU ban on titanium dioxide may lead to a decline in its use by industry, even for foods consumed in the UK. Given these reasons, the exposure estimates derived may overestimate current and future exposure to titanium dioxide in the UK population.

282. The exposure estimates do not account for exposures from medicines, toothpaste and other non-food sources. Exposure may be higher if these other sources are considered.

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Risk Characterisation

283. 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. Adverse health effects would not be expected for any of these populations.

284. 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. The remaining exposures are 1.3- to 2.6-fold the HBGV. However, for reasons discussed in the assumptions and uncertainties section, exposures are likely to be lower than calculated. Therefore, it is unlikely that there would be a risk to health from exposures of E171 TiO₂ from the diet.

Conclusions

285. 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 have all (100%) of their particles less than 100 nm in diameter and are colourless and would therefore be unsuitable for use as a pigment in food applications.

286. The Committee concluded that there is uncertainty over the effect that TiO₂ nanoparticles have on toxicity. The Committee therefore considered that if animals and/or humans are exposed to test substances which contain higher levels of NPs 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.

287. This statement has separated the assessment of the engineered nano form and the food grade TiO₂. The focus and conclusions of the statement are based on food grade TiO₂.

288. The COT concluded that the physical form of TiO₂ will affect the absorption and distribution of TiO₂. The wide variance of test materials used (nano, micro and

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mixtures of nano and micro) was noted. Due to this large variability, as well as the potential impact of the matrix of administration, the Committee could not ascribe a percentage for the absorption of TiO₂. However, the Committee considered that absorption of food grade TiO₂ (E171) is low, based on the available evidence.

289. The COM reviewed a number of studies to assess the genotoxicity of TiO₂.

290. The COM stated that a definitive assessment of the safety of food grade E171 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, and this led to conflicting data and uncertainty in the risk assessment for TiO₂. (COM, 2024b).

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

292. Overall, however, 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) (COM 2024b).

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

294. 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 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).

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295. A standard uncertainty factor of 100 (10 for inter species variability and 10 for 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 due to the low absorption of TiO₂ and because there is no metabolism of TiO₂ particles.

296. 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. All mean total dietary exposures were below or very close to the HBGV and would not be expected to lead to adverse health effects. 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.

297. Based on the uncertainties and assumptions of the exposure assessment as mentioned, the exposure estimates derived probably overestimate the current and future exposure to titanium dioxide in the UK population. It is therefore unlikely that there would be a risk to health from exposures of E171 TiO₂ in the diet.

298. 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 from the diet.

COT Secretariat

May 2024

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Abbreviations Table

To be updated

Abbreviation	
ABC	Aberrant Crypts
ACF	Aberrant Crypt Foci
COM	Committee on Mutagenicity
COT	Committee on Toxicity
E171	Titanium Dioxide (food additive)
EFSA	European Food Safety Authority
EOGRT	Extended One-Generation Reproductive Toxicity (study)
HBGV	Health-based Guidance Value
OECD	Organisation for Economic Co-Operation and Development (Guideline 443)
POD	Point of Delivery
TiO ₂	Titanium Dioxide
TiO ₂ -P25	Anatase:Rutile : 85:15, Diameter: 10-40nm
BET	Brunauer-Emmett-Teller

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TOX/2024/xx Annex B

Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment.

Third draft statement on the safety of Titanium Dioxide (E171) as a Food Additive

1. The search terms

- Search "In Vitro Techniques"[Mesh] OR "Caco-2 Cells"[Mesh] OR "Lysosomes"[Mesh] OR "Hypoxanthine Phosphoribosyltransferase"[Mesh] OR "Autopsy"[Mesh] OR "Micronucleus Tests"[Mesh] OR "Pathology"[Mesh] OR Autops*[tiab] OR histopath*[tiab] OR "in vivo"[tiab] OR "in vitro"[tiab] OR GALT[tiab] OR Caco 2 cell*[tiab] OR Colonic Epithelial Cell*[tiab] OR Colorectal epithelial cell*[tiab] OR Comet assay*[tiab] OR HPRT[tiab] OR "Hypoxanthine Phosphoribosyltransferase"[tiab] OR "hypoxanthin phosphoribosyl transferase"[tiab] OR Lysosom*[tiab] OR "M cell"[tiab] OR "M cells"[tiab] OR "Microfold cell"[tiab] OR "Microfold cells"[tiab] OR Micronucleus assay*[tiab] OR Micronucleus test*[tiab] OR "read across"[tiab] OR SCGE assay*[tiab] OR single-cell gel electrophoresis assay*[tiab] OR "xanthine guanine phosphoribosyl transferase"[tiab] OR "xanthine guanine phosphoribosyltransferase"[tiab] OR xprr[tiab] AND titanium dioxide (322; 19)
- Search "Aneugens"[Mesh] OR "Aneuploidy"[Mesh] OR "apoptosis"[Mesh] OR "Chromatids"[Mesh] OR "Chromosomes"[Mesh] OR "DNA Damage"[Mesh] OR "DNA Repair"[Mesh] OR "Mutagens" [Pharmacological Action] OR "Mutagens"[Mesh] OR "Mutagenesis"[Mesh] OR "Oxidative Stress"[Mesh] OR Aneugen*[tiab] OR Aneuploid*[tiab] OR Chromatid*[tiab] OR Chromosome*[tiab] OR Clastogen*[tiab] OR "DNA binding"[tiab] OR DNA fragmentation*[tiab] OR DNA damage*[tiab] OR "DNA lesion"[tiab] OR "DNA lesions"[tiab] OR "DNA repair" [tiab] OR genotox*[tiab] OR mutagen*[tiab] OR Mutat*[tiab] OR oxidative stress*[tiab] AND titanium dioxide (209; 16)

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- (Titanium dioxide AND Nanoparticles AND E171 AND toxicology) (1);
(Titanium dioxide AND Nanoparticles AND E171 AND genotoxicity) (8);
(Titanium dioxide AND Nanoparticles AND E171 AND human health) (13);
(Titanium dioxide AND Nanoparticles AND E171 AND adverse effects) (7);
(Titanium dioxide AND Nanoparticles AND E171 AND reproductive toxicity)
(5); (Titanium dioxide AND Nanoparticles AND E171 AND developmental
toxicity) (2); (Titanium dioxide AND Nanoparticles AND E171 AND
Distribution) (8); (Titanium dioxide AND Nanoparticles AND E171 AND
Metabolism) (4); (Titanium dioxide AND Nanoparticles AND E171 AND
Excretion) (1); (Titanium dioxide AND Nanoparticles AND E171 AND ADME)
(1); (Titanium dioxide AND Nanoparticles AND E171 AND
immunotoxicology) (0); (Titanium dioxide AND Nanoparticles AND E171
AND neurotoxicology) (0).

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TOX/2024/xx Annex C

Studies used to review the toxicokinetics and absorption of the nanoparticle form of TiO₂

Studies in rats and mice

Disdier et al., (2015)

299. The test material used was 75% anatase, 25% rutile titanium dioxide nanoparticles 21.5 ± 5 nm.

300. Adult Fischer rats were treated with 1 mg/kg titanium dioxide or saline buffer (control); no dispersion protocol was applied for the in vivo experiment. The dose was administered intravenously, and samples were taken at 30 minutes, 1, 2, 6 and 24 hours and 7-, 28-, 90- and 365-days post treatment from blood, liver, brain, spleen, kidney and lungs. Blood and brain samples were additionally collected at 5 and 15 minutes post injection. Titanium concentrations were determined by ICP-MS.

301. The authors reported that titanium burdens in the liver, spleen and lungs of the treated group were significantly higher for all time points post injection, however the levels declined over time. Levels in the liver were higher than the spleen and lungs. Titanium burden after a year remained high, suggesting biopersistence (approximately 33% of the titanium burden of the early time points). The titanium burden in the kidneys increased significantly from 30 minutes to 24 hours but decreased significantly 7 days after i.v. administration. No statistically significant results were reported for the blood samples. For the time points before 24 hours, there was a statistically significant increase in titanium concentrations in the brain. After 24 hours, titanium content did not differ from controls. No further details were given in the text, but graph 2 provides more details on the levels of titanium in the different organs. The authors estimated that they recovered approximately 44 % of

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the administered dose in the liver, 10 % in lungs and 2 % in spleen 6 hours post administration.

Kreyling et al., (2017a)

302. The test item used was titanium dioxide anatase nanoparticles, 7-10 nm.

303. Female Wistar- Kyoto rats were dosed with 10-20 µg of radiolabelled titanium dioxide given as a single i.v. injection of nanoparticles suspended in water.

304. The animals were sacrificed at 1, 4, 24 hours and 7 and 28 days post administration (n=4 per time point). All organs as well as blood and all excretions of the animals were collected. The results of a separate intravenous study performed to investigate the absorption and biodistribution of soluble ionic ⁴⁸V were used to correct ⁴⁸V release from [48V] titanium dioxide nanoparticle.

305. The highest [48V] titanium dioxide accumulations were found in liver (95.5% ID on day 1), followed by spleen (2.5%), carcass (1%), skeleton (0.7%) and blood (0.4%). Detectable nanoparticle levels were found in all other organs. The [48V] Titanium dioxide NP content in blood decreased rapidly after 24h while the distribution in other organs and tissues remained rather constant until day-28. Particularly, 4 hours post, administration, 99.5% of the radioactive dose was found in the liver and at 28 days 88.9% of the dose was detected in the liver. The spleen and the kidneys contained: spleen between 2.5% and 4%, and kidneys between 0.05% and 0.2%. All other tissues had lower contents. The bones (including the marrow) and the remaining tissues contained 1% and 0.7%. The radiolabelled compound was excreted in urine and within 28 days the excretion amounted to roughly 1%. Highest excretion occurred on day 1. Excretion by the faeces, indicative of biliary excretion, amounted to 3% over 28 days.

Kreyling et al., (2017b)

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306. A similar experiment to the one described above was also carried out, with the animals exposed via the oral route (a single dose of an aqueous, radiolabelled-nanoparticle suspension by intra-esophageal instillation) in female Wistar-Kyoto rats.

307. Titanium concentrations were determined at five retention time points 1 h, 4 h, 24 h, 7 d and 28 d after gavage in four rats for each time point. However, after observing in the seven-day experiment that fecal excretion of the test item was complete after 4–5 days, no further animals were sacrificed for a 28-day biodistribution.

308. Blood, all organs, tissues and excreta were collected, and the concentration of radioactive titanium dioxide was measured. Most of the radioactivity was excreted in the faeces. Absorption was calculated as the fraction of the dose that could not be accounted for by the radioactive content of the intestinal tract plus faeces. Approximately 0.6% of the dose was absorbed within an hour post-treatment. Seven days post treatment, roughly 0.05% of the dose administered was still present.

309. The authors noted that the distribution patterns between animals were variable and that several data were below the LOD during the first 4 hours. The spleen, kidneys, heart and uterus contained detectable levels even after 4 hours post treatment. Maximum retention was reached in the spleen, kidneys and heart at 24 hours post-treatment. In the liver, lung and blood, nanoparticle retention declined from 4 hours to 7 days. In the brain, uterus and kidneys, the highest concentrations were observed at day 7. The peak concentration in liver and spleen was 12.5% (4 hours) and 2.6% (24 hours) of the absorbed dose, respectively. According to the authors, due the slow excretion kinetics, accumulation of systemically circulating particles in specific cells and organs is likely to occur in subjects chronically exposed to titanium dioxide nanoparticles. When comparing the biodistribution of the radioactive titanium dioxide nanoparticles retained after oral administration with the results obtained after intravenous injection (Kreyling et al., 2017a), the authors concluded that the kinetics patterns are very different and intravenous injection does not appear an adequate surrogate for assessing the biodistribution occurring after oral exposure to titanium dioxide nanoparticles.

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Geraets et al., (2014)

310. In the EFSA 2021 Opinion, only the i.v. experiment was discussed; however, the paper also contains the result of an oral administration experiment.

311. The test items used were NM-100, NM101, NM-102, NM-103 and NM-104. More information is provided on these in paragraphs 123 – 127.

312. In the oral study, the single dose groups received a gavage dose of 2.3 mg/rat corresponding to 6.8-8.6 mg/kg bw. The repeat dose groups received five consecutive daily administrations of 2.3 mg titanium dioxide in one mL per rat resulting in a cumulative dose range of 34.1- 42.4 mg/kg bw for male rats and 54.5- 59.9 mg/kg bw for female rats.

313. In the i.v. study, the suspensions prepared contained 2.3 mg titanium dioxide/mL. The single i.v. dose treated rats received a dose of 8.4-9.8 mg/kg bw and 12.4- 14.1 mg/kg bw for male and female rats respectively, via the tail vein. The repeated dose treated rats received a cumulative dose that ranged from 42.3-49.4 mg/kg bw and 61.2-71.9 mg/kg bw for male and female rats, respectively. Thus, the actual dose in mg/kg bw depended on the weight of the rats.

314. After repeated oral exposure (overall dose of 11.5 mg Titanium dioxide) titanium levels were near or below the detection limit in liver and spleen, indicating a very low absorption. In two out of 30 liver/spleen samples of exposed animals (for NM-102 and NM-103) titanium levels were above the LOD, whereas all mesenteric lymph nodes (MLN) samples (including controls) contained titanium amounts above LOD. Only a small increase in titanium content was observed, because the background levels in MLN were 2–3 times the LOD. MLN from control rats contained 0.14 µg titanium whereas the highest titanium average was 0.36 µg and was located in MLN from NM-104 exposed rats. This gives an increase of 0.226 µg titanium in MLN or 0.003% of the 6895 µg Titanium exposure in the dose. The total recovery of dosed titanium in all tested organs (expressed as % of the total dose) was estimated to be approximately 0.02%.

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315. In the i.v. study, the highest levels were observed in the liver, but redistribution to the spleen was observed over the 90-day post-exposure period (Day 2/Day 6 and Day 90). Redistribution to remaining tissues was not identifiable. The authors hypothesised that release of particles from liver and possibly other organs may be responsible for the increase in spleen levels. Titanium was detected in all investigated tissues in the present study, i.e., blood, liver, spleen, kidney, lung, heart, brain, thymus and reproductive organs.

316. Both after single and repeated i.v. exposure, blood titanium levels in blood decreased rapidly during the first minutes after which the titanium levels slowly decreased and approached the limit of detection at 24 hours post exposure.

317. Based on the available data, the authors concluded that elimination of total Titanium dioxide has a long half-life. For the liver, which was considered the main target organ, the estimated half-life was 28–248 days.

318. The authors considered that the data showed that at the long run Titanium dioxide particles will accumulate in the spleen. Finally, they noted that the expected accumulation with daily exposure as a consequence of the negligible elimination might indicate a potential concern for human health risk.

Hendrickson et al., (2016)

319. The test materials used were NM-101 (5-10 nm) and NP-25 (20-25 nm).

320. Male Sprague Dawley rats were treated with 250 mg/kg bw/d of either one of the test materials, dispersed in an aqueous starch solution containing 0.1% Tween-80 and sonicated via intragastric administration for 28 days.

321. Within a day of the last exposure, the animals were sacrificed and blood samples as well as samples from the lungs, liver, spleen, testes, small intestine heart, stomach and kidneys were harvested.

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322. For animals treated with NM-101, titanium dioxide nanoparticles were detected in all organs and tissues. The organs with the highest concentrations were the spleen (0.227 µg/g) and liver (0.147 µg/g). In the kidneys, small intestine and testicles similar amounts of nanoparticles were detected (0.092, 0.098 and 0.089 µg/g respectively). Titanium dioxide nanoparticles were detected at 0.028 µg/g in the heart, 0.04 µg/g in the lungs and 0.049 µg/g in the brain.

323. In NP-25 treated animals' accumulation of titanium dioxide nanoparticles were detected in the small intestines and liver (0.29 µg/g in the liver) and at low levels (0.01 µg/g) in the kidneys. In the spleen, it was detected at levels of 0.11 µg/g of the organ. No titanium dioxide nanoparticles were detected in the lungs, brain, testicles, heart or blood.

324. The authors concluded that biodistribution differs between smaller (NM-101) and larger (NP-25) titanium dioxide nanoparticles, with the smaller particles showing a greater distribution spread and accumulation in all organs. The larger particles exhibited reduced but similar tendency. The main difference was that the larger nanoparticles could not overcome the blood brain barrier and penetrate the brain.

325. Due to the fact that the detected levels accounted for less than 1% of the administered dose, the authors concluded that the data was evidence of the limited bioavailability and efficient excretion of titanium dioxide.

Ammendolia et al. (2017)

326. The test material used was titanium dioxide nanoparticles (anatase, primary size < 25nm BET) surface area 45-55 m²/g, purity 99%, suspended; the suspensions were sonicated.

327. Sprague-Dawley rats (10/sex/group) were treated with 1 or 2 mg/kg bw/d titanium dioxide nanoparticles or vehicle only (ultrapure water) via gavage for 5 consecutive days.

328. Twenty four hours after the last dose, the animals were sacrificed, and the small intestine was excised. A piece of jejunum was used for histological analysis

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and the remaining part of small intestine was sampled for studying either tissue accumulation of titanium dioxide nanoparticles, determined as titanium by ICP-MS.

329. Titanium was detected in small intestine tissue at 0.08 ± 0.02 lg/g in the control, 0.09 ± 0.02 g/g in the low dose group and at 0.13 ± 0.03 lg/g at the high dose group.

Hendrickson et al., 2020

330. The test material used was titanium dioxide nanoparticles, rutile, rod/ needle like shape, 5 x 30 nm.

331. Wistar rats were treated with 50 mg/kg bw titanium dioxide using an isolated intestinal loop technique.

332. Three hours post treatment, the isolated loop was cut out. The liver and spleen were collected.

333. The presence of particles in tissues was studied by TEM and diffraction analysis. Loose agglomerates (100 nm and larger) were detected. Diffraction analysis was used to confirm that the particles were titanium dioxide. Titanium dioxide nanoparticles were detected on the surface and between the microvilli of the mucosal cells of the small intestine and also in the mucosal tissue. Nanoparticles were detected in the Peyer's patches, both as single nanoparticles and agglomerates of sizes ranging between 20 and 60 nm. In the liver, parenchymal tissue aggregates of titanium dioxide nanoparticles (150–200 nm) and up to 300 nm were seen. In the spleen red pulp, single nanoparticles (20–30 nm), agglomerates (up to 100 nm) and conglomerates (up to 800 nm) were observed.

Chen et al., 2020a

334. The test material used was titanium dioxide nanoparticles anatase, 29 nm (SEM).

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335. Sprague- Dawley rats were dosed with 0, 2, 10, 50 mg/kg bw by oral gavage for 90 days. The test material was sonicated prior to treatment.

336. The tissue distribution of the titanium dioxide nanoparticles was evaluated by determining the titanium content in blood and tissues including liver, stomach, small intestine, colon, spleen, heart, lung, kidneys and testicles by high resolution ICP-MS.

337. Significantly increased titanium dioxide nanoparticle levels were only detected in the colon of rats exposed to 50 mg/kg test material, compared with the control group. There was no dose-response relationship, however. The authors hypothesised that the significant increase of titanium dioxide in colon tissue was due to the titanium dioxide nanoparticles attaching on the surface of the colonic mucosa tissue and not in mucosa cells. As most of orally ingested test material was excreted through feces, it resulted to long-term retention in large intestine. The titanium dioxide nanoparticles did not enter the colon epithelial cells and were mainly deposited in the intestinal cavity or between villi. The content of Ti in all tissues was very low, which was approximately 0.0001%-0.00001% or 100-1000 ng/g tissue, except for the colon of the high dose group. All spleen and heart tissue samples from rats contained very low titanium levels, which were below the limit of detection (LOD) of 0.032 µg/g. Finally, they concluded that the results indicate that the absorption and distribution of titanium dioxide nanoparticles was very low after low-dose and long-term oral administration.

Warheit, Boatman and Brown, 2015

338. The COT noted that TiO₂ is able to move into the body through the GI tract. Warheit *et al.* (2015) evaluated the potential maternal or developmental toxicity of six different forms of predominantly NP form of TiO₂ in pregnant rats (22 or 23 rats per group). The dose levels for each form of TiO₂ were 0, 100, 300, and 1000 mg/kg bw per day. The TiO₂ formulations were in sterile water and delivered via oral gavage. This study noted that in a set of companion analyses, one was of the pigment-grade (pg) TiO₂ and the other was of the ultrafine (uf) TiO₂ test material (BET (Brunauer-Emmett-Teller) and the surface areas of pg and uf samples ranged from 7-17 m²/g

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and 50-82 m²/g respectively). Each material was evaluated for potential systemic exposure/uptake from the GI tract to the blood for circulation.

339. In three studies, Warheit et al. (2015b) dosed time-mated pregnant Sprague–Dawley, Crl:CD(SD), rats (n=22 per group), daily with TiO₂ by gavage on Gestational Days 6 to 20. Three additional studies included pregnant Wistar rats (n = 22 – 23 per group) exposed daily to TiO₂ by gavage from Gestational Days 5 to 19. The dose levels used in the studies were 0, 100, 300 or 1,000 mg/kg bw per day. The dose volume was 5 mL/kg bw per day with the TiO₂ specifications detailed below:

- i. anatase/rutile (89/11%)(uf-1), d50 = 43 nm (XSDC), d50 = 23 nm (TEM), irregular
- ii. anatase (100% nano) (uf-2), d50=42 nm (XSDC), d50=19 nm (TEM), irregular
- iii. rutile (100% nano) (uf-3), d50=47 nm (XSDC),d50=22 nm (TEM), rod-like
- iv. 4)anatase (27% nano) (pg-1), d50=153 nm (XSDC), d50=120 nm(TEM), irregular
- v. rutile (11% nano) (pg-2), d50=195 nm (XSDC), d50=165 nm (TEM), irregular.

340. No dose-dependent increase in TiO₂ was observed in the rat blood at 48 or 72 hours, or in the liver at 72 hours following a single exposure (2000 mg/kg bw TiO₂). These results suggested that there was little to no absorption of particles from the GI tract into the blood, which conflicted results from a previous study by Tassinari *et al.*, 2014.

Tassinari et al., 2014

341. In the Tassinari *et al.* (2014) study, rats were dosed with distilled water (controls) or TiO₂ NPs (anatase, primary size < 25nm, BET surface area 45–55 m²/g, purity 99%) by gavage for 5 consecutive days at 0, 1, or 2 mg/kg bw/day (7 rats/sex/group). Twenty-four hours after the last treatment (day 6), the animals were sacrificed, and the uterus, ovary, testes, thyroid and adrenals were excised and weighed. The spleen was sampled both for histopathological examination and for studying tissue deposition of Titanium dioxide nanoparticles.

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342. The highest total titanium concentration was detected in the thyroid. However, the difference in levels was not of statistical significance between the treated animals and the controls (0.24 ± 0.09 mg/g and 0.22 ± 0.04 mg/g of fresh thyroid weight in animals dosed with 2 mg/kg bw/day and controls, respectively). A significant increase was observed in the ovaries of animals dosed with 2 mg/kg bw/day TiO₂ NPs compared with control animals (0.28 ± 0.07 vs. 0.12 ± 0.04 mg/g fresh weight, respectively). Levels in uteri were low (0.051 ± 0.006 vs. 0.49 ± 0.04 mg/g fresh weight). A significant increase in the concentration of titanium in the spleen was observed in animals dosed with 2 mg/kg bw per day when compared to controls. (Tassinari *et al.* 2014). Overall, the authors considered that these results indicated the potential for titanium dioxide bioaccumulation. The COT considered that the strength of evidence for the absorption of TiO₂ from the GI tract into the blood was not clear.

[Bettini et al., 2017](#)

343. In addition to the rats dosed with E171 as described in paragraphs xx-xx, Bettini et al also assessed TiO₂-NPs using NM-105. As described previously, rats (10 per group) were dosed daily by gavage with NM-105 (10 mg/kg bs/day) for 7 days (the control group was given water). As described previously the NanoSIMS analyses of subcellular TiO₂ NM-105 showed a similar distribution in the immune cells of Peyer's patches, with the greatest concentration of particles in the central zones of the Peyer's patches which have a high concentration of immune cells. Ti was also located in the cytoplasm and nuclei of the Peyer's Patches cells.

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TOX/2023/xx Annex D

Summary table of studies

Absorption, Distribution, Metabolism and Excretion (ADME) – E171 animal studies

Reference	TiO ₂ characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls,	Results	Notes, comments, other
Talamini <i>et al.</i> , 2019	E171 (35% nano determined by TEM), 99.3% pure anatase, 201.2 ± 8.5 nm in suspension (NTA). No sonification or deagglomeration to simulate realistic conditions.	This work was reviewed by the Institute for Pharmacological Research Mario Negri IRCCS Animal Care and Used Committee (IACUC) and then approved by the Italian National Institute of Health (code:42/2016-PR).	Treatments were given 3 days per week for 3 weeks for a total of 9 treatments in 21 days. Average daily dose of ~2 mg/kg bw. Treatments were dripped slowly into the mice's mouths, allowing each drop to be swallowed.	NFR male mice (22/group) were administered either water (control) or 5 mg/kg bw E171 suspended in water. Ti concentrations in tissues were determined by single particle ICP-MS analysis.	Ti concentrations in the liver (0.94 ± 0.57 µg/g tissue) and large intestine (1.07 ± 0.38 µg/g tissue) were significantly higher in treated mice compared to controls. Ti concentrations in the brain, kidney, and testes were	This study aimed to investigate whether TiO ₂ is deposited in the digestive system and internal organs and whether there are any molecular and cellular alterations associated with an inflammatory response.

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Reference	TiO ₂ characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls,	Results	Notes, comments, other
					<p>below the quantification limit (<0.03 µg/g).</p> <p>Ti concentrations in lungs, spleen, stomach, and small intestine were not statistically significant between treated and control mice.</p>	
Riedle et al., 2020	E171, anatase, 119 nm.		Mice were exposed to 0, 1, 10, or 100 mg/kg bw/d E171 via the	Mice were divided into 4 groups of 18 and given 0, 6.25, 62.5, or 625 mg/kg diet	No evidence of gross alteration of immune-cell physiology or inflammation at doses up to 100	Author notes that '(OECD) guidelines for repeat dose oral toxicity testing (e.g., TG 408)

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Reference	TiO ₂ characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls,	Results	Notes, comments, other
			diet for 6, 12 and 18 weeks.	(equivalent to approximately 0, 1, 10, or 100 mg/kg bw). Then 6 mice per group were euthanized at 6, 12 and 18 weeks.	<p>mg/kg bw/d via the diet.</p> <p>Authors demonstrate E171 uptake by Peyer's patches, validating the delivery model.</p> <p>Presence of E171 particles detected by reflectance confocal microscopy (no quantification of particles completed).</p> <p>Weak signals observed at the base of Peyer's patches at low and mid-doses.</p>	do not mandate gavage but also allow for a test material to be incorporated in the diet or dissolved in drinking water, so any dietary approach could still be adopted within the OECD framework.'

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Reference	TiO ₂ characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls,	Results	Notes, comments, other
					Higher signals observed at highest dose, indicating evidence of dose-response.	

Allergenicity

Phue et al., (2022)	Food grade titanium dioxide nanoparticles and E171.		Used ELISA to study the alterations of the IgG binding, and mast cell degranulation assay to study allergenicity of milk and individual milk proteins (β-lactoglobulin and casein) in the presence of E171.	For ELISA, primary antibody for casein (Anti-casein rabbit antibody-cat # ab166596), primary antibody for β-lactoglobulin (Anti-LGB rabbit antibody-cat # ab112893) and secondary anti-rabbit antibody (cat # 6721) were used.	Significant enhancement in the allergenicity of milk proteins/ skimmed milk interacted with both E171 and food grade titanium dioxide nanoparticles. The presence of E171 showed the highest level of LAD2 degranulation (a proxy for allergenicity),	
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				Quebon skimmed milk was used.	followed by food grade titanium dioxide nanoparticles.	
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Inflammation and Immunotoxicity

Reference	TiO ₂ characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls,	Results	Notes, comments, other
Talamini <i>et al.</i>, 2019	E171 (35% nano determined by TEM), 99.3% pure anatase, 201.2 ± 8.5 nm in suspension (NTA). No sonification or deagglomeration to simulate realistic conditions.	This work was reviewed by the Institute for Pharmacological Research Mario Negri IRCCS Animal Care and Used Committee (IACUC) and then approved by the Italian National Institute of Health (code:42/2016-PR).	Treatments were given 3 days per week for 3 weeks for a total of 9 treatments in 21 days. Average daily dose of ~2 mg/kg bw. Treatments were dripped slowly into the mice's mouths, allowing each drop to be swallowed.	NFR male mice (22/group) were administered either water (control) or 5 mg/kg bw E171 suspended in water. Ti concentrations in tissues were determined by single particle ICP-MS analysis.	Ti concentrations in the liver (0.94 ± 0.57 µg/g tissue) and large intestine (1.07 ± 0.38 µg/g tissue) were significantly higher in treated mice compared to controls. Ti concentrations in the brain, kidney, and testes were below the	This study aimed to investigate whether TiO ₂ is deposited in the digestive system and internal organs and whether there are any molecular and cellular alterations associated with an inflammatory response.

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					<p>quantification limit (<0.03 µg/g).</p> <p>Ti concentrations in lungs, spleen, stomach, and small intestine were not statistically significant between treated and control mice.</p>	
<p>Pinget et al., 2019</p>	<p>E171, anatase, 30-300 nm.</p> <p>E171 was dispersed in drinking water using sonication.</p>		<p>Mice were exposed to E171 via drinking water for 4 weeks at doses of 0, 2, 10, 50 mg/kg bw/d. Dose is calculated based on water intake measured per cage.</p>	<p>Male C67BL/6JAusb mice were exposed to E171 via drinking water at doses of either 0, 2, 10, or 50 mg TiO₂/kg BW/day for 3 weeks to determine impact on colonic</p>	<p>At the highest dose tested, TiO₂ had minimal impact on the composition of the gut microbiota. Alterations in bacterial metabolites were observed</p>	

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			<p>Microbiota populations in fecal samples or the small intestine were examined through 16S rRNA sequencing.</p> <p>Canonical correspondence analysis (CCA) constrained to the 4 distinct TiO₂ concentrations used.</p>	<p>microbiota composition and on gut bacterial metabolites (10 mice/group).</p> <p>Incubated commensal bacteria derived from mouse colons anaerobically for 5 days with dose of 0, 2, 10, 50 µg/ml of TiO₂ biofilm formation (6 mice/group).</p> <p>Impact of TiO₂ on colonic epithelial function was determined by comparison of gene expression of key markers Muc2, Tjp1, Defb3, and Gzmb in colonic tissue of mice</p>	<p>from 10 mg/kg bw/d.</p> <p>Doses of 10 and 50 µg/ml TiO₂ significantly promoted biofilm formation by commensal bacteria.</p> <p>There was reduced expression of the colonic mucin 2 gene, a key component of the intestinal mucus layer, and increased expression of the beta defensin gene, indicating that TiO₂ significantly impacts gut homeostasis. These changes were associated with colonic</p>	
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				<p>administered 0, 2, 10, or 50 mg TiO₂/kg BW/day in drinking water (n = 5–8 mice per group).</p> <p>To investigate impact of TiO₂ on colonic inflammation, flow cytometric analysis, gene expression determined by qPCR and tissue staining were used on mice administered 0, 2, 10, or 50 mg TiO₂/kg BW/day in drinking water (n = 8–10 mice per group).</p> <p>TiO₂ impact on adaptive immune cell infiltration into the colon was investigated</p>	<p>inflammation, as shown by decreased crypt length, infiltration of CD8+ T cells, increased macrophages as well as increased expression of inflammatory cytokines.</p>	
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				using gene expression via qPCR and flow cytometric analysis on mice treated with 0, 2, 10, or 50 mg TiO ₂ /kg BW/day in drinking water (5-8 mice/group).		
Riedle <i>et al.</i> , 2020	E171, anatase, 119 nm.		Mice were exposed to 0, 1, 10, or 100 mg/kg bw/d E171 via the diet for 6, 12 and 18 weeks. E171 was formulated into diet.	6-week-old male and female C57BL/6 mice (6/sex/group) were exposed to E171 daily via diet for 6, 12 and 18 weeks. Mice were divided into 4 groups of 18 and given 0, 6.25, 62.5, or 625 mg/kg diet (equivalent to approximately 0, 1, 10, or 100 mg/kg bw). Then 6 mice per group were euthanized at 6,	No evidence of gross alteration of immune-cell physiology or inflammation at doses up to 100 mg/kg bw/d via the diet. Authors demonstrate E171 uptake by Peyer's patches, validating the delivery model. Presence of E171 particles detected by reflectance confocal	Author notes that '(OECD) guidelines for repeat dose oral toxicity testing (e.g., TG 408) do not mandate gavage but also allow for a test material to be incorporated in the diet or dissolved in drinking water, so any dietary approach could still be adopted within the OECD framework.'

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				12 and 18 weeks.	microscopy (no quantification of particles completed). Weak signals observed at the base of Peyer's patches at low and mid-doses. Higher signals observed at highest dose, indicating evidence of dose-response.	
Liu et al., 2020	This is a review, and is only mentioned once in the TiO ₂ statement in a quote from the Health Canada report.					
Han et al., 2020	E 171, anatase, 150 nm, 99.5% purity.	Study conducted according to OECD TG 408.	E171 suspended in distilled water, sonicated for at least 10 minutes. E171 administered by	Sprague–Dawley rats (10/sex/group) were administered E171 by oral gavage at doses of 0, 10, 100 or 1,000	Statistically significant decreases in GM-CSF plasma levels (~30% in females) and plasma IgM (~12% in	

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			<p>oral gavage at doses of 0, 10, 100 or 1,000 mg/kg bw/d for 90 days.</p> <p>Quantitative analysis in Sprague–Dawley rat’s tissues.</p>	<p>mg/kg bw/d for 90 days.</p> <p>Ti concentrations were measured in the colons, kidneys, and spleens harvested from all rats at necropsy.</p>	<p>females and 9% in males) were observed at the highest dose compared to controls.</p> <p>E171 accumulation in the stomach wall of several rats administered 1,000 mg/kg E171 for 90 days.</p> <p>Ti concentration increased in the colons of both sexes administered 1,000 mg/kg E171 compared with the control, while colonic, superoxide dismutases (SOD)-1 (male and female) and SOD-2 (female) protein levels</p>	
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					<p>were down-regulated.</p> <p>When exposed to AGS cells (human stomach epithelial cell line) for 24 h, E171 (40 µg/mL) was located in the perinuclear region. The E171 treatment affected expression of ER stress-related proteins but did not induce cell death up to the used maximum concentration (40 µg/mL). A gene profile analysis also showed that immune response-related microRNAs</p>	
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					were most strongly affected by E171 exposure.	
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Studies used to review the toxicokinetic and absorption of the nanoparticle form of TiO₂

Reference	TiO ₂ characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls,	Results	Notes, comments, other
Tassinari et al., 2014	TiO ₂ nanoparticles (anatase, primary size <25 nm, BET surface area 45-55 m ² /g, purity 99%).	All experiments on animals were performed according to the European Community Council Directive 86/609/EEC (EEC 1986).	TiO ₂ nanoparticles were administered by oral gavage over 5 consecutive days at a dose of 0, 1, 2 mg/kg body weight per day.	Sprague-Dawley rats were divided into 3 treatment groups (7 rats/sex/group). Treatment groups were high dose (2 mg/kg bw), low dose, (1 mg/kg bw), and controls (CTRL) (vehicle only (distilled water)).	In the high dose treatment group, significant increases in total Ti tissue levels were found in spleen (0.036 ± 0.009 vs. 0.046 ± 0.008 µg/g fresh weight; p ≤ 0.05) and ovaries (0.28 ± 0.07 vs. 0.12 ± 0.04 µg/g fresh weight; p ≤ 0.01). Sex-related histological alterations were observed at both dose levels in thyroid, adrenal medulla, adrenal cortex (females) and	

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					<p>ovarian granulosa, without general toxicity.</p> <p>Altered thyroid function was indicated by reduced T3 (males). Testosterone levels increased in high-dose males and decreased in females.</p> <p>In the spleen of treated animals TiO₂ aggregates and increased white pulp (high-dose females) were detected, even though Ti tissue levels remained low reflecting the low doses and the short exposure time.</p>	
Warheit et al., 2015	1) anatase/rutile (89/11%) (uf-1), d50=43 nm d50=23 nm	OECD Guideline 414	Sterile water-based TiO ₂ sample formulations were administered by oral gavage to time-	Three studies (Group size n=22): Time-mated pregnant Sprague–Dawley rats,	At 1,000 mg uf-1/kg per day, mean fetal sex ratio and the means for male	

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	<p>Methods: XSDC and TEM respectively Shape: Irregular</p> <p>2) anatase (100% nano) (uf-2) d50= 42 nm d50=19 nm Methods: XSDC and TEM respectively Shape: Irregular</p> <p>3) rutile (100% nano) (uf-3), d50=47 nm d50=22 nm Methods: XSDC and TEM respectively Shape: rod- like</p> <p>4) anatase (27% nano)</p>		<p>mated rats from the time of approximate implantation until the day prior to expected parturition.</p> <p>Dose levels: 0, 100, 300 or 1,000 mg/kg bw per day</p> <p>Dosage volume: 5 mL/kg bw per day.</p>	<p>(CrI:CD(SD)) exposed to TiO₂ (uf- 1, uf-3 and pg-1) by gavage on Gestational Days 6–20.</p> <p>Three additional studies (Group size n=22-23) pregnant Wistar rats exposed to TiO₂ (uf-2 and pg-2) by gavage from Gestational Days 5 to 19.</p> <p>Necropsy:</p> <ul style="list-style-type: none"> • gross examination of the dam • counting of corpora lutea • implantation sites • resorptions • live and dead fetuses • fetal sex • fetal weight. • fetal pathological external, 	<p>and female fetuses per litter were statistically significantly different from the control group means.</p> <p>Mean male fetuses: 7.2 Mean male fetuses control group: 5.5 Test facility historical control group data range: 5.2 to 7.4.</p> <p>Mean female fetuses: 4.8 Mean female fetuses control group: 6.7 Test facility historical control group data range: 5.8 to 8.3.</p> <p>Mean fetal sex ratio of the 1,000 mguf-1/kg bw per</p>	
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	<p>(pg-1), d50=153 nm d50=120 nm Methods: XSDC and TEM respectively Shape: irregular</p> <p>5) rutile (11% nano) (pg-2), d50=195 nm d50=165 nm Methods: XSDC and TEM respectively Shape: irregular</p>			<p>visceral and skeletal examinations for abnormalities</p>	<p>day group: 60% (males/females) Mean control group fetal sex ratio: 46% Test facility historical control group data range: 43% to 53%.</p> <p>A few incidental changes in body weight and feed intake were noted, no other changes were observed in the dams or the fetuses.</p>	
Bettini et al., 2017	<p>1) E 171, anatase, 20–340 nm (118 nm) (TEM); 44.7% particles < 100 nm;</p> <p>2) TiO₂ NPs (NM-105), anatase/rutile, 15–24 nm.</p>	OECD?	<p>Series One Dosage: 200 µ L with TiO₂ NM-105, E171 (10 mg/kg of BW/day) or water for 7 days by gavage.</p> <p>Series Two Dosage: E-171 at 200 µ g or 10 mg/kg of BW/day</p>	<p>Series One: rats (n = 10 rats/group) dosed daily by intragastric gavage (200 µ L) with TiO₂ NM-105, E171 (10 mg/kg of BW/day) or water for 7 days.</p>	<p>Titanium was detected in the immune cells of Peyer's patches. Dendritic cell percentage were increased, observed days after exposure but</p>	<p>Peyer's patches = clusters of subepithelial, lymphoid follicles found in the intestine</p>

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			<p>via drinking water for 100 day (with or without DMH treatment)</p> <p>Series Three Dosage: No treatment followed by a single dose of 10 mg/kg E-171</p>	<p>Tissue imaging, flow cytometry and cytokine assays, tissue inflammation and gut permeability measurements were conducted.</p> <p>Series Two: rats (n = 11 to 12 per group) were treated or not with 1,2-dimethylhydrazine (DMH) to induce colon carcinogenesis and exposed to E-171 at 200 µg or 10 mg/kg of BW/day via drinking water for 100 days. Control animals (n = 12) received water only.</p> <p>Flow cytometry and cytokine assays were assessed for gut inflammation and ACF.</p>	<p>no effect at 100 days.</p> <p>No effects in the spleen.</p> <p>Regulatory T cells and T-helper cells were significantly decreased days after exposure and at 100 days for rats exposed to E 171.</p> <p>Stimulation in vitro of immune cells isolated from Peyer's patches had a decrease in T-helper (Th)-1 IFN-γ secretion and splenic Th1/Th17 inflammatory responses increased.</p> <p>With exposure to TiO₂ NP there was an observed increase in the</p>	<p>Th = T-helper cells</p>
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				<p>Series Three: untreated rats (n = 4) were evaluated for ex-vivo cytotoxicity and proliferative assays on isolated immune cells.</p> <p>E-171 particle agglomeration was assessed in the luminal contents of the jejunum and colon collected from 4 rats 4 h after a single dose of 10 mg/kg was delivered.</p>	<p>percentage of dendritic cells in Peyer's patches with no decrease in the percentage of Tregs.</p> <p>E 171 exposure (1 week) did not initiate intestinal inflammation, but E 171 treatment (100-day) showed colon microinflammation - significantly increased IL-1β, IL-8 and TNF-α expression in the colon and increased IL-10.</p>	
Karimpour <i>et al.</i> , 2018	TiO ₂ NPs, anatase, 10–25 nm		<p>One dose of TiO₂ NP (100 mg/kg per day) or the test vehicle (control group) daily for 5 weeks</p> <p>NMRI = Naval Medical Research Institute</p>	<p>54 ten week old (25\pm2 g) adult female NMRI mice were divided into a control group which received vehicle (saline solution) orally and TiO₂ NP group which received 100 mg/kg</p>	<p>There was a significantly decreased pregnancy rate (70% vs. 100% in the control group), a 20% decrease in litter size and increases in circulating</p>	

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				<p>per day TiO₂ NP solution orally.</p> <p>Pregnancy and in vitro fertilization rates, histological changes in ovaries, malondyaldehyde and estrogen hormone levels in the blood serum were assessed after five weeks.</p> <p>24 hours post last administration of test item: 3 control or test female mice were housed with 3 male mice for 11 days. The percentage of pregnancy and numbers of newborns were evaluated.</p>	<p>oestrogen (20%) and MDA (25%).</p> <p>Degeneration and reduction of follicles, cyst formation and impairment of follicular development in the ovaries was observed in the TiO₂ NPs group but no quantitative data was provided. Additionally a lower number of oocytes was isolated from the TiO₂ NP group as well as a higher percentage of developmental arrest before the blastocyst stage after in vitro fertilisation. The authors proposed that this could have been an indirect effect of</p>	
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					TiO ₂ NPs through the generation of increased ROS levels.	
Khorsandi <i>et al.</i> , 2016	TiO ₂ NPs < 30 nm		<p>Test item: NTiO₂ nanopowder (TNP, Sigma) made with 100 ml BSA (bovine serum albumin) solution dissolve in Milli-Q water.</p> <p>Oral Dosage Groups:</p> <p>TNP-1: 75 mg/kg TNP</p> <p>TNP-2: 100 mg/kg TNP</p> <p>TNP-3: 300 mg/kg TNP</p> <p>Control: saline solution</p>	<p>32 adult 6–8 weeks old male NMRI mice (25–30 g)</p> <p>Four groups of 8 mice with a dosage of 75, 100 and 300 mg/kg TNP for 35 consecutive days respectively for each of the test groups and the control group received saline orally for 35 consecutive days.</p> <p>Testicular testosterone levels, testis weight, total volumes of testis, seminiferous tubules, interstitial tissue and total Leydig cell numbers were measured.</p>	<p>Body weight was unaffected by treatment.</p> <p>Dose-dependent decreases in testis weight were observed from a dose of 100 mg/kg bw per day.</p> <p>Mid- and high-dose groups showed decreases in serum and testicular testosterone levels, the diameter and total volume of seminiferous tubules, the height of the spermatogenic</p>	

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					epithelium and total Leydig cell numbers however the total volume of the interstitial tissue increased.	
Lee <i>et al.</i> , 2019	TiO ₂ NPs P25 (15–24 nm)	OECD Guideline 414 (Pre-natal Toxicity Study)	<p>Test item: Nanoparticles in deionised water.</p> <p>80/20 anatase/rutile.</p> <p>Mean diameter of approximately 21 nm (minimum of 100 particle sizes averaged) administered daily by oral gavage.</p> <p>Dosage: Test item was administered from Gestational Days 6 to 19 at dose levels of 0, 100, 300 and 1000 mg/kg with a dose volume of 10 mL/kg.</p>	<p>Sprague–Dawley rats (12 females per group)</p> <p>Quantitative analysis in blood/tissues.</p> <p>Four groups of twelve females per group in the toxicology group (total test animals: 48) and four groups of four females in the tissue distribution group (total test animals: 16)</p>	<p>No statistically significant differences in general clinical signs, body weight, organ weights (absolute and relative to body weight), macroscopic findings except a statistically significant decrease in food intake but no correlated decreased body weight or body weight gain during the study period of the females of the high-dose group.</p>	

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					<p>No statistically significant differences in caesarean section parameters and fetal external and visceral examination.</p> <p>A small but statistically significant increase (4%) was observed in the number of ossification centres in the metatarsals of both hindlimbs of the fetuses of 100 mg/kg bw per day group which may have been incidental.</p>	
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Aberrant Crypt Foci (ACF) as a marker for carcinogenicity

Reference	TiO ₂ characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls,	Results	Notes, comments, other

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<p>Blevins et al., 2019</p>	<p>E 171, anatase, 110–115 nm (SEM), 36% particles < 100 nm.</p>		<p>E-171 concentrations: 0 mg/kg* dose (22.3 ± 1.2, 23.7±1.8 ppm) 40 ppm dose (59.6 ±1.1, 61.0±2.6 ppm) 400 ppm dose (384 ± 8, 387±13 ppm) 5000 ppm dose (4310 ± 132, 4610±160 ppm).</p>	<p>Six-week-old male Wistar Han IGS (Cri:WI (Han)) rats.</p> <p>Test material: Food grade sample E-171. Different grades of commercially-available E-171 were averaged to produce the test material supplied. Test material was added to feed.</p> <p>Two feed batches: batch one was fed throughout the 7-day study and through week 10 of the 100-day study. Batch two was fed post-week 10 of the 100-day study.</p> <p>7-day study: 4 groups of 5 animals</p>	<p>E-171 consumption did not alter T-cell-mediated mechanisms of immune control.</p> <p>Dietary E-171 did not induce inflammation peripherally or in the GI tract.</p> <p>An increase was observed in the relative spleen weight in 22.4 mg E-171/kg bw per day + DMH compared to not initiated animals and an increase in IL-17A in colon (22.4 mg E 171/kg bw per day + DMH) and IL-12p70 in plasma (3.5 mg E 171/kg bw per day + DMH), with no dose-related effects.</p>	
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				<p>(randomised based on weight). Total food and water consumption were calculated at the end of the study. Body weights measured at the start and end of the 7-day exposure period.</p> <p>100-day study: 8 groups of 16 animals.</p> <p>Groups 1-4 were dosed with 180 mg/kg BW dimethylhydrazine dihydrochloride (DMH · 2HCl) in 1.5% EDTA-0.9% NaCl, pH 6.5 by intraperitoneal injection (the highest dose that showed no signs of toxicity in pilot studies).</p>	<p>No changes were observed in spleen cellularity.</p> <p>No changes were observed in the percentage of CD103+ DC, CD4+ T helper cells or total or activated Treg in peripheral blood, spleen or Peyer's patches in animals exposed to E-171 + DMH compared to animals treated with only DMH.</p> <p>No treatment related histopathological changes were observed in the duodenum, jejunum, ileum, spleen, liver, lung and testes</p>	
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				<p>Animals in groups 5-8 were given a dose of 1.5% EDTA-0.9% NaCl, pH 6.5. by intraperitoneal injection.</p> <p>Seven days post-injection, feeding of the test material was commenced at 0, 40, 400, or 5000 ppm E-171 in groups 1-4 and 5-8 for 100 days.</p>	<p>in animals exposed only to E-171.</p> <p>Rats treated with DMH only and those which received E-171 in the diet after the initiation displayed several histopathological abnormalities</p> <p>A significant surface area of the epithelial surface of the sampled colon (proximal, middle and distal) was obscured when observed by light microscopy therefore the entire surface of the colon samples for ACF and ABC could not be examined.</p>	
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					No change in the number of ACF and ABC were observed due to E-171 exposure alone.	
Akagi et al., 2023 – 28 Day Study	6 nm TiO ₂ nanoparticles		5 female and 5 male F344/DuCrI CrIj rats	TiO ₂ NPs with a crystallite size of 6 nm were examined in male and female F344/DuCrI CrIj rats by repeated oral administration of 10, 100, and 1000 mg/kg bw/day (5/sex/group) for 28 days	No mortality was observed in any group, and no treatment-related adverse effects were observed in body weight, urinalysis, haematology, serum biochemistry, or organ weight. Histopathological examination revealed TiO ₂ particles as depositions of yellowish-brown material. The particles observed in the gastrointestinal lumen were also	

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					<p>found in the nasal cavity, epithelium, and stromal tissue in the 28-day study.</p> <p>Overall, No effects were observed after repeated oral administration of TiO₂ with a crystallite size of 6 nm at up to 1000 mg/kg bw/day regarding general toxicity, accumulation of titanium in the liver, kidneys, and spleen, abnormality of colonic crypts, and induction of DNA strand breaks and chromosomal aberrations.</p>	
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Reproductive toxicity

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Reference	TiO ₂ characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls,	Results	Notes, comments, other
TDMA, 2020 – Main Study	<p>Test substance: Anatase E-171, median particle diameter 99.9 (± 2.0 nm), 51% of particles < 100 nm.</p> <p>Dietary particle size: 109.2 (± 1.4) to 113.7 (± 4.9 nm), 31-43% of particles < 100 nm.</p>	OECD Test Guideline 443	<p>Dosages (by oral diet, dependent on endpoint for each cohort):</p> <p>F0, F1-1A and 1B - 0, 100, 300, and 1000 mg/kg bw/day over 10 weeks (prior to mating and up to the end of weaning periods)</p> <p>F1-2A and 2B: indirectly exposed to test item via milk of feed-dosed rats</p> <p>Additional background levels of TiO₂ in feed were estimated to be approximately 1.4 mg/kg bw/day</p> <p>Diet mixtures were prepared weekly. Food intake was monitored and volumes of dosed feed was adjusted</p>	<p>96 male and 96 female CD® (Sprague Dawley) IGS Rat (CrI:CD(SD))</p> <p>Test Grouping Sizes:</p> <p>F0 – 4 groups, 20 male, 20 female per group.</p> <p>F1-1A and 1B – 20 male, 20 female</p> <p>F1-2A, 2B and 3–4 groups, 10 male, 10 female</p> <p>Endpoint Groupings:</p> <p>F1-1A and 1B - reproductive & developmental toxicity</p>	<p>Results:</p> <p>F0 - Dose-dependent marginal increase in TiO₂ blood and urine concentration in rats dosed with 1000 mg/kg bw/day</p> <p>No test item-related effects on sexual function or fertility in males or females. No test item-related pre- or postnatal loss observed</p> <p>No test item-related thyroid hormone or haematological effects..</p>	KLH (Keyhole Limpet Haemocyanin)

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			<p>according to previous consumption weekly.</p> <p>Administration of KLH and cyclophosphamide KLH via intravenous bolus injection in F3 cohort.</p>	<p>F1-2A and 2B - developmental neurotoxicity</p> <p>F3 - developmental immunotoxicity</p> <p>Test Item Exposure:</p> <p>F0 - Exposure to test item was from 10 wks prior to mating until F1 generation weaning.</p> <p>F1 – Exposure to test item was from weaning to PND 4/PND 8 of F2 generation</p> <p>F2 – Exposure from birth until PND 4 or PND 8 (via milk)</p>	<p>No test item-related differences in splenic lymphocyte subpopulation distribution</p> <p>No test item-related changes related to histopathology examinations including the testis and epididymides and intestinal examinations for ACF.</p> <p>Reproductive & developmental toxicity F1-1A and 1B</p> <p>No test item-related effects on sexual function or fertility in males (F1-1A – sexual hormones and</p>	
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					<p>maturation and sperm) or females (F1-!B - sexual maturation and hormones, number and length of estrous cycles, follicle number or corporea lutea). No treatment-related pre- or postnatal loss . No external or internal abnormalities detected in pups.</p> <p>No test item-related effects on growth or sexual development.</p> <p>No test item-related differences in body weight or food consumption.</p> <p>No test item-related thyroid</p>	
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					<p>hormone or haematological effects reported at any dose for F1-1A cohort.</p> <p>No test item-related differences in weight or histopathology (spleen, thymus, lymph nodes, bone marrow, white blood cell count, and splenic lymphocyte subpopulation distribution in F1-1A.</p> <p>No test item-related effects on pathology or histopathology.</p> <p>There was a statistically significant decrease in the antigen specific</p>	
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					<p>IgM level was measured at the highest dose tested (1,000 mg/kg bw/d) in males only (-9%) and without an apparent dose-response.</p> <p>No dose-dependent increase in TiO₂ blood concentration in rats up to 1000 mg/kg bw/day</p> <p>Observation of pale faeces not of toxicological relevance.</p> <p>Developmental neurotoxicity F1-2A and 2B</p> <p>No test item-related pre- or postnatal loss</p>	
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					<p>observed. No external or internal abnormalities detected in pups.</p> <p>No test item-related effects on growth or sexual development.</p> <p>No test item-related differences in body weight or food consumption.</p> <p>No test item-related effects on neurofunctional endpoints (F1-2A)</p> <p>Dose-dependent elevations of blood TiO₂ levels were found exceeding 300 mg/kg</p> <p>No test item-related effects on</p>	
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					<p>pathology or histopathology.</p> <p>Observation of pale faeces not of toxicological relevance.</p> <p>Developmental immunotoxicity F3</p> <p>No test item-related differences in body weight or food consumption.</p> <p>No mortality and no test item-related effects reported at any dose for any generation.</p> <p>No ACF were found in the colons of animals in any dose group.</p>	
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					Observation of pale faeces not of toxicological relevance.	
LPT, 2020 – Satellite study	Test substance: Anatase E-171, 51% of particles < 100 nm. Dietary particle size: 31-43% of particles < 100 nm.	OECD Test Guideline 443	F0 satellite group: 0, 100, 300, and 1000 mg/kg bw/day over 10 weeks (prior to mating and up to the end of weaning periods)	CD® (Sprague Dawley) IGS Rat (CrI:CD(SD)) F0 satellite group – 30 male, 30 female per group + additional 40 (20 male, 20 female) for use as an F1 generation of satellite animals to be used as the positive control group in the KLH-assay (?) Endpoint: ACF	No test item-related effects in behaviour or external appearance. No test item-related thyroid hormone effects. No test item-related effects on body weight, food consumption and water consumption. No test item-related effects on haematology and biochemical parameters or urinalysis.	

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					<p>No test item-related effects on thyroid and sexual hormones or sperm.</p> <p>No test item-related changes in bone marrow or organ weights. No test item-related histopathological effects in the high dose group.</p> <p>No test item-related induction of aberrant crypt foci (ACF) in rat colons.</p> <p>Satellite animals of F1 used as the positive control for the KLH assay (at a different time and ages from F1-3 cohort). No additional controls were tested.</p>	
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					<p>No test animals died prior to sacrifice.</p> <p>Observation of pale faeces not of toxicological relevance.</p>	
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Immunotoxicity

Reference	TiO ₂ characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls,	Results	Notes, comments, other
Han <i>et al.</i> , 2020	E171, anatase, 150 nm, 99.5% purity.	Study conducted according to OECD TG 408.	<p>E171 suspended in distilled water, sonicated for at least 10 minutes.</p> <p>E171 administered by oral gavage at doses of 0, 10, 100 or 1,000 mg/kg bw/d for 90 days.</p>	Sprague–Dawley rats (10/sex/group) were administered E171 by oral gavage at doses of 0, 10, 100 or 1,000 mg/kg bw/d for 90 days.	Statistically significant decreases in GM-CSF plasma levels (~30% in females) and plasma IgM (~12% in females and 9% in males) were observed at the highest dose	

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			Quantitative analysis in Sprague-Dawley rat's tissues.	Ti concentrations were measured in the colons, kidneys, and spleens harvested from all rats at necropsy.	compared to controls. E171 accumulation in the stomach wall of several rats administered 1,000 mg/kg E171 for 90 days. Ti concentration increased in the colons of both sexes administered 1,000 mg/kg E171 compared with the control, while colonic, superoxide dismutases (SOD)-1 (male and female) and SOD-2 (female) protein levels were down-regulated.	
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					<p>When exposed to AGS cells (human stomach epithelial cell line) for 24 h, E171 (40 µg/mL) was located in the perinuclear region. The E171 treatment affected expression of ER stress-related proteins but did not induce cell death up to the used maximum concentration (40 µg/mL). A gene profile analysis also showed that immune response-related microRNAs were most strongly affected by E171 exposure.</p>	
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<p>NCI, 1979 – see link -></p>	<p>TR-097: Titanium Dioxide (CASRN 13463-67-7) (nih.gov)</p> <p>Titanium dioxide anatase Purity: 98%</p>		<p>Groups of 50 rats of each sex and 50 mice of each sex were administered titanium dioxide in the diet at one of two doses, either 25,000 or 50,000 ppm, for 103 weeks and then observed for 1 additional week. Matched controls consisted of 50 untreated rats of each sex and 50 untreated mice of each sex. All surviving rats and mice were killed at 104 weeks</p>	<p>Administration of the titanium dioxide had no appreciable effect on the mean body weights of rats or mice of either sex. With the exception of white feces, there was no other clinical sign that was judged to be related to the administration of titanium dioxide. Survival of the rats and the male mice at the end of the bioassay was not affected by the test chemical; mortality in female mice was dose related. Sufficient numbers of dosed and</p>	<p>In the male and female mice, no tumours occurred in dosed groups at incidences that were significantly higher than those for corresponding control groups. It is concluded that under the conditions of this bioassay, titanium dioxide was not carcinogenic by the oral route for Fischer 344 rats or B6C3F1 mice.</p>	
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				control rats and mice of each sex were at risk for development of late-appearing tumors.		
Akagi et al., 2023 – 28 Day Study	6 nm TiO ₂ nanoparticles		5 female and 5 male F344/DuCrI CrIj rats	TiO ₂ NPs with a crystallite size of 6 nm were examined in male and female F344/DuCrI CrIj rats by repeated oral administration of 10, 100, and 1000 mg/kg bw/day (5/sex/group) for 28 days	No mortality was observed in any group, and no treatment-related adverse effects were observed in body weight, urinalysis, haematology, serum biochemistry, or organ weight. Histopathological examination revealed TiO ₂ particles as depositions of yellowish-brown material. The particles observed in the gastrointestinal lumen were also found in the	

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					nasal cavity, epithelium, and stromal tissue in the 28-day study. Overall, No effects were observed after repeated oral administration of TiO ₂ with a crystallite size of 6 nm at up to 1000 mg/kg bw/day regarding general toxicity, accumulation of titanium in the liver, kidneys, and spleen, abnormality of colonic crypts, and induction of DNA strand breaks and chromosomal aberrations.	
Akagi et al., 2023 – 90 Day Study	6 nm TiO ₂ nanoparticles		10 female and 10 male F344/DuCrIcrIj rats		TiO ₂ NPs with a crystallite size of 6 nm were examined in male and female	No mortality was observed in any group, and no treatment-related adverse effects

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					<p>F344/DuCrI CrIj rats by repeated oral administration of 100, 300, and 1000 mg/kg bw/day (10/sex/group) for 90 days</p>	<p>were observed in body weight, urinalysis, haematology, serum biochemistry, or organ weight. In addition, they were observed in Peyer's patches in the ileum, cervical lymph nodes, mediastinal lymph nodes, bronchus-associated lymphoid tissue, and trachea in the 90-day study.</p> <p>Overall, no effects were observed after repeated oral administration of TiO₂ with a crystallite size of 6 nm at up to 1000 mg/kg bw/day regarding</p>
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						general toxicity, accumulation of titanium in the liver, kidneys, and spleen, abnormality of colonic crypts, and induction of DNA strand breaks and chromosomal aberrations.
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Neurotoxicity

Reference	TiO ₂ characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls,	Results	Notes, comments, other
<i>Sofranko et al., 2021</i>	10 mg/g TiO ₂ , 2 mg/g polyvinylpyrrolidone-coated Ag	OECD 424 Neurotoxicity study in the rodents		10 female and 10 male C57BL/6J mice	The mice were fed ad libitum with food pellets dosed with 10 mg/g TiO ₂ , 2 mg/g polyvinylpyrrolidone-coated Ag or control pellets for 28 days	The neurotoxicity of TiO ₂ and Ag NMs, applied in food pellets, in male and female C57BL/6 J mice in a 28-day oral exposure study with or without a 14-day post-exposure recovery period. No major neuropathological changes regarding

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						<p>neuroinflammation in biochemical and immunohistochemical analyses could be observed and behavioural changes in anxiety and cognition were absent. However, in the Ag NM exposed mice motor performance effects were observed by the rotarod test that differed between sexes. The female mice that were exposed to Ag NM for 28 days, showed a consistent diminished motor coordination and increased cortical activity of specific tyrosine kinases.</p> <p>Female mice that were exclusively investigated in a subsequent toxicokinetic study also revealed whole</p>
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						brain levels of Ag that steadily increased during the 28 days of exposure and persisted up to 4 weeks post-exposure. Our study demonstrates that subacute exposure to foodborne TiO ₂ and Ag NMs does not cause marked neurotoxicity in mice. However, our toxicokinetic and specific toxicodynamic findings with Ag NMs indicate that long-term oral exposures to this nanomaterial may cause adverse effects on the central nervous system in a sex dependent manner.
Grissa et al. (2016)	TiO ₂ NPs, anatase, 5–12 nm (TEM, XRD).		Internal exposure: quantitative in male Wistar rat		There was a statistically significant dose-related increase in	

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			tissues; methodology with important flaws.		the level of NO in 100 and 200 mg/kg bw per day TiO ₂ NPs groups observed and a statistically significant dose- related increase in brain TNF- α in 200 mg/kg bw per day TiO ₂ NPs group.	
<i>Gerber et al., 2022</i>	TiO ₂ NPs, average primary particle size of 26.2 \pm 10.7 nm			The aim of the study was to investigate the effects of two common types of NP, titanium dioxide NP (TiO ₂ NP) and silver NP (AgNP), on neuronal function following acute (0.5 h), sub-chronic (24 h and 48 h) and chronic (14 days) exposure in vitro rat cortical cells. Acute and		

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				sub-chronic exposure to TiO ₂ NP is without effects, whereas chronic exposure only modestly reduces neuronal function without affecting morphology.		
Ciu et al., 2021			36 male Sprague Dawley rats aged postnatal day 21 (PND 21) were injected intraperitoneally with TiO ₂ NPs (20 mg/kg) and/or BEO (200 mg/kg).	TiO ₂ NPs exposure during the adolescent period induced anxiety-like behaviour, cognitive impairment, neuroinflammation and oxidative damage in hippocampus, and BEO treatment could significantly ameliorate the neurotoxicity induced by TiO ₂ NPs exposure.		

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Naima et al., 2021			Rats were injected intravenously with a single dose of TiO ₂ -NPs (20 mg/kg body weight) and were subjected to cognitive and emotional tests using Morris water maze and elevated plus maze.	Acute intravenous injection of TiO ₂ -NPs impaired behaviour performances through brain biochemical and structural changes and precautions should be taken to their usage in food additive and medical applications.		
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TOX/2023/xx Annex E

Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment.

Third draft statement on the safety of Titanium Dioxide (E171) as a Food Additive

Occurrence levels of E171 TiO₂ used in the exposure assessment scenarios.

Table 1: Occurrence levels of titanium dioxide (E171) used in the exposure assessment scenarios (mg/kg or mg/L as appropriate)

EFSA Food category number	Food category name	Concentration levels used in the exposure assessment (Maximum reported)	MPL (mg/L or mg/kg as appropriate)
01.4	Flavoured fermented milk products including heat-treated products*	48	QS
01.5	Dehydrated milk as defined by Directive 2001/114/EC	-	QS
01.6.3	Other creams	-	QS
01.7.1	Unripened cheese, excluding products falling in category 16.	-	QS
01.7.3	Edible cheese rind	-	QS
01.7.4	Whey cheese	-	QS
01.7.5	Processed cheese	-	QS
01.7.6	Cheese products, excluding products falling in category 16	-	QS
01.8	Dairy analogues, including beverage whiteners*	125	QS
03	Edible ices*	857	QS
04.2.4.1	Fruit and vegetable preparations, excluding compote	-	QS
04.2.4.1	Fruit and vegetable preparations, excluding compote	-	QS
04.2.5.3	Other similar fruit or vegetable spreads	-	QS

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05.2	Other confectionery including breath-refreshening microsweets*	4,500	QS
05.3	Chewing gum*	16,000	QS
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4*	20,000	QS
06.3	Breakfast cereals	-	QS
06.5	Noodles	-	QS
06.6	Batters	-	QS
06.7	Pre-cooked or processed cereals	-	QS
07.2	Fine bakery wares*	318	QS
08.3.3	Casings and coatings and decorations for meat	-	QS
09.2	Processed fish and fishery products, including molluscs and crustaceans	-	QS
09.2	Processed fish and fishery products, including molluscs and crustaceans	-	QS
09.2	Processed fish and fishery products, including molluscs and crustaceans	-	QS
09.2	Processed fish and fishery products, including molluscs and crustaceans	-	QS
09.3	Fish roe	-	QS
12.2.2	Seasonings and condiments	-	QS
12.4	Mustard	-	QS
12.5	Soups and broths*	193	QS
12.6	Sauces*	4,000	QS
12.7	Salads and savoury-based sandwich spreads*	3,000	QS
12.9	Protein products, excluding products covered in category 1.8*	5,000	QS
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	-	QS
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	-	QS

This is a draft statement for discussion. This does not represent the views of the Committee and should not be cited.

13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009	-	QS
14.1.4	Flavoured drinks*	70	QS
14.2.3	Cider and perry	-	QS
14.2.4	Fruit wine and made wine	-	QS
14.2.5	Mead	-	QS
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	-	QS
14.2.7.3	Aromatised wine-product cocktails	-	QS
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol	-	QS
15.1	Potato-, cereal-, flour- or starch-based snacks	-	QS
15.2	Processed nuts*	7,000	QS
16	Desserts, excluding products covered in categories 1, 3 and 4*	200	QS
17.1	Food supplements supplied in a solid form, excluding food supplements for infants and young children*	26,950	QS
17.2	Food supplements supplied in a liquid form, excluding food supplements for infants and young children*		QS

QS – *quantum satis* (no maximum numerical level is specified, and substances will be used in accordance with good manufacturing practice, at a level not higher than is necessary to achieve the intended purpose and provided the consumer is not misled).

* the 16 food categories used in the exposure assessment