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SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 25 March 2021

doi: 10.2903/j.efsa.2021.6585

Safety assessment of titanium dioxide (E171) as a food additive

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Abstract

The present opinion deals with an updated safety assessment of the food additive titanium dioxide (E 171) based on new relevant scientific evidence considered by the Panel to be reliable, including data obtained with TiO_2 nanoparticles (NPs) and data from an extended one-generation reproductive toxicity (EOGRT) study. Less than 50% of constituent particles by number in E 171 have a minimum external dimension < 100 nm. In addition, the Panel noted that constituent particles < 30 nm amounted to less than 1% of particles by number. The Panel therefore considered that studies with TiO_2 NPs < 30 nm were of limited relevance to the safety assessment of E 171. The Panel concluded that although gastrointestinal absorption of TiO_2 particles is low, they may accumulate in the body. Studies on general and organ toxicity did not indicate adverse effects with either E 171 up to a dose of 1,000 mg/kg body weight (bw) per day or with TiO_2 NPs (> 30 nm) up to the highest dose tested of 100 mg/kg bw per day. No effects on reproductive and developmental toxicity were observed up to a dose of 1,000 mg E 171/kg bw per day, the highest dose tested in the EOGRT study. However, observations of potential immunotoxicity and inflammation with E 171 and potential neurotoxicity with TiO_2 NPs, together with the potential induction of aberrant crypt foci with E 171, may indicate adverse effects. With respect to genotoxicity, the Panel concluded that TiO_2 particles have the potential to induce DNA strand breaks and chromosomal damage, but not gene mutations. No clear correlation was observed between the physico-chemical properties of TiO_2 particles and the outcome of either *in vitro* or *in vivo* genotoxicity assays. A concern for genotoxicity of TiO_2 particles that may be present in E 171 could therefore not be ruled out. Several modes of action for the genotoxicity may operate in parallel and the relative contributions of different molecular mechanisms elicited by TiO_2 particles are not known. There was uncertainty as to whether a threshold mode of action could be assumed. In addition, a cut-off value for TiO_2 particle size with respect to genotoxicity could not be identified. No appropriately designed study was available to investigate the potential carcinogenic effects of TiO_2 NPs. Based on all the evidence available, a concern for genotoxicity could not be ruled out, and given the many uncertainties, the Panel concluded that E 171 can no longer be considered as safe when used as a food additive.

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Keywords: Titanium dioxide, E 171, CAS No 13463-67-7

Requestor: European Commission

Question number: EFSA-Q-2020-00262

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Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific output: Ana Campos Fernandes, Laura Ciccolallo, Esraa Elewa, Galvin Eyong, Christina Kyrkou, Irene Munoz, Giorgia Vianello, the members of the SCER Cross-cutting WG nanotechnologies: Jacqueline Castenmiller, Mohammad Chaudhry, Roland Franz, David Gott, Stefan Weigel and the former member of the SCER Cross-cutting WG Genotoxicity Maciej Stepnik. The FAF Panel wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

Suggested citation: EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes M, Aquilina G, Castle L, Engel K-H, Fowler P, Frutos Fernandez MJ, Fürst P, Gundert-Remy U, Gürtler R, Husøy T, Manco M, Mennes W, Moldeus P, Passamonti S, Shah R, Waalkens-Berendsen I, Wölfle D, Corsini E, Cubadda F, De Groot D, FitzGerald R, Gunnare S, Gutleb AC, Mast J, Mortensen A, Oomen A, Piersma A, Plichta V, Ulbrich B, Van Loveren H, Benford D, Bignami M, Bolognesi C, Crebelli R, Dusinska M, Marcon F, Nielsen E, Schlatter J, Vleminckx C, Barmaz S, Carfí M, Civitella C, Giarola A, Rincon AM, Serafimova R, Smeraldi C, Tarazona J, Tard A and Wright M, 2021. Scientific Opinion on the safety assessment of titanium dioxide (E171) as a food additive. EFSA Journal 2021;19(5):6585, 130 pp. https://doi.org/10.2903/j.efsa.2021.6585

ISSN: 1831-4732

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The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

Summary

At the request of the European Commission, the Panel on Food Additives and Flavourings (FAF Panel) of EFSA provides an updated safety assessment of the food additive titanium dioxide (E 171) taking into account all new relevant data available to EFSA since the completion of its re-evaluation in 2016. These include the data generated by a consortium of interested business operators (IBOs) in response to the follow-up call launched by the European Commission further to the 2016 re-evaluation by the EFSA Panel on Food Additive and Nutrient Sources added to Food (EFSA ANS Panel) under Regulation (EC) No 257/2010. New data retrieved from the published literature and considered to be in line with the data requirements specified in the 2018 EFSA 'Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain' were also included.

The safety of E 171 was re-evaluated by EFSA in 2016 under Regulation (EU) No 257/2010, as part of the re-evaluation programme for food additives authorised in the EU before 20 January 2009. On the basis of the information available at that time, the EFSA ANS Panel considered that E 171 mainly consisted of micro-sized TiO_2 particles, with a nano-sized (< 100 nm) fraction less than 3.2% by mass. Uncertainties around the identity and characterisation of E 171 were however highlighted, noting that no limits for the particle size of E 171 were set in the EU specifications. The ANS Panel concluded that, based on the data available at that time, E 171 when used as a food additive did not raise concern with respect to genotoxicity and that it was not carcinogenic after oral administration. Taking into account the presumed limited absorption of TiO_2, the ANS Panel concluded that, based on a margin of safety (MoS) calculated from the no-observed-adverse-effect level (NOAEL) of 2,250 mg TiO_2/kg bw per day (identified from a carcinogenicity study in rats) and the exposure, calculated based on the reported use levels and analytical data, E 171 would not be of concern. However, given the toxicological data set at that time, the ANS Panel identified data gaps and uncertainties that required follow-up by the European Commission by means of a call for data aimed at gathering information from interested business operators. In particular, in order to address concerns related to the lack of adequate data on reproductive and developmental toxicity, the ANS Panel recommended that an extended one-generation reproduction toxicity (EOGRT) study be performed. An EOGRT study was commissioned by interested business operators and its study protocol was later amended to accommodate the investigation of additional parameters related to the occurrence and TiO_2-related induction of aberrant crypt foci (ACF) in the colon; these are preneoplastic lesions that had been reported by Bettini et al. (2017) shortly after the completion of the ANS Panel re-evaluation of E 171.

Subsequent to the evaluation of data submitted by interested business operators, in 2019, the Panel recommended that the EU specifications for E 171 include the parameter of median minimum external dimension by particle number > 100 nm (measured by electron microscopy), which is equivalent to less than 50% of constituent particles by number with a minimum external dimension < 100 nm.

Based on the presence of a fraction of nanoparticles in E 171, the food additive falls under the scope of the EFSA Guidance on nanotechnology, which was broadened in its 2018 revision to also cover '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'.

For the reason given above, the proposed amendment to the specifications of the food additive E 171 in 2019 was accompanied by a recommendation from the Panel for a re-assessment of the toxicological data set in line with the data requirements specified in the 2018 EFSA Guidance on nanotechnology.

Accordingly, the Panel considered that studies with TiO_2 NPs were relevant in the current risk assessment of E 171. TiO_2 particles in pristine E 171 likely form large agglomerates. When dispersion procedures are applied, these agglomerates may de-agglomerate, resulting in increased numbers of 'free' nanoparticles. The extent of agglomeration and the number of 'free' nanoparticles present may be further affected by the conditions in food and the gastrointestinal tract (GIT) environment. The data available to EFSA showed that the percentage by number of constituent particles < 30 nm was in the order of 1% or less in samples of pristine E 171 or in E 171 extracted from foods analysed after dispersion. The Panel therefore considered that studies with TiO_2 NPs < 30 nm were of limited relevance to the safety assessment of E 171.

In mice, E 171 has a low oral systemic availability, probably not greater than 0.5%. In studies in rats with TiO_2 NPs, the oral systemic availability was also low (most probably < 1%) but higher than that of E171 and TiO_2 NPs were detected in blood and tissues. For absorbed TiO_2 particles, half-lives of 200–450 days were estimated by the Panel.

Concerning general and organ toxicity, the Panel concluded that the available information in the literature did not indicate adverse effects with either E 171 up to a dose of 1,000 mg/kg bw per day or with TiO_2 NP > 30 nm up to the highest dose tested of 100 mg/kg bw per day. No reliable studies were found in the literature addressing reproductive and developmental toxicity of E 171 and no effect was reported up to a dose of 1,000 mg/kg bw per day for TiO_2 containing a fraction of nanoparticles. Concerning neurotoxicity, no reliable studies performed with E 171 were found in the literature. In studies with TiO_2 NP > 30 nm, neurotoxic effects were observed at the only dose tested of 100 mg/kg bw per day in rats exposed in embryonal life and at the only dose tested of 500 mg/kg bw per day in rats exposed in adult life. In studies using TiO_2 NPs < 30 nm, effects were seen at doses as low as 2.5 mg/kg bw per day. The findings in studies with E 171 on immunotoxicity and inflammation were considered inconsistent; in studies with TiO_2 NPs > 30 nm effects were seen at a dose of 20 mg/kg bw per day whereas in studies with TiO_2 NPs < 30 nm effects were observed at doses as low as 2.5 mg/kg bw per day.

Regarding the newly performed EOGRT study with E 171, the Panel concluded that there were no indications of general toxicity, no effect on thyroid or sex hormone levels, no effect on reproductive function and fertility in either male or female rats. Furthermore, no effects were observed on pre- and postnatal development. No effects on neurofunctional endpoints in F1 offspring were observed either. Concerning immunotoxicity, a marginal but statistically significant decrease in antigen-induced IgM levels (−9%) in males of the F1 Cohort 3 only was noted, with no apparent dose-response. However, the Panel noted that there were methodological shortcomings in the design of this part of the EOGRT study. Therefore, the Panel could not conclude on immunotoxicity. In a satellite group of that study, E 171 at doses up to 1,000 mg/kg bw per day did not induce ACF in the colon. The Panel considered that there was uncertainty regarding the extent of the internal exposure to TiO_2 nanoparticles (present in E 171) across the range of tested doses.

The Panel considered that the effect of E 171 in producing ACF reported by Bettini et al. (2017) was not replicated in later investigations (EOGRT study and Blevins et al., 2019), but noted that the investigation by Blevins et al. had methodological limitations. Furthermore, it is unclear to what extent animals were exposed to TiO_2 NPs in the EOGRT and in the study by Blevins et al. The Panel concluded that E 171 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 NPs prior to administration by gavage.

Concerning the genotoxicity studies, combining the available lines of evidence, the Panel concluded that TiO_2 particles have the potential to induce DNA strand breaks and chromosomal damage, but not gene mutations. No clear correlation was observed between the physico-chemical properties of TiO_2 particles – such as crystalline form, size of constituent particles, shape and agglomeration state – and the outcome of *in vitro* or *in vivo* genotoxicity assays. The Panel concluded that several modes of action (MOA) may operate in parallel and the relative contributions of the different molecular mechanisms resulting in the genotoxicity of TiO_2 particles are unknown. Based on the available data, no conclusion could be drawn as to whether the genotoxicity of TiO_2 particles is mediated by a mode (s) of action with a threshold(s). Therefore, the Panel concluded that a concern for genotoxicity of TiO_2 particles cannot be ruled out.

Concerning absorption and toxicity of TiO_2 particles that are present in E 171, the Panel concluded that:

- the absorption of TiO_2 particles is low; however, they may accumulate in the body due to their long half-life;
- studies on general and organ toxicity, including the newly performed EOGRT study with E 171, 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 TiO_2 NP > 30 nm up to the highest dose tested of 100 mg/kg bw per day;
- no effects on reproductive and developmental toxicity up to a dose of 1,000 mg/kg bw per day, the highest dose tested, were observed in the EOGRT study E 171. No other reliable studies were found in the literature addressing these effects with E 171;
- some findings regarding immunotoxicity and inflammation with E 171 as well as neurotoxicity with TiO_2 NPs may be indicative of adverse effects;
- there are indications of an induction of ACF with E 171;
- no studies appropriately designed and conducted to investigate the potential carcinogenicity of TiO_2 nanoparticles were available;

- combining the available lines of evidence on genotoxicity, TiO_2 particles have the potential to induce DNA strand breaks and chromosomal damage, but not gene mutations. No clear correlation was observed between the physico-chemical properties of TiO_2 particles – such as crystalline form, size of constituent particles, shape and agglomeration state – and the outcome of either *in vitro* or *in vivo* genotoxicity assays;
- a concern for genotoxicity of TiO_2 particles that may be present in E 171 could not be ruled out;
- several modes of action for the genotoxicity may operate in parallel. The relative contributions of different molecular mechanisms elicited by TiO_2 particles are unknown and there is uncertainty as to whether a threshold mode of action could be assumed;
- a cut-off value for TiO_2 particle size with respect to genotoxicity could not be identified.

Overall, on the basis of all currently available evidence along with all the uncertainties, in particular the fact that genotoxicity concern could not be ruled out, the Panel concluded that E 171 can no longer be considered as safe when used as a food additive.

This conclusion applies to E 171 as described in Commission Regulation (EU) No 231/2012 as well as to E 171 specified in the EFSA FAF Panel opinion in 2019.

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