EFSA Journal

SCIENTIFIC OPINION

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.

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

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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1. Introduction

In the present opinion, the EFSA Food Additives and Flavourings (FAF Panel) provides an updated safety assessment of the food additive titanium dioxide (E 171) on the basis of newly available scientific evidence. The principles of the EFSA Guidance on nanotechnology (EFSA Scientific Committee, 2018a,b) have been followed in the assessment.

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Background

The use of food additives is regulated under the European Parliament and Council Regulation (EC) No 1333/2008 on food additives.[1] Only food additives that are included in the Union list, in particular in Annex II to that Regulation, may be placed on the market and used in foods under the conditions of use specified therein. Moreover, food additives shall comply with the specifications as referred to in Article 14 of that Regulation and laid down in Commission Regulation (EU) No. 231/2012[2].

Titanium dioxide (E 171) is authorised for use as food additive (food colour) in the Union. Since titanium dioxide (E 171) was permitted in the Union before 20 January 2009, it belongs to the group of food additives which are subjected to a new risk assessment by the European Food Safety Authority (EFSA), according to Commission Regulation (EU) No 257/2010[3], and in line with the provision of Regulation (EC) No 1333/2008.

The re-evaluation of titanium dioxide (E 171) as food additive was completed by EFSA in June 2016 and a scientific opinion was published on 14 September 2016.[3] In that opinion, EFSA concluded, on the basis of the available evidence that titanium dioxide used as a food additive (E 171) did not raise a concern with respect to genotoxicity, was not carcinogenic after oral administration and exposure from the reported use/analytical levels would not be of concern. EFSA recommended that additional reproductive toxicity testing could be performed to enable EFSA to establish a health-based guidance value (e.g. an accepted daily intake – ADI) for titanium dioxide (E 171). Therefore, the Commission issued in January 2017 a call for data[5] requesting business operators to submit new reproductive toxicity data for titanium dioxide (E 171), as well as data addressing other recommendations made by EFSA concerning the specifications for titanium dioxide (E 171). In reply to this call for data, business operators committed to submitting by June 2020 data from a new extended one-generation reproduction toxicity (EOGRT) study carried out according to the current OECD guidelines.

On 4 April 2017, the French Agency for Food, Environment and Occupational Health and Safety (ANSES) published an opinion on dietary exposure to nanoparticles of titanium dioxide[6] assessing, in particular, the study of Bettini et al. (2017) and concluded that the data available do not bring into question the risk assessment performed by EFSA.

On 22 March 2018, the Commission requested EFSA to evaluate four new studies describing potential adverse health effect of titanium dioxide used as food additive (E 171). The EFSA opinion, published on 4 July 2018, concluded that the outcome of the four studies did not merit re-opening the existing opinion of EFSA related to the safety of titanium dioxide (E 171) as food additive[7]. In that opinion EFSA, however recommended that biomarkers for putative pre-cancerous lesions in the colon should be examined, as additional parameters, in the reproductive toxicity study recommended by EFSA in 2016. Business operators have followed this recommendation and consequently the EOGRT study that is expected for submission in June 2020 will also cover the outcome of theses examinations.

[1] Available online: https://www.efsa.europa.eu/en/food-consumption/comprehensive-database
[2] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) no 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1.
[3] Arrêté du 21 décembre 2020 portant suspension de la mise sur le marché des denrées contenant l'additif E 171 (dioxyde de titane - TiO_2) - Légifrance (legifrance.gouv.fr).
[4] 2020/2795(RPS) – 8/10/2020 – Objection pursuant to Rule 112(2) and (3) and (4)(c): Specifications for titanium dioxide (E 171) (europa.eu).
[5] EFSA-Q-2010-01522.
[6] Commission Delegated Regulation (EU) 2020/217 of 4 October 2019 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures and correcting that Regulation. C/2019/7227. OJ L 44, 18.2.2020, p. 1–14. ELI: http://data.europa.eu/eli/reg_del/2020/217/oj
[7] Where X refers to the percentage of particles < 100 nm as described in the publication.

On 15 April 2019, ANSES published a review of the risk related to the ingestion of food additive titanium dioxide (E 171)[8] taking into account the most recent scientific studies available and referring to its earlier opinion of 2017. On 5 May 2019, the European Commission requested EFSA to assess the ANSES review and to indicate whether it includes any and major findings showing that titanium dioxide (E 171), when used as food additive, is of safety concern and thus whether it overrules the conclusion of the previous EFSA safety evaluations of titanium dioxide (E 171). EFSA was also requested to indicate whether the ANSES review identified additional uncertainties that could be addressed in the follow-up work undertaken by the interested business operators.

On the 13 May 2019, the European Food Safety Authority (EFSA) published a statement[9] on the review of the risk related to the exposure to the food additive titanium dioxide (E 171) performed by the French Agency for Food, Environment and Occupational Health Safety (ANSES). In that statement EFSA concluded that the ANSES opinion does not identify any major new finding that would overrule the conclusions made in the previous EFSA scientific opinion on the safety of titanium dioxide (E 171) as a food additive. The ANSES opinion reiterates the previously identified uncertainties and data gaps, which are currently being addressed in the context of the follow-up activities originating from the previous EFSA evaluation and their recommendations. In addition, ANSES recommended further investigations of *in vivo* genotoxicity. EFSA considered that this recommendation should be revisited once the work on the physico-chemical characterisation of the food additive titanium dioxide (E 171) is completed.

On the 7 August 2018, the European Commission requested EFSA to assess new data provided by interested food business operators in response to the call for data published as a follow-up of the re-evaluation of titanium dioxide (E 171), and addressing the uncertainties identified with respect to the characterisation of this food additive, including its particle size and particle size distribution. This led to the publication on 12 July 2019 of a scientific opinion on the proposed amendment of the specifications of titanium dioxide (E 171) with respect to the inclusion of the additional parameters related to its particle size distribution. In that opinion EFSA indicated that the conclusions made, and the uncertainties identified, in the previous EFSA assessment of the food additive titanium dioxide (E 171) remain valid. Moreover, EFSA indicated that the characterisation of titanium dioxide (E 171) does not provide a reason to revise the conclusion on the genotoxicity of titanium dioxide (E 171) drawn by EFSA in the previous opinions on titanium dioxide (E 171). Nevertheless, EFSA concluded that based on the proposed change in the specifications, the toxicological database on titanium dioxide (E 171) as a food additive should be revisited 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". Based on this latest EFSA opinion, the specifications for the food additive titanium dioxide (E 171) in Commission Regulation (EU) No 231/2012 will be updated.

The legislation on food additives envisage that food additives should be kept under continuous observation and re-evaluation whenever necessary in the light of new scientific information. Therefore, it is appropriate to ask EFSA to reassess the safety of the food additive titanium dioxide (E 171) taking into account all new relevant data available to EFSA since the completion of the re-evaluation of titanium dioxide (E 171) as a food additive by EFSA in 2016, including the new EOGRT study recommended by EFSA. The new assessment should take into account the relevance of the data 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".

1.1.2. Terms of Reference

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2012, the European Commission requests the European Food Safety Authority (EFSA) to provide an updated scientific opinion as regards the safety of the food additive titanium dioxide (E 171).

In particular, EFSA is requested to reassess the safety of food additive titanium dioxide (E 171) taking into account all new relevant data available to EFSA since the completion of its re-evaluation of titanium dioxide (E 171) as a food additive in 2016. These included the data generated by a consortium of interested

[8] Total Ti corresponds to the analytical determination of the Ti element from all sources after digestion of tissues and application of appropriate analytical techniques and may be used as a proxy estimate for TiO_2 mass concentration. In the current opinion, the term titanium (Ti) is used to indicate total Ti.

[9] The content of Ti in the liver and the large intestine was estimated by multiplying the measured concentrations with the organ weights (Ronald et al., 1997). To obtain the percentage of the dose, the sum of both contents was related to the dose applied.

business operators in response to the follow-up call lunched by the European Commission further to the re-evaluation of the food additive completed by EFSA in the context of Regulation (EC) No 257/2010, once available, as well as any 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".

2. Data and methodologies

2.1. Data

The present evaluation is based on the following data:

- Information from publications retrieved in the literature search (see Section 2.2).
- Data submitted in response to the call for data from European Commission as follow-up of the re-evaluation of E 171 (Documentation provided to EFSA No 1, 2, 3, 4, 5 and 6).
- Toxicokinetic and genotoxicity studies considered in the re-evaluation of titanium dioxide (E 171) (EFSA ANS Panel, 2016).
- Exposure data available in the re-evaluation 2016 and additional relevant information that came available since then (see Section 4.4).
- *In vitro* and *in vivo* studies reported in the OECD dossier (2016) and submitted to EFSA (Documentation provided to EFSA No 7, 8, 9 and 10).
- *In vitro* genotoxicity studies submitted to EFSA (Documentation provided to EFSA No 14 and 15).
- Food consumption data used to estimate the dietary exposure to titanium dioxide (E 171) were derived from the EFSA Comprehensive European Food Consumption Database (Comprehensive Database[1]). Dietary data from the UK were included in the EFSA Comprehensive European Food Consumption Database for the period in which UK was a member of the European Union.
- The Mintel's Global New Products Database (GNPD) was used to verify the use of titanium dioxide (E 171) in food and beverage products and food supplements within the EU's food market. The Mintel's GNPD is an online database that contains the compulsory ingredient information present on the label of numerous products.

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA Guidance on transparency in the scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing Guidance from the EFSA Scientific Committee, in particular the Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health (EFSA Scientific Committee, 2018a).

A literature search was performed following the approach described in Appendix A. Information on the criteria for inclusion and exclusion of publications based on information from the abstract and title, and kind of material used in the study is available in Appendix B.

Toxicokinetic and toxicity studies considered 'included' according to Appendix B were assessed for their relevance and reliability taking into account the criteria described in Appendix C.

Genotoxicity studies considered 'included' according to Appendix B were assessed taking into account the criteria described in Appendix D.

Nanoscale considerations for the assessment of the study design and study results in toxicity studies classified with reliability 1 and 2 (see Section 4.1), and genotoxicity studies (see Section 4.3), were assessed according to criteria described in Appendix E.

Dietary exposure to E 171 from its use as a food additive was estimated combining the food consumption data available within the Comprehensive Database with reported use levels submitted to EFSA (EFSA ANS Panel, 2016) and information extracted from a report of the Netherlands National Institute for Public Health and the Environment (RIVM) (Sprong et al., 2015). The exposure was estimated according to different exposure scenarios (EFSA ANS Panel, 2017). Uncertainties in the exposure assessment were identified and discussed (Section 4.4.3).

After receiving the mandate from the European Commission, the support from the cross-cutting Working Group (ccWG) on Genotoxicity to review the evidence and conclude for the genotoxicity of E 171 was requested. Accordingly, the assessment of the data (referred to in Section 4.3) has been conducted independently by the ccWG Genotoxicity and submitted to the Panel for its consideration and decision.

3. Background information and previous evaluations

Titanium dioxide (E 171) is authorised as a food additive in the EU according to Annex II of Regulation (EC) No 1333/2008 and specifications have been defined in Commission Regulation (EU) No 231/2012[2]

In June 2016, the EFSA Panel on Food Additives and Nutrient sources added to Food (ANS Panel) completed the re-evaluation of the safety of the food additive E 171, performed under the frame of Regulation (EC) No 257/2010 (EFSA ANS Panel, 2016).

In that opinion, the ANS Panel had concluded that the food additive did not raise concerns with respect to genotoxicity and carcinogenicity but the ANS Panel was unable to establish a health-based guidance value (HBGV) because of certain deficiencies identified in the available toxicological data set, in particular with respect to the investigation of potential reproductive toxicity. Another important source of uncertainty identified during the re-evaluation concerned the characterisation of the material used as the food additive E 171.

The European Commission followed up on the recommendations issued by the ANS Panel and, in January 2017, published a call for data addressed to interested business operators and requesting their commitment to provide the data requested to reduce the uncertainties underpinning the conclusions of the ANS Panel opinion. These included data on the characterisation of the material and the performance of a new EOGRT study in rodents, to be conducted in accordance with the latest OECD Guidance applicable and with test material representative of the food additive on the EU market.

In January 2017, the publication of a study by Bettini et al. (2017) raised some concerns on the potential tumour promoting effect of dietary intake of E 171, which led to an opinion of the ANSES in April 2017 on the dietary exposure to nanoparticles of titanium dioxide (ANSES, 2017). In that opinion, the ANSES concluded that the data available did not put the 2016 EFSA assessment in question.

A subsequent scientific opinion was issued by the ANS Panel in June 2018, to address a request from the European Commission for the evaluation of four publications – among them, Bettini et al. (2017) previously considered by ANSES – raising concerns on the safety of E 171. Having reviewed these publications, the ANS Panel maintained the conclusions reached in 2016 but recommended the inclusion of biomarkers for putative pre-cancerous lesions in the colon to be included in the ongoing EOGRT study as additional parameters to be investigated (EFSA ANS Panel, 2018).

In July 2018, the safety assessment of food additives was handed over from the EFSA ANS Panel to the newly constituted EFSA Food Additives and Flavourings (FAF) Panel.

In July 2018, the EFSA Scientific Committee published a Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health (EFSA Scientific Committee, 2018a) updating the 2011 Guidance Document on nanomaterials (EFSA Scientific Committee, 2011a), and clarifying that conventional materials containing a fraction of nanoparticles require specific risk assessment considerations, which were detailed in the document.

While the follow-up activities for the generation of new data were ongoing, in April 2019, the French Government decided to take risk management action introducing a ban on foods containing the food additive E 171. The French decree, that entered into force on 1 January 2020, was based by the application of the precautionary principle to the latest advice issued by ANSES (2019). The ban on foods containing the food additive E 171 in France has been reconfirmed for the current year, pending the finalisation of the present assessment by EFSA.[3]

As a follow-up to the re-evaluation of E 171 completed by the ANS Panel, in 2018, the European Commission requested EFSA to assess a proposal for an amendment of the EU specifications for the food additive E 171 based on the data on particle size and particle size distribution that had been provided by the interested business operators in response to the first part of the European Commission call for data. The related scientific opinion was adopted by the FAF Panel in June 2019 and published shortly afterward (EFSA FAF Panel, 2019). The FAF Panel, while recommending the inclusion of additional parameters related to the particle size distribution in the EU specifications for E 171, also concluded that the toxicological database should be revisited 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' (EFSA Scientific Committee, 2018a). Scope of this guidance document is not only engineered nanomaterials but also those materials containing a fraction of particles that is less than 50% in the number–size distribution, with one or more external dimensions in the size range 1–100 nm, a definition which could be applicable to the case of the food additive titanium dioxide (E 171).

Based on the recommendations from the 2019 scientific opinion of the FAF Panel, the European Commission made a proposal for amending the definition and specifications of E 171, introducing limits with respect to the particle size and particle size distribution in the food additive in Regulation (EU) No 231/2012. In October 2020, the European Parliament called on the Commission to withdraw its draft regulation, to apply the precautionary principle and to remove E 171 from the list of food additives authorised by the Union.[4]

In 2019, the Office for Risk Assessment and Research of the Netherlands Food and Consumer Product Safety Authority (NVWA) delivered an opinion on possible health effects of the food additive E 171 (NVWA, 2019). The opinion concluded that studies conducted since 2016 in mice and rats provide an indication of tumour promotion by E 171 in the intestinal tract but should be considered 'exploratory' since they were not conducted in accordance with OECD guidelines. With regard to the EOGRT study (ongoing at the time), the opinion concluded that an examination of immunotoxicological effects was important given recent studies, in addition to potential reprotoxicological effects. The opinion also concluded that an examination for potential promotion of colon cancer by E 171 should be examined but considered it doubtful whether the performance of an EOGRT study or chronic exposure test would be suitable test system. In addition to this, further research in humans was considered required to establish any relevance of experimental findings to man.

In parallel to the re-evaluation of titanium dioxide as a food additive, the EFSA FEEDAP Panel was also evaluating the safety of titanium dioxide in feed for all animal species. This assessment has been put on hold, awaiting submission of the data requested as a follow-up of the re-evaluation of the food additive.[5]

The substance evaluation for titanium dioxide under REACH was started in 2018. The Competent Authority of France (the evaluating Member State Competent Authority) was appointed to carry out the evaluation. Currently the decision-making is under the registrants' comment review period.

The European Chemicals Agency (ECHA) Committee for Risk Assessment (RAC) concluded in its scientific opinion of 14 September 2017 that titanium dioxide met the criteria in Regulation (EC) No 1272/2008 for classification as a carcinogen in category 2 by inhalation (ECHA, 2017). The adopted harmonised classification and labelling was later included in an amendment of Regulation (EC) No 1272/2008 as indicated in Commission Delegated Regulation (EU) 2020/217.[6] The new entry in Annex VI to Regulation (EC) No 1272/2008 applies to titanium dioxide in powder form containing 1% or more of particles with aerodynamic diameter $\leq$ 10 μm.

Titanium dioxide is widely used as an excipient in medicinal products, mainly as a colour/opacifier in oral and cutaneous dosage forms. Titanium dioxide for use in medicinal products needs to meet the requirements defined in the European Pharmacopoeia. Colouring matter should comply with the requirements of European Union Directive 2001/83/EC.[10] Current EU legislation laying down specific purity criteria concerning colours for use in foodstuffs (Commission Regulation (EU) No 231/2012) also applies to medicinal products (as detailed in Directive 2009/35/EC[11]).

4. Assessment

Relevant studies with (i) the food additive titanium dioxide (E 171), (ii) titanium dioxide – other than E 171 – containing a fraction of particles < 100 nm (TiO_2 (X% nano))[7] or (iii) nano titanium dioxide (TiO_2 NPs) have been evaluated when considered reliable, i.e. scoring 1 or 2 according to the approach described in Appendix C for toxicity or the approach described in Appendix D for genotoxicity. Unless otherwise indicated by the Panel (Appendices G, H), the constituent particles of these materials investigated in the assessed studies had a nearly spherical shape and the purity of the material was considered acceptable. The constituent particle size is indicated in the summaries below (Appendices G, H, J, K, L, M, N, O, P), followed by the analytical technique in parenthesis.

The characterisation of E 171 was previously evaluated by the Panel and it was concluded that, according to data received from interested business operators, less than 50% of constituent particles in E 171 have a minimum external dimension below 100 nm by number (EFSA FAF Panel, 2019)

Information on the physico-chemical characteristics of the representative test materials considered relevant for the assessment of E 171, e.g. those from the JRC repository (NM-100, NM-102 and NM-105) is available in the JRC report (Rasmussen et al., 2014)) and in specific publications (Taurozzi and Hackley, 2012). As opposed to commercially manufactured TiO_2 materials such as 'P25'

[10] Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. OJ L 311, 28.11.2001, p. 67–128.

[11] Directive 2009/35/EC of the European Parliament and of the Council of 23 April 2009 on the colouring matters which may be added to medicinal products. OJ L 109, 30.4.2009, p. 10–13.

(AEROXIDE® TiO_2 P 25) that are also applied as intralaboratory standards (Taurozzi and Hackley, 2012)), NM-100 and NM-102 originate from single batches of commercially manufactured TiO_2 materials and have been demonstrated to be sufficiently homogeneous and stable to be used as reference materials in chemical analysis and toxicological testing. The Panel noted that NM-105 is produced from a batch of the P25 material.

The Panel considered that studies performed with TiO_2 NPs that predominantly consist of particles smaller than 30 nm (e.g. P25) are of limited relevance to the safety assessment of E 171, since in samples of E 171 and in E 171 extracted from foods the percentage by number of particles below 30 nm is in the order of 1% or less (Verleysen et al., 2020, 2021; Geiss et al., 2021; Appendix W). However, data from toxicity studies performed with $TiO_2 < 30$ nm have been considered for completeness of the database and may be relevant with respect to whether a minimum limit for particle size should be included in the EU specifications for E 171.

In several studies described in the open literature, there was an inadequate description of the test material (size or crystalline form is not reported). In all cases, EFSA requested additional information about the test material by contacting the corresponding authors reported in the publications. No responses were received.

4.1. Toxicokinetic and toxicity studies from the literature search (January 2015–November 2020)

More than 11,000 publications retrieved according to the literature search (Appendix A) were screened based on the criteria agreed in advance (Appendix B). After the first screening based on the description of title and abstract of the publication, around 200 *in vivo* studies and 300 *in vitro* studies were identified as potentially relevant for the current assessment.

Information on the assessment of *in vitro* and *in vivo* genotoxicity studies is provided in Appendix D.

For the current toxicokinetic and toxicological assessment, it was decided to focus on *in vivo* studies (Appendix F). The relevance of the *in vivo* toxicity studies for the different key areas of concern (Table 1) was assessed following the approach described in Appendix C. Around 30 studies were considered relevant for hazard characterisation and 33 additional studies were considered only relevant for providing supporting evidence (Table 1).

In a second step, an assessment of the reliability of the relevant publications took place according to the criteria described in Appendix C. Publications were classified from 1 to 4 and only those publications considered sufficiently reliable with respect to their internal validity, i.e. the extent to which the design and conduct of a study are likely to have prevented bias (reliability 1 and 2) were further examined for the safety assessment of E 171 (Appendix H).

Table 1: Summary of the number of *in vivo* toxicity studies evaluated for their relevance and reliability

	Studies assessed for their relevance	Studies considered relevant	Studies considered with reliability 1 and 2	
			Relevant for hazard characterisation	Relevant for providing supporting evidence
Studies examining toxicity effect in the liver, spleen and pancreas	56	32	3	3
Studies examining toxicity effect in heart and kidney	27	10	3	2
Studies examining other organs	37	14	5	3
Reproductive and developmental toxicity studies	39	29	4	3
Neurotoxicity and developmental neurotoxicity studies	24	17	6	6
Inflammation and immunotoxicity studies	38	30	9	8
Gut microbiota	16	13		8

4.1.1. Toxicokinetic studies

The toxicokinetics of TiO_2 was reviewed by the ANS Panel in the 2016 opinion on the re-evaluation of E 171 (EFSA ANS Panel, 2016). According to the ANS Panel, the absorption of orally administered TiO_2 is low. Its oral systemic availability (measured either as particles or as titanium) is estimated to be 0.02–0.1%, and the vast majority being eliminated unchanged in the faeces. The ANS Panel had noted that the small amount of orally ingested TiO_2 appeared to be absorbed by Peyer's patches, a group of cells in the gut-associated lymphoid tissue (GALT). It is subsequently distributed to various organs (by order of decreasing concentration: mesenteric lymph nodes, liver, spleen, kidney, lungs, heart and reproductive organs), from which the material disappears with variable half-lives. The ANS Panel noted the potential for tissue accumulation based on the slow elimination of titanium from tissues after intravenous administration with calculated half-lives ranging between 28 and 650 days in different organs (EFSA ANS Panel, 2016). Interpretation of these findings was, however, complicated by the extent of the variability in the background levels of Ti in animals and humans which also prevented the accurate determination of kinetic parameters such as the elimination half-life.

The focus of the current updated assessment was to gather from the newly available evidence, any relevant information that could be used to refine the risk assessment and reduce the uncertainties identified by the ANS Panel in its earlier evaluation. In particular, the Panel examined whether new data from the published literature could provide better estimates of the oral systemic availability, the distribution in tissues and the elimination half-life of TiO_2 after oral administration.

The estimate of the oral systemic availability of TiO_2 was updated by multiplying the reported concentration with the respective organ or tissue weights. Subsequently, the sum of the calculated amounts in the different organs was compared to the dose applied to estimate the percentage absorbed. Data were extracted only from those publications in which the analytical method used for the measurement of internal exposure was evaluated as reliable or reliable with some limitations (see Appendix C). In addition, two references from the previous ANS Panel opinion were also re-examined because their results could contribute to a quantitative estimate of absorption (Geraets et al., 2014; Tassinari et al., 2014).

The issue of the variability in the environmental, dietary and tissue background levels of Ti in the studies, already flagged by the ANS Panel in its previous opinion, remains one of the main critical aspects to be taken into consideration when evaluating the toxicokinetics of TiO_2. Challenges in the analytical determination of low concentrations of Ti in tissues, primarily related to detection limits, contamination issues and spectral interferences in inductively coupled plasma mass spectrometry (ICP-MS) determination, further complicates obtaining accurate and reliable tissue concentrations and toxicokinetic data.

Further information on the description of the test materials, scoring for nanoscale considerations (NSC) and internal exposure for the following studies is reported in Appendix G.

Studies on E 171

Mice

One of the aims of the study of Talamini et al. (2019) was to investigate whether repeated administration of E 171 to mice would result in accumulation in tissues. Mice (n = 4) were administered 5 mg E 171/kg body weight (bw) per day, 3 days per week, for 3 weeks (nine treatments in 21 days, providing an average daily dose of 2 mg E 171/kg bw). On day 21, the animals were sacrificed and Ti concentration was measured by triple quadrupole ICP-MS in tissues from four mice.

Total Ti[8] concentrations in the stomach, small intestine, large intestine and liver were one order of magnitude greater than those in the other organs. Differences in Ti concentrations in lungs, spleen, stomach and small intestine of treated animals compared to controls were not statistically significant. The Ti concentration in liver (0.94 ± 0. 57 μg Ti/g tissue) and in the large intestine (1.07 ± 0.38 μg Ti/g tissue) was significantly higher in the treated animals than in the controls (ca. 0.2 μg Ti/g tissue, liver, and 0.6 μg/g tissue, large intestine). Ti concentrations in brain, kidney and testis were below the limit of quantification (LOQ) of 0.03 μg Ti/g tissue.

The Panel adopted a conservative approach and considered that all colon-associated Ti was absorbed. On this basis, the Panel calculated that 0.1% of the total dose of TiO_2 was absorbed in the Talamini et al. (2019) study.[9]

In the study of Coméra et al. (2020) in C57BL/6 mice, the processes associated with the absorption of TiO_2 in the gastrointestinal tract (GIT) were investigated. In the first series of experiments, mice were given a single dose of a suspension of E 171 (40 mg/kg) in water by gavage, while control mice

received water. Segments of the jejunum, ileum and colon were prepared 2, 4, 8 and 24 h after administration following intraluminal content recovery through gentle scraping. Particle diameters in tissues were measured by transmission electron microscopy (TEM). The existence of particles in gut tissue and blood was analysed by laser-reflective confocal microscopy. ICP-MS analysis was performed to measure Ti concentration. In the second series of experiments, performed under anaesthesia, a closed mid-jejunal loop of 10 cm was pretreated with inhibitors of tight junctions, micropinocytosis, clathrin-mediated endocytosis or raft-dependent endocytosis. Thereafter, the loops were filled with sonicated E 171 (300 μg/mL) in buffer or buffer as control, followed by incubation for 30 min. Confocal microscopic evaluation was performed on cryosectioned tissue slices to detect TiO_2 particles by light reflection. The same detection method was applied to examine the sizes of particles present in the E 171 water suspension prepared for the gavage, in the gastrointestinal (GI) luminal contents and in blood. In mice having received 40 mg E 171/kg, increases in reflective particle content was observed in the ileal and jejunal villi and colon crypts. In jejunal and ileal villi, maximal reflective particle content occurred 4 h after administration and returned to basal values at 8 h. In the colon, a small non-significant increase in reflective particle content was observed at 4 h with normalisation at 8 h. The presence of Ti and O in particles was confirmed in jejunal goblet cells and enterocytes by transmission electron microscopy energy-dispersive X-ray spectroscopy (TEM-EDX). In the Peyer's patches, a statistically significant increase in laser-reflecting particles was found only at 8 h and not at 4 h. In blood, the number of particles significantly increased by 3.5- and 4.1-fold at 4 and 8 h, respectively. In blood, the Ti concentrations remained below the limit of detection (LOD $<$ 0.02 ng Ti/kg) at all time points. From the content in the intestines and the weight of the mice tissues, the authors calculated that approximately 0.007% of the Ti administered was present in the entire intestine at the 4 h time point. The authors concluded that the TiO_2 was absorbed primarily/predominantly in the ileum, partly in jejunum with a small amount absorbed in the colon. The authors considered – based on surface area considerations – that TiO_2 is primarily/predominantly absorbed in the area of the small intestinal villi and to a less extent, through Peyer's patches. From the *ex vivo* experiments, inhibiting the paracellular pathway reduced the absorption of TiO_2. Blockers of transepithelial passage did not influence the absorption of TiO_2 to a significant extent. As the absorption was not totally blocked by paracellular pathway inhibitors, the authors concluded that besides a paracellular pathway, endocytosis could also be involved in the transport of TiO_2 from the intestinal lumen to blood.

The Panel noted that TiO_2 can be taken up from the small intestine by the paracellular pathway and by endocytosis. Specialised cells in the Peyer's patches may also play a role in the uptake. The authors indicated that 0.007% of the total TiO_2 dose at 4 h is present in the intestine. The Panel noted high agglomeration of TiO_2 in the exposure medium.

In the study of Riedle et al. (2020), primarily aimed at studying effects of E 171 on immune cells in Peyer's patches, C57BL/6 mice at 6 weeks of age were randomly exposed to 0 and $\approx$ 1, 10 and 100 mg E 171/kg bw per day for 6, 12 and 18 weeks. After 18 weeks, the presence of particles in the basal regions of Peyer's patches was examined via confocal microscopy and *scanning electron microscopy* (SEM) with EDX analysis. According to the authors, GI harvest and the method used to prepare tissue sections took care to avoid inadvertent contamination. Reflectance confocal microscopy was employed to examine for the presence of E 171 in Peyer's patches. Subsurface particles were identified by reflectance confocal microscopy which were rich in Ti based on SEM/EDX analyses. The lowest and mid-dose groups (1 and 10 mg E 171/kg bw per day) showed very weak signals from impacted cells at the base of the Peyer's patch, whereas higher signals were observed in the highest dose group (100 mg E 171/kg bw per day). No quantitative information was given by the authors.

The Panel noted the uptake of TiO_2 particles into cells in the Peyer's patches after 18-week exposure to E 171.

Human

A study on the oral systemic availability of E 171 was performed by Pele et al. (2015) in eight volunteers (seven completed the study). Participants in the study ingested two capsules each containing 50 mg of E 171 with 250 ml water. Blood samples were taken at 0, 30 min and 1, 1.5, 2, 3, 6, 8 and 10 h after ingestion. Tea and coffee with milk and/or sugar were allowed from 2 h after ingestion. Blood drops were spread on a glass slide and protected from drying. Particles were detected by light microscopy with a 100-fold and then 400-fold magnification using a dark field condenser. This method could only be carried out in the samples of five out of the seven subjects due to blood clotting in the samples from two of the subjects. Reflectance grade (0, 1, 2 and 3) was used as a semiquantitative measure of the number of TiO_2 particles. Total Ti concentration was measured in blood via isotope

dilution analysis by high resolution (HR)-ICP-MS. Significant increases in positive signals by dark field microscopy were observed in blood films from 2 h onwards, with a peak at 6 h. Blood Ti concentrations were not statistically significantly different from the baseline value up to 3 h. The highest blood concentration of Ti determined in any participant was approximately 11 ng/mL (at 6 h). Thereafter the blood Ti-concentration declined to about 5 ng/mL at 10 h after administration. Reflectance grades, indicating the number of particles in blood, correlated with the levels of Ti measured by HR-ICP-MS. The authors considered that two routes of particle uptake appear to exist in the human gut, one proximal (in the duodenum/jejunum) and one distal (Peyer's patch uptake in the ileum).

The Panel concluded that after oral administration of 100 mg E 171 in human volunteers, blood Ti concentration and the number of particles increased, demonstrating oral systemic availability of TiO_2.

The study of Guillard et al. (2020) consisted of two parts. In the first part, Ti and TiO_2 particle concentrations were measured in placentas collected from 22 women given birth at term without complications. Ti and TiO_2 particle concentrations were also measured in 18 meconium samples of newborn infants. The placentas and the meconium samples used in this study were not related. Ti concentrations were measured by ICP-MS and the morphology, size and chemical characterisation of the particles identified in the placental tissue sections and in the meconium samples were prepared for electron microscopy. Ti concentrations in the placentas ranged between 0.01 and 0.48 mg/kg, the median being 0.05 mg/kg. In only 9 of the 18 meconium samples, Ti concentrations were above the LOQ (0.01 mg/kg). The median concentration was 0.25 mg/kg. Scanning transmission electron microscopy coupled to energy dispersive X-ray (STEM-EDX) analysis in two placentae and two meconium samples confirmed the presence of TiO_2 particles. The Panel noted that the meconium samples were collected within the first few days after birth.

In the second part of the study, a well-established *ex vivo* human placenta perfusion model was used to quantify the transplacental transfer of TiO_2 across the placenta. Integrity and functionality of the placental membranes were confirmed by antipyrine testing. The perfusion medium of a cotyledon contained either 15 μg E 171/mL (n = 13) or E 171 (n = 2) controls, with confirmed dispersion of the TiO_2 particle. In two of the placentae, particles were identified and their size measured; 53% of the 34 particles sizes measured were above 100 nm and 47% below. Using confocal microscopy for particle visualisation in the fetal effluent and SEM-EDX to ascertain the chemical nature of detected particles, it was shown that the number of particles in the fetal exudate increased for the first 40 min of the 1-h placental perfusion. The number of particles (depicted as mean ± SEM of four to six independent experiments) was between 6 and 8 per microscopic field.

From these studies, the Panel considered that Ti is found in the placenta in low concentrations, indicating that TiO_2 is systemically available, is distributed to the placenta and is capable of crossing placental membranes. The Panel estimated the amount of Ti in the placenta.[12] The resulting total median amount of Ti in the placenta was 35.75 μg (19.5–84.5 μg, 25–75 percentiles).

From the *ex vivo* study, the Panel noted that the extent of TiO_2 transfer is small and not measurable within the short experimental period, in conformity with the *in vivo* findings. However, particles were present in the fetal exudate and 53% of the 34 particles had a size above 100 nm and 47% below. The process by which TiO_2 particles enter the meconium was not considered by the authors. Given the fact that the meconium collection period was up to 48 h after birth and that in 50% of the meconium samples, no Ti was measurable and no particles identified, the Panel considered that some of the infants would have been nursed before the meconium was collected. The Panel noted that the authors did not control for the possibility that TiO_2 particles could have come from the diapers used to collect the meconium or from other sources, e.g. mothers' milk.

Studies with TiO_2 NPs or TiO_2 other than E 171

Rat – Intravenous administration

In the study of Disdier et al. (2015), male Fisher rats were injected with a TiO_2 NPs suspension (1 mg/kg), controls received saline buffer. From six controls and six treated animals, samples were taken at 30 min, 1, 2, 6 and 24 h, and 7, 28, 90 and 356 days following injection encompassing blood, liver, brain, spleen, kidney and lungs. Additional blood and brain samples were obtained at 5 and 15 min following injection. Ti concentrations in the samples were determined by ICP-MS. In blood cells and plasma, the Ti concentration in the treated and control groups did not differ at any time point. In

[12] The content of Ti in placenta was estimated by multiplying the measured concentrations with the organ weight of 650 g (Valentin, 2002).

the brain, the Ti concentrations were higher than in the control in the first 24 h. From the next sampling time on (7 days), no difference in brain Ti concentrations existed between controls and treated animals. The amount of Ti in the brain was very low. In kidneys, the Ti concentration was several-fold higher than in the control in the first 24 h. From 7 days on, the next sampling time, no difference existed between controls and treated animals. In spleen, liver and lung, the Ti concentrations declined over time, with the concentrations of Ti being higher in the treated group than in the control animals at 356 days after injection. The authors estimated that at 6 h about 44% of the total dose was in the liver, 10% in the lungs and 2% in the spleen.

The Panel estimated the half-lives and the accumulation factors (R).[13] The estimate for the half-life was 83 days for the liver and lung and 350 days for the spleen. The Panel considered these estimates have high level of uncertainty. However, for the liver, the Panel calculated an accumulation factor of 134.7. The steady state for Ti/TiO_2 NPs in the liver would be reached after approximately 1.5 years. These figures are roughly the same for the lungs. For the spleen, the accumulation factor would be 350. The steady state would be reached after 5 half-lives which would be roughly 5 years.

The Panel noted that this study identifies a potential for TiO_2 NP accumulation in the liver, lung and spleen.

Kreyling et al. (2017a) investigated the fate of an intravenously administered aqueous [^{48}V]TiO_2 nanoparticle suspension, primary particle size 7–10 nm, particle size in water/tissue 88 nm in female Wistar-Kyoto rats. This preparation was obtained by irradiation of TiO_2 NPs with a proton beam current of 5 μA. The radioactive product (1.0 MBq/mg (^{48}V-activity per TiO_2 mass)) was used for the experiments lasting up to 24 h, whereas the product after irradiation for 5 days with a higher activity concentration of 2.35 MBq/mg was used for the experiment of 7 days and 28 days duration. At 1 h, 4 h, 24 h, 7 days and 28 days, four rats per time point were euthanised by exsanguination under anaesthesia. Blood, all organs, tissues and excreta were collected and ^{48}V-radioactivities were measured. The authors reported the nanoparticle quantities as percentages of the total intravenously injected ^{48}V radioactivity, which was calculated as the sum of all measured radioactivities, including faecal and urinary samples, corrected for background and radioactive decay during the experiments (^{48}V-half-live: 16.0 days). The percentage was the average over the group of four rats per time point. The detection limit was 0.2 Bq. The results of a separate intravenous study performed to investigate the absorption and biodistribution of soluble ionic ^{48}V were used to correct ^{48}V release from [^{48}V]TiO_2 nanoparticle. At 4 h following injection, 99.5% of the radioactive dose was found in the liver and at 28 days 88.9% of the dose was found in the liver. The spleen and the kidneys contained a few per cent of the dose (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%. ^{48}V was excreted in urine, within 28 days the excretion amounted to roughly 1%, the highest amount being excreted on day 1. Excretion by the faeces, indicative of biliary excretion, amounted to 3% over 28 days.

The Panel did not estimate the half-life of the [^{48}V]TiO_2 as the last measurement was at day 28 after administration when the decline in radioactivity was only 10% and the extrapolation would have a high degree of uncertainty. However, the data indicate that the half-life is long and likely of the order of months rather than weeks.

The study by Geraets et al. (2014) has already been described in the EFSA opinion on the re-evaluation of E 171 published in 2016. In the current opinion, the results of an analysis of the data with respect to an estimate of the whole body half-life and the resulting accumulation factor are presented. Geraets et al. (2014) investigated the fate of four TiO_2 nanomaterials after single and repeated (five times on consecutive days) intravenous administration. In the context of this evaluation, only NM-100 and NM-102 were considered of relevance for the assessment of E 171. The authors had reported half-lives for several organs they had investigated (not for the whole body) and they reported on the body recovery. Recovery of Ti was measured 24 h after the last dosing, on day 2 (single administration) and 6 (repeated administration), respectively, and for both dosing schedules on day 90 by summing up the contents of all organs investigated.

From these data, the Panel estimated a half-life for TiO_2 NPs for the whole body of roughly 200 and 450 days, with an accumulation factor of 290 and 450, for NM-100 and NM-102, respectively, and the time to steady state as 3 and 5 years, for NM-100 and NM-102, respectively.

[13] The authors did not give numbers for the tissue concentration but depicted their results in a figure with bars from which a rough estimate could be made at which time the concentration was 50% of the concentration after the end of distribution (24 h). Accumulation was estimated by using the textbook formula $R = 1/1 - e^{-ke \times \tau}$ with ke = ln 2/half-life with the assumption that the same dose would be applied every day.