EFSA statement on the review of the risks related to the exposure to the food additive titanium dioxide (E 171) performed by the French Agency for Food, Environmental and Occupational Health and Safety (ANSES)

EFSA (European Food Safety Authority)

Abstract

On 15 April 2019, the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) published an opinion on the risks related to the exposure to the food additive titanium dioxide (E 171) taking into account the most recent scientific studies available. Further to this publication, EFSA was requested by the European Commission to provide urgent scientific and technical assistance regarding the opinion issued by ANSES. In the ANSES opinion, 25 new relevant publications published between 2017 and 2019 were reviewed together with previous opinions by EFSA and ANSES and a systematic review on in vitro genotoxicity of nano titanium dioxide. In this statement, EFSA concludes that the ANSES opinion published in April 2019 does not identify any major new findings that would overrule the conclusions made in the previous two scientific opinions on the safety of titanium dioxide (E 171) as a food additive issued by the EFSA ANS Panel in 2016 and 2018. 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 evaluations and their recommendations. In addition to the aspects for which the follow-up work is currently ongoing, ANSES recommends further investigation of in vivo genotoxicity. EFSA considers this recommendation should be revisited once the ongoing work on the physico-chemical characterisation of the food additive titanium dioxide (E 171) is completed.

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The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.
1. **Introduction**

The current statement has been prepared by EFSA to provide urgent scientific and technical assistance regarding the review of the risks related to the exposure to the food additive titanium dioxide (E 171) performed by the French Agency for Food, Environmental and Occupational Health and Safety (ANSES).

1.1. **Background and Terms of Reference as provided by the requestor**

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. Only food additives that are included in the Union list, in particular Annex II of 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.

Titanium dioxide is authorised for use as a 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 subject to a new risk assessment by the European Food Safety Authority (EFSA), according to Commission Regulation (EU) No 257/2010, and in line with the provisions of Regulation (EC) No 1333/2008.

The re-evaluation of titanium dioxide as a food additive (E 171) was completed by EFSA in June 2016 and a scientific opinion was published in September 2016 (EFSA ANS Panel, 2016). In that opinion, EFSA concluded, on the basis of the available evidence, that titanium dioxide used as a food additive (E 171) does not raise concern with respect to genotoxicity; is not carcinogenic after oral administration; and exposure from the reported use/analytical levels would not be of concern. However, some recommendations were made, which are being followed up by the European Commission, to address data gaps highlighted in the scientific opinion.

In April 2017, ANSES published an opinion on dietary exposure to nanoparticles of titanium dioxide assessing, in particular the study of Bettini et al. (2017). It concluded that the data do not question the risk assessment performed by EFSA and cannot be used before having been confirmed by additional studies (ANSES, 2017).

In March 2018, the Commission requested EFSA to evaluate four new studies describing a potential adverse health effect of titanium dioxide used as a food additive. The EFSA opinion completed in June 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 a food additive.

On 15 April 2019, ANSES published a review of the risks related to the ingestion of the food additive titanium dioxide (E 171) taking into account the most recent scientific studies available and referring to its opinion of 2017.

The legislation on food additives envisages that food additives should be kept under continuous observation and be re-evaluated whenever necessary in the light of new scientific information. Therefore, it is appropriate to ask EFSA for a scientific assistance to scrutinise the review of new scientific information made by ANSES.

1.1.2. **Terms of Reference**

In accordance with Article 31 of Regulation (EC) No 178/2002, the European Commission requests the European Food Safety Authority (EFSA) to provide a scientific statement in relation to the review performed by ANSES on titanium dioxide (E 171).
In particular, EFSA is requested to consider whether the review made by ANSES:

i) includes any new and major findings showing that titanium dioxide (E 171), when used as a food additive, is of safety concern and thus overrules the conclusions made in the scientific opinion re-evaluating safety of titanium dioxide (E 171) as a food additive (2016) or in the evaluation of four studies on the potential toxicity of titanium dioxide used as a food additive (E 171) (2018);

ii) identifies further uncertainties which are worth addressing in addition to the aspects for which the follow up work is currently ongoing.

1.2 Previous evaluations of titanium dioxide (E 171) as a food additive

As stated in the background to this mandate, the food additive titanium dioxide (E 171) was re-evaluated for its safety under Regulation (EC) No 257/2010 in 2016 by the former EFSA Panel on Food Additives and Nutrient Sources (EFSA ANS Panel, 2016).

On the basis of the data available at the time, the ANS Panel concluded that titanium dioxide (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 anticipated limited absorption of titanium dioxide, the ANS Panel also concluded that the margins of safety (MoS) calculated from the no observed adverse effect level (NOAEL) of 2,250 mg titanium dioxide/kg body weight (bw) per day identified from a carcinogenicity study in rats (NTP, 1979) and the exposure, calculated based on the reported use levels and analytical data of titanium dioxide (E 171), would not be of concern (EFSA ANS Panel, 2016).

In re-evaluating the safety of titanium dioxide (E 171) as a food additive, the ANS Panel had however identified data gaps and uncertainties that required follow-up by the European Commission by means of a subsequent call for additional data.11

Among the recommendations issued by the ANS Panel was the request for data on the characterisation of the particle size distribution of the food additive E 171, in order to update the EU specifications of titanium dioxide when used as a food additive (see Section 1.3).

In addition, the Panel had identified the possible adverse effects in the reproductive system reported in two studies (Jia et al., 2014; Tassinari et al., 2014). Because of deficiencies in the study design and inadequate data reporting, the ANS Panel considered that the relevance of these findings was uncertain for the risk assessment of titanium dioxide as a food additive (EFSA ANS Panel, 2016). Therefore, the ANS Panel recommended an extended 90-day study or a multigeneration or extended-one generation reproduction toxicity study conducted with a test material representative for the food additive (E 171), to be performed in order to fill the data gap identified in the course of the assessment.

In 2017, ANSES issued a scientific opinion assessing the publication by Bettini et al. (2017) which investigated the potential for titanium dioxide to cross the gut barrier and distribute to other organs, its possible effects on immunological parameters, and its genotoxic and carcinogenic (initiator, promotor) potential. ANSES concluded that the findings from the Bettini et al. (2017) study did not call into question the risk assessment of titanium dioxide (E 171) performed by EFSA in 2016 and would require to be confirmed by additional studies with titanium dioxide (E 171) administered to animals in the food matrix. The potential promoting effect of titanium dioxide (E 171) in the colon must be confirmed by studies of longer duration, which should evaluate more biomarkers. Inclusion of an additional treated group with a sufficient number of animals was recommended to investigate the tumour-initiating potential of titanium dioxide (E 171) (ANSES, 2017).

In 2018, the ANS Panel issued a scientific opinion on four publications (Heringa et al., 2016; Bettini et al., 2017; Guo et al., 2017; Proquin et al., 2017) identified by the European Commission as relevant for the safety assessment of titanium dioxide (E 171). One of the four publications was the study by Bettini et al. (2017) mentioned above. Having reviewed the four publications, and after having received additional clarifications from their authors, the ANS Panel concluded that these studies highlighted some concerns although with uncertainties. Therefore, their relevance for the risk assessment was considered limited and the need for further research to decrease the level of uncertainties was identified. Altogether, the ANS Panel concluded that the outcome of the four studies did not merit re-opening of its 2016 opinion (EFSA ANS Panel, 2018).

10 Referred as NCI, 1979 in EFSA 2016 opinion.
11 Available online: https://ec.europa.eu/food/sites/food/files/safety/docs/fs_food-improvement-agents_reeval_call_20170130_E171_data.pdf
In line with the evaluation by ANSES (2017), the ANS Panel also recommended that the findings reported by Bettini et al. (2017) should be further investigated (EFSA ANS Panel, 2018). Inclusion of additional and relevant endpoints, such as biomarkers for putative preneoplastic lesions in the colon, to the parameters already included in the extended one-generation reproductive toxicity study was recommended. Interested business operators had already planned to generate data to be submitted to the European Commission in response to the follow-up call launched in January 2017.

1.3. Ongoing evaluation of titanium dioxide (E 171) as a food additive

In August 2018, EFSA was requested by the European Commission 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 the food additive, including its particle size and particle size distribution.12

The Panel on Food Additives and Flavourings (FAF) has been mandated with this assessment and is currently aiming at finalising its scientific opinion in the coming months.

2. Data and methodologies

The data considered in this statement are limited to the latest scientific advice published by ANSES in April 2019 in response to a request received at the end of February 2019 from the Ministers of the Economy, Health, Agriculture and the Environment to analyse the most recent knowledge on the toxicity of E 171 (ANSES, 2019).

3. ANSES opinion April 2019

ANSES set up an expert group which carried out a literature review on the oral toxicity of titanium dioxide covering the time span since the previous ANSES opinion issued in 2017. As part of its work, 25 new publications were considered relevant for the assessment (Table 1).

Table 1: Publications considered in the ANSES (2019)(a)

| Type of study          | Authors                                      |
|------------------------|----------------------------------------------|
| Literature review      | Winkler et al. (2018)                        |
|                        | Sohal et al. (2018a)                         |
| *In vitro* digestion   | Yusoff et al. (2018a)                        |
|                        | Yusoff et al. (2018b)                        |
|                        | Zhang et al. (2019)                          |
|                        | Sohal et al. (2018b)                         |
| Microbiota and gut barrier | Radziwill-Bienkowska et al. (2018)           |
|                        | Dufdefoi et al. (2017)                       |
|                        | Talbot et al. (2018)                         |
| Inflammation           | Riedle et al. (2017)                         |
| Genotoxicity           | Jensen et al. (2019)                         |
|                        | Gha et al. (2019)                            |
|                        | Dorier et al. (2018)                         |
|                        | Dorier et al. (2017)                         |
|                        | Proquin et al. (2017)                        |
| Carcinogenicity         | Proquin et al. (2018a)                       |
|                        | Proquin et al. (2018b)                       |
|                        | Proquin et al. (2018c)                       |
| Developmental toxicity | Jovanovic et al. (2018)                      |
|                        | Savčić-Zdravkovic et al. (2018)              |
|                        | Ma et al. (2019)                             |
| Cardiovascular toxicity | Jensen et al. (2018a)                       |
|                        | Jensen et al. (2018b)                        |
|                        | Freyre-Fonseca et al. (2018)                 |

(a): In addition to these 24 publications, the EFSA ANS Panel (2018) was considered.

12 EFSA-Q-2018-00625.
According to the ANSES expert group, new findings were identified from the 25 newly published studies reviewed, however none of them was robust enough to confirm or rule out the effects reported in the Bettini et al. (2017) study with respect to the potential tumour-promoting effects of titanium dioxide.

The need for a precise physico-chemical characterisation of titanium dioxide (E 171) is acknowledged as the first step needed for completing the risk assessment of the food additive. It is understood that this recommendation refers to the percentage of particles of the food additive titanium dioxide (E 171) that would fall in the nano range.

With respect to the new findings identified in the current review by ANSES, there are reportedly gene expression changes in an initiation/inflammation model in mice as well as epigenetic effects suggested primarily by histone expression changes that may ultimately facilitate tumour induction and promotion (Proquin et al., 2018a,b,c). Although with several limitations, ANSES considered that some of the effects seem consistent with previous observations (Urrutia-Ortega et al., 2016; Bettini et al., 2017) of a potential tumour-promoting potential of titanium dioxide and, like those two studies, the study by Proquin et al. (2018a,b,c) would deserve further investigation in specifically designed and adequately conducted studies.

The ANSES opinion acknowledges the conclusions of the ANS Panel on the lack of evidence of carcinogenicity based on the NTP10 (1979) studies although with a remark on the deficiency of the characterisation of the test material. Therefore, the relevance of the studies for the assessment of the carcinogenicity of the food additive is called into question by ANSES.

In their current opinion, previous recommendations issued by ANSES in 2017 are also reiterated with respect to the generation of new toxicological data to investigate the tumour-promoting potential of titanium dioxide (E 171) in the colon. These recommendations include studies with longer duration of exposure, which should integrate several biomarkers (such as aberrant crypt foci (ACF), mucin depleted foci, beta catenin accumulated crypt). The need to investigate the tumour initiator potential of titanium dioxide (E 171) is also reiterated.

ANSES reported findings of developmental abnormalities from studies in invertebrate species after exposure to titanium dioxide (E 171) (Jovanović et al., 2018; Savić-Zdravković et al., 2018; Ma et al., 2019). Although ANSES acknowledges in its opinion that these models are not suitable for the risk assessment of the food additive (because of difficulties in inter-species extrapolation), it still considers their results as indicative of a potential adverse effect that would warrant further investigation in relevant developmental toxicity animal models (i.e. mammals). This recommendation is supported by the lack of knowledge about the effects on reproductive organs and endocrine disruption highlighted by Heringa et al. (2016).

With respect to genotoxicity, the ANSES opinion makes reference to a previous systematic review of in vitro genotoxicity studies on nano titanium dioxide (Charles et al., 2018). According to the authors of this review, the majority of the publications analysed have shown that the in vitro genotoxic effect of nano titanium is linked to a secondary mechanism consequent to oxidative stress, although primary genotoxic effects cannot be excluded.

The new in vitro studies identified and assessed by ANSES in the current opinion do not call into question the outcome of the review by Charles et al. (2018).

The only additional in vivo study identified (Jensen et al., 2019) was considered by ANSES to have important methodological limitations (with respect to the doses and the protocol used and reporting of data) that limit the reliability of the negative results observed.

According to the ANSES opinion, although there are no studies showing direct interaction of titanium dioxide (E 171) with the DNA and/or the mitotic apparatus, a direct effect of titanium dioxide (E 171) on genetic material or other molecules interacting with the genetic material cannot be excluded. A recommendation for further investigation of in vivo genotoxicity is included in the ANSES (2019).

ANSES (2019) reported additional effects but did not consider them relevant for the safety assessment of the food additive: in vitro studies on the interaction between titanium dioxide and gut microbiota (Dudefoi et al., 2017; Radziwill-Bienkowska et al., 2018; Talbot et al., 2018); in vivo study in rats and ex vivo in human subcutaneous artery segments suggesting that exposure to titanium dioxide (E 171) at high doses may lead to cardiovascular effects (Jensen et al., 2018a,b), and in vitro studies investigating local inflammation in macrophages (Riedle et al., 2017).
4. **EFSA assessment**

The re-evaluation of titanium dioxide (E 171) finalised in 2016 was based on data available in the open literature and information provided by industry in response to EFSA public calls for scientific data or specific requests (referred as ‘Documentation provided to EFSA’ ANS Panel, 2016).

Within the information provided by industry, the following statement was provided ‘There has been no significant change in the particle size of products supplied for the food market, however, as with other particulate materials, there will be a distribution of primary particle sizes around the average value and it is possible that a small fraction of the primary particles would be below 100 nm. It is indicated that in practice any products supplied would be aggregated so the actual particle size would be larger than the primary particle size’. It was further noted that the percentage of nanoparticles – by number – in samples of titanium dioxide (E 171), analysed by TEM, varied from 11% to 39% (Table 3, ANS Panel, 2016).

On the basis of the information available at the time of the re-evaluation of titanium dioxide (E 171), the ANS Panel considered that ‘the food additive (E 171) mainly consists of microsized TiO2 particles, with a nanosized (< 100 nm) fraction less than 3.2% by mass’ (EFSA ANS Panel, 2016).

The ANS Panel noted that there were no set limits for the particle size of titanium dioxide (E 171) in its EU specifications and, therefore, at the time recommended that the characterisation of the particle size in the food additive E 171 should be included among the specifications. A similar recommendation for a precise physico-chemical characterisation of titanium dioxide used as a food additive in order to assess its safety is also reiterated in the ANSES opinion (2019).

EFSA is currently evaluating data submitted by interested business operators on the characterisation of titanium dioxide (E 171) focused on the particle size distribution of the materials used as a food additive in order to propose a modification of the EU specifications for titanium dioxide (E 171) that will define which titanium dioxide can be used as the food additive E 171.

With respect to the indication of developmental abnormalities identified in the literature review, the ANSES opinion makes reference to the gap in the reproductive toxicity database previously identified by EFSA in the course of the re-evaluation of titanium dioxide (E 171). ANSES recommends that the reproductive toxicity study should be conducted following the latest version of the OECD TG 443 (OECD, 2018), including all the three cohorts.

As a follow-up of the re-evaluation of titanium dioxide (E 171), in 2017 the European Commission had launched a call for data requesting interested parties to perform an extended one-generation reproductive toxicity (EOGRT). Interested business operators have informed that the planned study will include: cohort 1 (extension by mating of F1 animals to the F2 generation), cohort 2 (for developmental neurotoxicity) and cohort 3 (for developmental immunotoxicity). Following the recommendation from the 2018 ANS Panel, interested business operators have indicated that the ongoing EOGRT study has been modified to include 160 additional animals for the investigation of treatment-related and circadian variations of sexual hormones as well as the scoring of ACF. This modification partially addresses the recommendation from the ANSES opinion (2019). The final report of the EOGRT study is expected to be available by the end of July 2020 (Documentation provided to EFSA n. 1).

In addition, a subchronic dietary (100-day) study with E 171 to investigate the concerns raised by Bettini et al. (2017), effects of administration of E 171 on the formation of aberrant crypt foci in the intestine and on dendritic and T-cell tissue distribution and function with and without pretreatment with 1,2-dimethylhydrazine has been completed. This study was run jointly by Michigan State University and University of Nebraska Medical Center. Furthermore, a toxicokinetic study with E 171 to investigate the blood kinetics, tissues distribution and elimination/excretion mass balance will be initiated in 2019 (Documentation provided to EFSA n. 1).

No new carcinogenicity studies with titanium dioxide (E 171) have been identified in the literature review conducted by ANSES (2019). On the basis of the effects reported in the Proquin et al. (2018a, b, c) and, previously, in other studies (Urrutia-Ortega et al., 2016; Bettini et al., 2017), ANSES recommended to further investigate the tumour-promoting potential of E 171 in the colon. This concern is expected to be addressed by the above mentioned 100-day study commissioned by industry.

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13 Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/fs_food-improvement-agents_reeval_call_20170130_E171_outcome-2.pdf

14 Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/fs_food-improvement-agents_reeval_call_20170130_E171_outcome-2.pdf
In addition to the systematic review by Charles et al. (2018) on in vitro genotoxicity studies with nano titanium dioxide, ANSES has assessed three in vitro studies, among them Proquin et al. (2017) previously reviewed by EFSA (EFSA ANS Panel, 2018) and one in vivo genotoxicity study (Jensen et al., 2019).

According to Charles et al. (2018), non-consistent results are observed in the in vitro genotoxicity studies with nano titanium dioxide. Although secondary genotoxic effects consequent to oxidative stress seem to be the major mechanism responsible for the genotoxicity of nano titanium dioxide reported in some studies, primary genotoxic effects cannot be excluded. The authors recommended further in vivo studies to clarify the exact mode of action of nano titanium dioxide.

As highlighted by ANSES, the new in vitro genotoxicity studies present some methodological and reporting limitations (e.g. the absence of positive controls, deviation from OECD guidance) and do not allow drawing firm conclusions on in vitro genotoxicity. The potential for titanium dioxide to elicit positive results in in vitro genotoxicity tests, possibly associated with a mechanism involving oxidative stress, was already reported in the re-evaluation of titanium dioxide (E 171) as a food additive (EFSA ANS Panel, 2016) and further reiterated in the assessment of the Proquin et al. (2017) study (EFSA ANS Panel, 2018).

With respect to the in vivo study by Jensen et al. (2019), titanium dioxide (E 171) did not induce DNA strand breaks in a comet assay in lung and liver tissues of rats. The results from this in vivo study show a general lack of genotoxicity and of Reactive oxygen species (ROS) generation, however, these findings are hampered by some limitations (lack of a positive control, deviation from OECD guidance, no investigation in tissues at first site of contact). A potential effect on telomere length was also investigated in this study. The reported effect on telomere length, although statistically significant, is small and of unclear biological significance. While it is known that the telomere length is relevant for DNA stability, its relevance for ageing is still under debate. Thus, the biological relevance of their finding on telomere length shortening is questionable.

Overall, the new genotoxicity studies assessed in the ANSES opinion do not add new elements to the previous conclusions by the ANS Panel and do not provide any reason to revise the conclusion on genotoxicity of titanium dioxide (E 171) previously drawn by the ANS Panel (EFSA ANS Panel, 2016, 2018).

ANSES, however, supported the conclusions stated by Charles et al. (2018) and recommended new in vivo genotoxicity data to be generated.

EFSA considers that a review of the overall genotoxicity database may be needed once the characterisation of titanium dioxide used as the food additive E 171 is completed and has addressed one of the main uncertainties highlighted during the re-evaluation.

Conclusions

In addressing the Terms of Reference as part of this mandate, EFSA concludes that:

- The latest ANSES opinion published in April 2019 does not identify any major new findings that would overrule the conclusions made in the previous two scientific opinions on the safety of titanium dioxide (E 171) as a food additive issued by the EFSA ANS Panel in 2016 and 2018.
- The latest 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 evaluations and their recommendations.
- In addition to the aspects for which the follow-up work is currently ongoing, ANSES recommends further investigation of in vivo genotoxicity. EFSA considers this recommendation should be revisited once the ongoing work on the physico-chemical characterisation of the food additive titanium dioxide (E 171) is completed.

Documentation as provided to EFSA

1) CEFIC, 2019. Personal communication on the status of the toxicological testing with titanium dioxide (E 171). 6th May 2019.

References

ANSES, 2017. Avis de l’Agence nationale de sécurité sanitaire de l’alimentation, de l’environnement et du travail relatif à une demande d’avis relatif à l’exposition alimentaire aux nanoparticules de dioxyde de titane, 2017; Saisine n. 2017-SA-0020. Available online: https://www.anses.fr/fr/system/files/ERCA2017SA0020.pdf
ANSES, 2019. Avis de l’Agence nationale de sécurité sanitaire de l’alimentation, de l'environnement et du travail relatif au risques liés à la ingestion de l’additif alimentaire E 171. Saisine n° 2019-SA-0036. Available online: https://www.anse.fr/fr/system/files/ERCA2019SA0036.pdf

Bettini S, Boutet-Robinet E, Cartier C, Coméra C, Gaultier E, Dupuy J, Naud N, Taché S, Grysan P, Reguer S, Thieriet N, Réfrégier M, Dravet C, Cravedi J-P, Carrierre M, Audinot J-N, Pierre FH, Guzyack-Piriiu L and Houdeau E, 2017. Food-grade TiO2 impairs intestinal and systemic immune homeostasis, initiates preneoplastic lesions and promotes aberrant crypt development in the rat colon. Scientific Reports, 7, 40373.

Charles S, Jomini S, Fessard V, Bigorgne-Vizee E, Rousselle C and Michel C, 2018. Assessment of the in vitro genotoxicity of TiO2 nanoparticles in a regulatory context. Nanotoxicology, 12, 357–374. https://doi.org/10.1080/17435390.2018.1451567

Dorier M, Béa D, Marie-Desvergne C, Dubosson M, Barreau F, Houdeau E, Herlin-Boime N and Carrierre M, 2017. Continuous in vitro exposure of intestinal epithelial cells to E171 food additive causes oxidative stress, inducing oxidation of DNA bases but no endoplasmic reticulum stress. Nanotoxicology, 11, 751–761.

Dorier M, Tissery C, Dussert F, Béal D, Arnal ME, Douki T, Valdigliesis V, Laffon B, Fraga S, Brandão F, Herlin-Boime N, Barreau F, Rabilloud T and Carrierre M, 2018. Toxicological impact of acute exposure to E171 food additive and TiO2 nanoparticles on a co-culture of Caco-2 and HT29-MTX intestinal cells. Mutation Research - Genetic Toxicology and Environmental Mutagenesis, https://doi.org/10.1016/j.mrgentox.2018.11.004

Dudefoot W, Moniz K, Allen-Vercoe E, Ropers MH and Walker VK, 2017. Impact of food grade and nano-TiO2 particles on a human intestinal. Community. Food and Chemical Toxicology, 106, 242–249.

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), 2016. Opinion on the re-evaluation of titanium dioxide (E 171) as a food additive. EFSA Journal 2016;14(9):4545, 83 pp. https://doi.org/10.2903/j.efsa.2016.4545

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), Younes M, Aggett P, Aguilar F, Crebelli R, Dusemund B, Filippic M, Frutos MJ, Galltier P, Gott D, Gundert-Remy U, Kuhnle GG, Lambré C, Leblanc J-C, Lillegaard IT, Moldeus P, Mortensen A, Oskarsson A, Stankovic I, Vaalkens-Berendsen I, Wright M, Lodi F, Rincon AM, Smeraldi C and Woutersen RA, 2018. Scientific Opinion on the evaluation of four new studies on the potential toxicity of titanium dioxide used as a food additive (E 171). EFSA Journal 2018;16(7):5366, 27 pp. https://doi.org/10.2903/j.efsa.2018.5366

Freyre-Fonseca V, Medina-Reyesa EI, Téllez-Medina DJ, Paniagua-Contreras GL, Monroy- Pérez E, Vaca-Paniagua F, Delgado-Buenrostroa NL, Flores-Flores JQ, López-Villegas EO, Gutiérrez-López GF and Chirinoa YI, 2018. Influence of shape and dispersion media of titanium dioxide nanostructures on microvesSEL network and ossification. Colloids and Surfaces B: Biointerfaces, 162, 193–201.

Gea M, Bonetta S, Iannarelli L, Giovannozzi AM, Maurino V, Bonetta S, Hodoroaba VD, Armato C, Rossi AM and Schiliro T, 2019. Shape-engineered titanium dioxide nanoparticles (TiO2- NPs): cytotoxicity and genotoxicity in bronchial epithelial cells. Food and Chemical Toxicology, 127, 89–100.

Guo Z, Martucci N, Moreno-Olivas F, Tako E and Mahler G, 2017. Titanium dioxide nanoparticle ingestion alters nutrient absorption in an in vitro model of the small intestine. NanoImpact, 5, 70–82, janvier 2017.

Heringa MB, Geraets L, vanEijkeren JCH, Vandebriel RJ, deJong W and Oomen AG, 2016. Risk Assessment of Titanium Dioxide Nanoparticles via Oral Exposure, Including Toxicokinetic Considerations., Nanotoxicology, 10.

Jensen DM, Christophersen DV, Sheykzhade M, Skovsted GF, Lykkefeldt J, Münter R, Roursgaard M, Loft S and Moller P, 2018a. Vasomotor function in rat arteries after ex vivo and intragastric exposure to food-grade titanium dioxide and vegetable carbon particles. Particle and Fibre Toxicology, 15, 12.

Jensen DM, Skovsted GF, Lykkefeldt J, Dreier R, Berg JO, Jeppesen JL, Sheykzhade M, Loft S and Moller P, 2018b. Vasomotor dysfunction in human subcutaneous arteries exposed ex vivo to food-grade titanium dioxide. Food and Chemical Toxicology, 120, 321–327.

Jia F, Sun Z, Yan X, Zhou B and Wang J, 2014. Effect of pubertal nano-TiO2 exposure on testosterone synthesis and spermatogenesis in mice. Archives of Toxicology, 88, 781–788.

Jensen DM, Lahr M, Sheykzhade M, Lykkefeldt J, Wils RS, Loft S and Moller P, 2019. Telomere length and genotoxicity in the lung of rats following intragastric exposure to food grade titanium dioxide and vegetable carbon particles. Mutationesis, geo003. https://doi.org/10.1080/17435390.2019.1648371

Jovanovic B, Jovanovic N, Cvetkovic VJ, Matic S, Stanic S, Whitley EM and Mitrovic TL, 2018. The effects of a human food additive, titanium dioxide nanoparticles E171, on Drosophila melanogaster – a 20 generation dietary exposure experiment. Scientific Reports, 8, 17922.

Ma H, Lenz A, Gao X, Li S and Wallis LK, 2019. Comparative toxicity of a food additive TiO2, a bulk TiO2, and a nano-sized P25 to a model organism: the nematode C. elegans. Environmental Science and Pollution Research, 26, 3556–3568.

NTP (National Toxicology Program), 1979. Bioassay of titanium dioxide for possible carcinogenicity. Technical Report Series No 97. NCI, Rockville, MD.

OECD (Organisation for Economic Co-operation and Development), 2018. Test No. 443: Extended One-Generation Reproductive Toxicity Study, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. https://doi.org/10.1787/9789264185371-en
Proquin H, Rodríguez-Ibarra C, Moonen CGJ, Urrutia Ortega IM, Briedé JJ, de Kok TM, van Loveren H and Chirino YI, 2017. Titanium dioxide food additive (E171) induces ROS formation and genotoxicity: contribution of micro and nano-sized fractions. Mutagenesis, 32, 139–149.

Proquin H, Jetten MJ, Jonkhout MCM, Garduño-Balderas LG, Briedé JJ, de Kok TM, Chirino YI and van Loveren H, 2018a. Gene expression profiling in colon of mice exposed to food additive titanium dioxide (E171). Food and Chemical Toxicology, 111, 153–165.

Proquin H, Jetten MJ, Jonkhout MCM, Garduño-Balderas LG, Briedé JJ, deKok TM, Chirino Y and vanLoveren H, 2018b. Time course gene expression data in colon of mice after exposure to food-grade E171. Data in Brief, 16, 531–600.

Proquin H, Jetten MJ, Jonkhout MCM, Garduño- Balderas LG, Briedé JJ, de Kok TM, van Loveren H and Chirino YI, 2018c. Transcriptomics analysis reveals new insights in E171-induced molecular alterations in a mouse model of colon cancer. Scientific Reports, 8, 9738.

Radziwill-Bienkowska JM, Talbot P, Kamphuis JB, Robert V, Cartier C, Fourquaux I, Lentzen E, Audinot JN, Jamme F, Refregiers M, Bardowski JK, Langella P, Kowalczyk M, Houdeau E, Thomas M and Mercier-Bonin M, 2018. Toxicity of Food-Grade TiO2 to Commensal Intestinal and Transient Food-Borne Bacteria: New Insights Using Nano-SIMS and Synchrotron UV Fluorescence Imaging. Frontiers in Microbiology, 9, 794.

Riedle S, Pele LC, Otter DE, Hewitt RE, Singh H, Roy NC and Powell JJ, 2017. Pro-inflammatory adjuvant properties of pigment-grade titanium dioxide particles are augmented by a genotype that potentiates interleukin 1β processing. Particle and Fibre Toxicology, 14, 51.

Savić-Zdravković D, Jovanović B, Durčević A, Stojković-Piperac M, Savić A, Vidmar J and Milošević D, 2018. An environmentally relevant concentration of titanium dioxide (TiO2) nanoparticles induces morphological changes in the mouthparts of Chironomus tentans. Chemosphere, 211, 489–499.

Sohal IS, O’Fallon KS, Gaines P, Demokritou P and Bello D, 2018a. Ingested engineered nanomaterials: state of science in nanotoxicity testing and future research needs. Particle and Fibre Toxicology, 15, 29.

Sohal IS, Cho YK, O’Fallon KS, Gaines P, Demokritou P and Bello D, 2018b. Dissolution Behavior And Biodurability Of Ingested Engineered Nanomaterials Gastrointestinal Environment. ACS Nano, 12, 8115–8128.

Talbot P, Radziwill-Bienkowska JM, Kamphuis JB, Steenkeste K, Bettini S, Robert V, Noordine ML, Mayeur C, Gaultier E, Langella P, Robbe-Masselot C, Houdeau E, Thomas M and Mercier-Bonin M, 2018. Food-grade TiO2 is trapped by intestinal mucus in vitro but does not impair mucin O-glycosylation and short-chain fatty acid synthesis in vivo: implications for gut barrier protection. Journal of Nanobiotechnology, 16, 53.

Tassinari R, Cubadda F, Moracci G, Aureli F, D’Amato M, Valeri M, De Berardis B, PASSERI D, Rossi M and Maranghi F, 2014. Oral, short-term exposure to titanium dioxide nanoparticles in Sprague-Dawley rat: focus on reproductive and endocrine systems and spleen. Nanotoxicology, 8, 654–662.

Urrutia-Ortega IM, Garduño-Balderas LG, Delgado-Buenrostro NL, Freyre-Fonseca V, Flores- Flores JO, González-Robles A, Pedraza-Haverri J, HERNÁNDEZ-PANDO R, Rodriguez-Sosa M, LEÓN-CABRERA S, TERRAZAS LL, van Loveren H and Chirino YI, 2016. Food-grade titanium dioxide exposure exacerbates tumor formation in colitis associated cancer model. Food & Chemical Toxicology, 93, 20–31.

Winkler HC, Notter T, Meyer U and Naegeli H, 2018. Critical review of the safety assessment of titanium dioxide additives in food. Journal of Nanobiotechnology, 16, 1–19.

Yusoff R, Nguyen LTH, Chiew P, Wang ZM and Ng KW, 2018a. Comparative differences in the behavior of TiO2 and SiO2 food additives in food ingredient solutions. Journal of Nanoparticle Research, 20, 76.

Yusoff R, Kathavala MH, Nguyen LTH, Setyawatia MI, Chiew P, Wub Y, Ch'ng AL, Wang ZM and Nga KW, 2018b. Biomolecular interaction and kinematics differences between P25 and E171 TiO2 nanoparticles. NanoImpact, 12, 51–57.

Zhang Z, Zhang R, Xiao H, Bhattacharya K, Dimitrios B, Demokritou P and Mc Clements DJ, 2019. Development of a standardized food model for studying the impact of food matrix effects on the gastrointestinal fate and toxicity of ingested nanomaterials. NanoImpact, 13, 13–25.

Abbreviations

ACF aberrant crypt foci
ANS EFSA Panel on Food Additives and Nutrient Sources added to Food
ANSES Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail/French Agency for Food, Environmental and Occupational Health and Safety
bw body weight
EOGRT extended one-generation reproductive toxicity
FAF EFSA Panel on Food Additives and Flavourings
MoS margin of safety
NCI National Cancer Institute
NOAEL no observed adverse effect level
NTP National Toxicology Program
OECD Organisation for Economic Co-operation and Development
ROS Reactive Oxygen Species