EFSA Re-Assessment of Titanium Dioxide (E 171), 2021

In this guide

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This is a paper for discussion.

This does not represent the views of the Committee and should not be cited.

50. The following section of this paper discusses the EFSA re-evaluation. It briefly addresses the data considered by EFSA and presents the main conclusions. The underlying data on the endpoints of toxicokinetics and absorption, developmental and reproductive toxicity and aberrant crypt foci are further discussed in detail in paragraphs 81 onwards to allow the COT to independently assess them. While a comment has been included on the conclusions around

genotoxicity, these will not be considered further in this paper.

51. Concerning absorption and toxicity of TiO2 particles that are present in E 171, the Panel concluded that:

- The absorption of TiO2 particles is low but they may accumulate in the body due to their long half-life.
- No studies appropriately designed and conducted to investigate the potential carcinogenicity of TiO2 nanoparticles were available.

Data & Methodology of the EFSA 2021 Opinion

52. 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 relevant existing Guidance from the EFSA Scientific Committee, including 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).

53. The 2021 EFSA evaluation is based on the following data:

- Information from publications retrieved in the literature search (see Annex B for criteria).
- Data submitted in response to the call for data from European Commission as follow-up of there-evaluation of E 171.
- Toxicokinetic studies considered in the re-evaluation of titanium dioxide (E 171)
- Exposure data available in the re-evaluation 2016 and additional relevant information published since that time.
- In-vitro and in-vivo studies reported in the OECD dossier (2016) and submitted to EFSA

54. 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.

55. 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.

56. With regards to toxicity, a literature search was performed following the approach and information on the criteria for inclusion and exclusion of publications based on information from the abstract and title, and material used in the study is described in Annex B. Toxicokinetic and toxicity studies considered ‘included’ were assessed for their relevance and reliability. The Panel further assessed the EOGRTS data submitted by industry, which also included an endpoint investigating aberrant crypt foci induction, following the Panel’s recommendations in the 2016 evaluation.

57. Nanoscale considerations for the assessment of the study design and study results in toxicity studies classified with reliability 1 and 2 (see Annex B for criteria).

Dietary Exposure Data

58. 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 the 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. The current paper does not expand on this information due to the fact that, because of the Panel conclusions on genotoxicity, the exposure information was not further considered in the risk assessment.

Toxicity

59. With regard to the genotoxicity studies, combining the available lines of evidence, the FAF Panel concluded that “TiO2 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 TiO2 particles – such as crystalline form, size of constituent particles, shape and 7 agglomeration state – and the outcome of in vitro or in vivo genotoxicity assays” (i.e a cut-off value for TiO2 particle size with respect to genotoxicity could not be identified). The Panel also 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 TiO2 particles are unknown. Based on the available data, no conclusion could be drawn as to whether the genotoxicity

of TiO2 particles is mediated by a mode (s) of action with a threshold(s)". Therefore, the Panel concluded that a concern for genotoxicity of TiO2 particles cannot be ruled out. The underlying data on genotoxicity is currently being reviewed by the COM, as part of the UK independent review of the safety of TiO2.

60. With regards to other endpoints the FAF Panel concluded "that the absorption of TiO2 particles is low, however they can accumulate in the body due to their long half-life; studies on general and organ toxicity, including the newly performed EOGRT study with E171, did not indicate adverse effects up to a dose of 1,000 mg/kg bw per day. In addition, no effects were seen in literature studies employing TiO2 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 with E171. No other reliable studies were found in the literature addressing these effects with E171; some findings regarding immunotoxicity and inflammation with E171 as well as neurotoxicity with TiO2 NPs may be indicative of adverse effects. They also considered that there are indications of the induction of aberrant crypt foci in the small intestine with E171 and that no studies appropriately designed and conducted to investigate the potential carcinogenicity of TiO2 nanoparticles were available."

Uncertainty

61. The Panel identified uncertainties related to the following points:

- The size distribution of the particles in marketed E171 that consumers are exposed to, related to the different types of E171, as presented in the EFSA ANS Panel (2019) opinion.
- The processes used by industry when using E171 in food and to what extent these processes may affect the degree of agglomeration and thus internal exposure.
- The state of agglomeration i.e., presence of 'free' (non-agglomerated) particles of tested material in gastrointestinal tract of the animals and its effect on absorption.
- The representativity of different tested materials used in toxicity studies for the food additive E171 when used in food.
- Differences in the physico-chemical properties of the different tested materials and the extent of their impact on the observed results.
- Interference in the measurements of Ti/TiO2 in blood, tissues or organs with the most widely used analytical technique, i.e., ICP-MS, and its impact on the

reliability of tissue concentration data.
- Confidence in the limited kinetic data as the basis for estimating half-lives and accumulation and for assessment of internal exposure and, related to that, the extent of systemic availability based on the proposed amendment for EU specifications of titanium dioxide.
- None of the rodent studies were sufficiently long to cover the time needed for reaching the steady state for accumulation and this impacted the interpretation of the study results.

62. The Panel identified uncertainties regarding the EOGRT study with respect to its validity to fully identify all potential adverse effects of E 171 when used as a food additive:

- The extent to which the particle size distribution of the E 171 used in the EOGRT study is reflective of the particle size distributions of E 171 when added to foods.
- The extent to which the particle size distribution of E 171 in transit through the gastrointestinal tract in the EOGRT study was affected by the concentration in the diet (i.e. dose).The selected test material was representative of E 171 containing a large proportion (around 50% by number) of constituent particles below 100 nm (E 171 sample E reported in EFSA FAF Panel, 2019).The particle size distribution of the E 171 in samples of the test diet was also analysed after applying a sample dispersion protocol that aims to extract E 171 particles from the feed matrix and the results show that the particle size distribution of the constituent Safety assessment of the food additive titanium dioxide (E 171) was similar to that of pristine E 171 after dispersion (EFSA FAF Panel, 2019; Verleysen et al.,2020). However, neither of these procedures were considered by the Panel to reliably determine the particle size distribution of E 171 in the feed.

63. The Panel acknowledged that the methods for determining particle size distributions in complex foods and feeds in-situ are not currently available. Accordingly, the Panel considers that the extent to which the particle size distribution of the E 171 used in the EOGRT study is sufficiently reflective of the particle size distributions of E 171 when added to foods remains uncertain. The interested business operator considered that mixing of two dry components (feed and E 171) was the best possible option to retain the particle size distribution properties of the original E 171 sample, and that the use of liquid dispersion would add further superfluous unknowns.

64. The Panel considered that E 171 has a broad size distribution of constituent particles (from about 40 to 250 nm); considered that in dry form, this size distribution of the constituent particles is expected to be stable and further, that homogenous mixing of E 171 with dry diet is a pragmatic approach to adopt in terms of performing an animal study over an extended time frame such as the EOGRT study. The Panel considered this approach to be representative of some uses of E 171 in food (e.g., E 171 in confectionary coatings and fillings and in ready to use sauces. However, the Panel also noted that this approach may not be fully representative for all uses of E 171 in food since liquid dispersion of E 171 was reported to be used, potentially along with additional processes, to reduce the formation of agglomerates in suspension in some products (e.g., incorporation of E 171 into a tablet coating or capsule.

65. The Panel considered that investigations of TiO2 levels in tissues would have reduced uncertainty regarding dose dependency of internal exposure. However, the Panel noted that the EOGRT study demonstrated unequivocally low levels of internal exposure to TiO2 in animals that were fed a diet prepared by addition of E 171 to dry feed. Dispersed Nanoparticles show a greater tendency to agglomerate when suspended in liquid media at higher concentrations. This concentration effect on agglomeration and/or resistance to de-agglomeration may also exist in the gastro-intestinal tract at high-dose levels The Panel therefore considered that there remains an uncertainty regarding the effects of dose levels/concentrations in feed and the extent to which agglomeration occurred in the gastrointestinal tract. However, the Panel considered the propensity for this agglomeration is likely reduced when exposure is via feed rather than through bolus gavage administration of E 171

Conclusion:

66. Considering all currently available evidence and uncertainties, the Panel concluded that E 171 can no longer be considered as safe when used as a food additive due to genotoxicity considerations. This applies to E 171 as described in Commission Regulation (EU) No 231/2012 and E 171 specified in the EFSA Food Additive and Flavourings Panel opinion in 2019.

COT comments on the 2021 EFSA opinion

67. The COT considered the EFSA Opinion on titanium dioxide at their July 2021 meeting. The Committee considered a summary of the EFSA opinion as well as the preliminary comments from the COM meeting; these are noted in the

introduction and not considered further in this paper.

68. The COT also noted that in several parts of the Opinion, published papers were presented at face value, and there was no discussion of the results nor the overall Weight of Evidence to support the conclusions being made. They furthermore noted discrepancies and conflicts between the results of the studies reported and the overall conclusions.

69. Overall, the COT considered that there was a lack of internal consistency and of objective weighing of the evidence. While some of this might have been due to differences in the nature of the TiO2 tested, this was not clear in the Opinion.

70. Members also noted that it was difficult to draw any conclusions from the studies and a closer look in terms of material characterisation was needed in order to understand some of the effects reported. Members also considered that follow up was needed on the reproductive toxicity study as only the presence or absence of an effect was measured.

71. The large variation in the specifications of E 171 was also discussed based on the analytical data for pristine E 171 that indicated that more than 50% of the constituents were in the nano-range so the COT considered that more clarification was needed on the actual composition of E 171. It was noted that the EFSA definition of nanomaterials lacked clarity with regard to materials that were not engineered as nanomaterials but contained particles in the nano range. The possibility and plausibility of removing the nano fraction from E 171 in order to mitigate the risk was also discussed by the COT.

72. With regard to absorption, it was noted that there was no reason to believe that titanium dioxide particles behaved differently to other particles in the gastrointestinal tract.

73. Members were advised that newer studies used in the previous evaluation were re-considered (evidence from deceased humans and indications that titanium dioxide could cross the placenta). The duration of the animal studies was not sufficient to evaluate at which levels steady state would be reached and therefore it was considered that absorption had previously been underestimated.

74. The extended one generation reproductive toxicity (EOGRT) study provided indirect evidence for systemic exposure following administration of titanium dioxide. Members were informed that EFSA had indications that when used by industry, E171 was dispersed into nanoparticles by sonication and

therefore also considered data on materials made solely of nanoparticles for the assessment. However, this was questioned by Members as it was noted that pure nano titanium dioxide would lose its technical function in the food (as it would not provide colour) and would therefore not be of use.

75. The COT also questioned the conclusions with regards to the ability of TiO2 to induce aberrant crypt foci. On this point, the Committee were advised that because of the above consideration by EFSA, only one study that used sonication of the material was considered, as the material tested was undispersed in the other available studies.

76. The findings of the studies on neurotoxicity were considered inconsistent by the COT. It was noted that the EOGRT study did not report any effects and that most of the other studies on this endpoint were of nanomaterials.

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

78. On balance, the Committee considered that the weight of evidence did not support the conclusions drawn by EFSA. The COT also agreed with the comments of the COM with regards to risk communication that “As it stands the conclusion is highly risk adverse based on the weak evidence available, and it might create unnecessary concern to the public.” They considered that care should be taken when expressing the conclusions as they might cause unnecessary concern and they were uncomfortable with EFSA’s binary communication on a dataset with a lot of uncertainties. They highlighted that the COT does not follow the precautionary approach.

79. When considering whether they agreed with EFSA’s conclusion that no differentiation could be made with regards to size/form of titanium dioxide and different aspects of toxicity, the COT erred towards the view that nanoparticles were driving the toxicity. It was decided that an interim position paper, capturing the COT’s view and the proposed next steps should be published.