

Fifth draft statement on the safety of Titanium Dioxide (E171) as a Food Additive- EFSA review and conclusions on ADME of TiO₂

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EFSA review and conclusions on ADME of TiO₂

93. “On the basis of the data available, the EFSA ANS Panel concluded that the absorption and oral bioavailability of titanium dioxide was low, independent of size. EFSA noted that the toxicokinetics of E 171 was assessed in three mouse studies and two human studies. The studies by Comera et al., (2020) and Talamini et al., (2019) allowed the derivation of estimates of internal exposure of 0.01 and 0.1% of the external dose, respectively.” (EFSA, 2021).

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

95. “The Panel noted that in mice, TiO₂ can be taken up from the small intestine by the paracellular pathway and by endocytosis. Furthermore, in two of the studies (Comera et al., 2020; Riedle et al., 2020), uptake of TiO₂ particles was demonstrated into M-cells of the Peyer’s patches, whereby the quantitative contribution to the systemic exposure seems to be low. In humans, the Panel considered that after oral administration of 100 mg E 171, Ti concentration in blood increased ca. 5- to 10-fold from 6 to 10 h post-dosing (Pele et al., 2015), demonstrating some oral systemic availability. TiO₂ particles were found in human placenta in low concentrations (Guillard et al., 2020), indicating that TiO₂

is systemically available after ingestion and also can distribute to the placenta. In an ex vivo human placenta model, particles were transferred and the size distribution of the particles was similar to the E 171 present in the perfusate. The Panel noted that the extent of transfer across placental membranes was small. The Panel noted that materials other than E 171, mainly TiO₂ NPs, were investigated in rats and humans. In rats, two intravenous studies (Disdier et al., 2015; Kreyling et al., 2017a) demonstrated long half-lives and, hence the potential for accumulation. Together with data from an intravenous study (Geraets et al. (2014) already addressed in the EFSA opinion on the re-evaluation of E 171 (EFSA ANS Panel, 2016), half-lives of 83 days (for liver) and of 450 days (for whole body) were estimated and accumulation factors between 135 and 450. Based on these data, the steady state would be reached between 1.5 and 5 years. Out of five oral rat studies, one provided an estimate for oral systemic availability of 0.0002% based on a limited number of organs (Hendrickson et al., 2016) and another study provided an estimate of 0.6% (Kreyling et al., 2017b). The Panel noted that in a study employing the model of isolated loop technique, the authors could provide data indicating the presence of TiO₂ NPs either as single particles or as smaller and larger agglomerates in intestine, liver and spleen (Hendrickson et al., 2020). The other studies did not give data suitable to quantify absorption and/or accumulation. The Panel considered that in two studies analysing tissues from deceased subjects, deposition of Ti-containing nanoparticles was observed in liver, spleen and kidney as well as in intestine. After quantification of the Ti amount in the organs and comparison with the estimated mean daily intake of E 171, the Panel concluded that the oral systemic availability of TiO₂ NP ingested from a number of sources, including dietary exposure to E 171, would be low (less than 1% by mass).” (EFSA, 2021).

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

Health Canada review and conclusions

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

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

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

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

FSANZ review and conclusions

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

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

COT review and conclusions

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

104. The COT further noted concerns around absorption, especially relating to nanoparticles/nanofractions as these were variable in the test substances and hence varied in absorption in the studies described above. The COT could not separate the impact of the TiO₂ form and matrix of administration to conclude on the observed variability in absorption.

105. The COT noted that TiO₂ particles can cross the placenta. However, because absorption is low, this would be a very small number. It was unclear whether this was by passive diffusion or active uptake and, what form the TiO₂ was in by the

time it got to these barriers. Once intracellular, materials can change in response to different pH and be transferred elsewhere.

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

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

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

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

Review of toxicity for endpoints identified by the COT

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

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