Detailed breakdown on studies considered by EFSA

In this guide

In this guide

This is a paper for discussion.

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

80. This section expands on the underlying data of toxicokinetics and absorption, the EOGRT study and ACF. For context, the EFSA Panel conclusions have been included in the relevant sections, for the COT's information.

Toxicokinetics and Absorption

81. The toxicokinetics of E 171 was addressed in five studies in total, three studies in mice and in two studies in humans.

E171 studies in mice

Talamini et al (2019)

82. The test material used was: E171 (food grade titanium dioxide), 99.3% pure anatase, 35% nanoparticles.

83. Groups of 8-week-old male NRF mice (n=22/group) were treated with 5 mg/kg bw of E171 dispersed in water (no sonication or deagglomeration). The animals were treated for 3 days/week for 3 weeks receiving a total of 9 treatments in 21 days. The average daily dose of 2 mg/kg bw. The actual treatment concentration was verified by inductively coupled plasma mass spectrometry (ICP-MS). The test material: E171 or water (control) was slowly dripped with pipette in the mouths of the mice, allowing for each drop to be swallowed.

84. The animals were weighed at the beginning of the experiment and observed daily. No signs of general toxicity were observed. On day 21, the animals were sacrificed and the lungs, liver, stomach, spleen. Kidney, brain, testes and whole intestine were removed. The concentrations of titanium were determined in 4 animals.

85. In the brain, kidney and testes, titanium levels were <0.03 µg/g, the quantification limit of the analytical method for solid tissue samples. In lungs and spleen the levels were low, with a not statistically significant, but slightly higher deposition in spleen of E171 treated animals compared to the controls. The authors reported that “Titanium concentration was one order of magnitude greater in the small intestine compared to the above tissues and distinctly higher in the stomach, large intestine and liver”. The concentrations of titanium in treated animals were: 1.07 ± 0.38 µg Ti/g tissue in the large intestine and 0.94 ± 0.57 µg Ti/g tissue in the liver. These levels were 1.8 and 3.6 times higher compared to the controls, respectively.

Comera et al (2020)

86. The test material used was: E 171, > 95% anatase, 20–340 nm (transmission electron microscopy- TEM); 44.7% nanoparticles.

87. In the first round of experiments adult C57BL/6 mice (12–18 weeks) were treated with a single gavage dose of 40 mg/kg of E171 suspended in water and sonicated or water. In the second round of experiments, 300µg/mL of E171 was suspended in buffer and used to fill a closed mid- jejunal loop of 10cm, pre-treated with inhibitors of tight junctions, micropinocytosis, clathrin-mediated endocytosis or raft-dependent endocytosis.

88. Animals were sacrificed at 2-, 4-, 8- and 24-hours following treatment. Confocal microscopy and micro x-ray fluorescence imaging was used to analyse the existence of particles in both the first and second round of experiments, whilst ICP-MS was used to determine titanium concentration in blood and tissues (jejunum, ileum, colon). The jejunal or colonic intraluminal contents were recovered by gentle scraping.

89. In mice treated with a single dose of E171, the number of titanium dioxide reflective particles in the lumen of the upper intestine was significantly increased, with analysis of the particles suggesting that no further agglomeration of titanium dioxide occurred during its transit through the intestinal tract, and that as it moved in the distal intestine, there was a decrease in its agglomeration state (as indicated by smaller particle size in the colonic versus the jejunal lumen).

90. An increase in the reflective particle content was observed in the jejunal and ileal villi, Peyer’s Patches and colon crypts. The overall particle content in jejunal villi increased from 2 hours after gavage, peaked at 4 hours, and returned to basal values at 8 hours. A statistically significant increase ($p < 0.001$) 4 hours after E171 administration was observed in the titanium dioxide particle density in the jejunal mucosa (increased by 3.4-fold over the controls). A lower and non-significant trend of increased particle content was also observed at 4 hours in the ileum and colon, with the values decreasing close to control levels at the time 8 hours in all three intestinal sections. In the jejunum, the reflective TiO2 particle spots displayed a mean diameter of 700 ± 59 nm ($n = 70$) and were mostly observed in the lamina propria and in goblet cells (GCs) distributed in the epithelium, with some of them also found in enterocytes lining the gut lumen. Analysis by transmission electron microscopy coupled with energy-dispersive X-ray spectroscopy (TEM-EDX) indicated the presence of both Ti and O in particles detected in jejunal GCs and enterocytes. These appeared as primary particles or aggregates with respective sizes of 450 and 170 nm. In the Peyer’s patches, a statistically significant increase in laser-reflecting particles was found only at 8 hours (increased by 5.4-fold over that of controls ($p < 0.001$)). In blood, the

number of particles significantly increased by 3.5- and 4.1-fold at 4 and 8 hours, respectively, but the titanium concentrations remained below the limit of detection (LOD<0.02 ng Ti/kg) at all timepoints. From the content in the intestines and the weight of the mice tissues, the authors calculated that approximately 0.007% of the titanium administered was present in the entire intestine at the 4 hours timepoint. The authors concluded that titanium dioxide was absorbed predominantly in the ileum, partly in jejunum and that small amount absorbed in the colon. Based on the surface area information it was concluded that titanium dioxide is predominantly absorbed by the small intestinal villi and to a lesser extent through Peyer's patches.

91. In the *ex vivo* experiment, under anaesthesia, a closed mid-jejunal loop (10cm) was isolated and pre-treated with either just PBS (control) or a PBS solution with inhibitors as described above for 30 minutes. The contents were then rinsed and replaced with PBS (control) or sonicated E171 and incubated for a further 30 minutes. A significant inhibition of TiO2 absorption (by 66%) was observed and the authors considered that the paracellular route is a major pathway governing transepithelial TiO2 passage. However, 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 titanium dioxide from the intestinal lumen to blood.

Riedle et al., (2020)

92. The test material used was: E171, anatase, 119nm (EFSA 2021).

93. C57BL/6 mice were treated with titanium dioxide doses of 0, 6.25, 62.5 and 625 mg/kg of diet. These were equivalent to 0 and approximately equal to 1, 10 and 100 mg E 171/kg bw per day.

94. The animals were sacrificed at 6, 12 and 18 weeks. Animals sacrificed at 18 weeks were also used to validate that the diet permitted uptake in the intestinal lumen. The basal regions of the Peyer's patches were surveyed, and reflectance confocal microscopy was used to determine the presence of titanium dioxide.

95. Reflectant foci, indicative of titanium dioxide presence, were found at the base of the Peyer's patches at all dose groups. SEM coupled to energy-dispersive X-ray (EDX) confirmed that the tissue contained subsurface particles rich in titanium. In the low and mid dose groups, weak signals were detected in the impacted cells at the base of the Peyer's patches, whereas higher signals

were observed at the highest dose group.

E171 studies in humans

Pele *et al.,* (2015)

96. The test material used was: E171, anatase, d50=250nm (EFSA, 2021)

97. Eight healthy volunteers (self reported) with normal intestinal permeability were given a permeability solution. At 7am, following an overnight fast baseline, urine samples were collected. After consumption of the solution, urine samples were collected for 5 hours.

98. Baseline blood samples were also taken at 9 am. Following that, the subjects received two tablets containing 50 mg of E171 (total dose 100 mg). Blood samples were collected at: 30 minutes, 1, 1.5, 2, 3-, 6-, 8- and 10-hours post E171 ingestion. Of the 8 volunteers, only 7 completed the study as blood could not be withdrawn from the cannula of 1 subject.

99. Dark field microscopy was used to identify titanium dioxide in the blood. Random areas were visualised and the estimation of particles within each field was based on four reflective grades: 0 (<5 particles/field), 1(5-10 particles/field), 2(10-20 particles/field), 3 (>20 particles/field). This analysis was only performed in 5/7 subjects due to blood clotting in two subjects. ICP-MS was used to quantify titanium in the blood for 0-10 hours, except in two subjects were samples could not be collected at 8hours (2 subjects) and 10 hours (1 subject).

100. Based on the results of the dark field microscopy, it was determined that some of the ingested titanium dioxide was absorbed directly into the blood. A significant increase in positive signals was observed from 2 hours onwards and both dark field microscopy and ICP-MS demonstrated a peak in absorption at 6 hours, reaching up to 11 ng/mL and decreasing to around 5ng/mL by 10 hours post exposure. Only the titanium levels from 6 hours post exposure onwards were significantly different than the baseline. A positive correlation between reflective grades and total titanium levels was observed.

101. The authors hypothesised that two routes of uptake in the gut were involved: one proximal (in the duodenum/jejunum) and one distal (Peyer’s patches in ileum). This was based on the fact that at two hours the uptake was visible in the dark field microscopy and the levels peaked at 6 hours as determined by ICP-MS (I*.e.*early absorption and late peak).

Guillard *et al.*, (2020)

102. The test material used was: titanium dioxide particles with a mean particle size of 104.9 ± 44.9 nm and a particle size distribution ranging from 20 to 440 nm, with 55% of NPs by number.

103. Human placentae and meconium were collected at term from normal pregnancies. The samples were analysed using ICP-MS and scanning transmission electron microscopy (STEM) coupled to EDX spectroscopy for content analysis of titanium and analysis of titanium dioxide particle deposition, respectively. Transplacental passage of titanium dioxide was determined using an *ex vivo* placental transfusion model.

104. All placental samples (n=22) contained titanium with the total content ranging from 0.01 to 0.48mg/kg of tissue. STEM-EDX confirmed the presence of titanium and oxygen in the particle deposits seen by TEM, as well as aluminium, silicone, iron, zinc and tin trace elements. Most of the analysed titanium dioxide particles were below 100nm. Size particle analysis of all particles indicated that 50% were below 100nm in diameter.

105. In 50% of the meconium samples (total of 18 samples), titanium was detected (0.02-1.5 mg/kg). TEM-EDX analysis confirmed the presence of titanium and oxygen elements in the particle deposits, alongside silicone, aluminium, iron and zinc. Analysis of all particles indicated a diameter of 5-194nm, with 26/33 (80%) in the nano range.

106. In the transplacental passage experiment, of the 7 *ex vivo* isolated perfused placentae, round shaped or small particle aggregates of titanium dioxide were observed. Titanium dioxide particles were recovered in the syncytiotrophoblast microvilli and had translocated in deeper areas of the placental chorionic mesenchyme surrounding foetal vessels. The particles had a diameter of below 250 nm, with 17 of them in the nano range.

107. The authors concluded that the results indicated the passage of titanium dioxide particles across the human placenta with potential local accumulation during pregnancy, depending on the individual. The findings of the perfused placenta experiment indicated, according to the authors, that the human placental barrier is unable to completely prevent the passage of titanium dioxide from dietary sources and protect the fetus.

108. Based on both experiments (results of perfused placenta study and the titanium levels in the placenta and meconium), the authors noted that there was

a need to assess the risk of titanium dioxide nanoparticle exposure in pregnant women and warranted specific attention for oral exposure to the nanosized fraction of the E171 food additive.