Absorption, Distribution, Metabolism and Excretion (ADME)

In this guide

16. Abbreviations Table - Statement on the safety of Titanium Dioxide (E171) as a Food Additive
17. References - Statement on the safety of Titanium Dioxide (E171) as a Food Additive
18. Annex A - Statement on the safety of Titanium Dioxide (E171) as a Food Additive
19. Annex B - Summary table of studies
20. Annex C - Statement on the safety of Titanium Dioxide (E171) as a Food Additive
21. Annex D - Statement on the safety of Titanium Dioxide (E171) as a Food Additive

51. The COT reviewed a number of studies to assess the ADME of TiO2, which had also been reviewed by EFSA, Health Canada, JECFA and/or FSANZ. These studies are described below and a brief overview of the review and conclusions of EFSA, Health Canada, FSANZ, and JECFA are included. The COT review and conclusions are presented at the end of each section.

Studies used to review the toxicokinetics of the E171 form of TiO2

52. The toxicokinetics of E171 has been addressed in a few studies. The COT have assessed 8 studies in total in their review, 6 studies in mice and rats and 2 studies in humans.

E171 studies in rats and mice

Talamini et al (2019)

53. Groups of NRF mice (n = 22/group) were treated with 5 mg/kg bw per day of E171 (99.3% pure anatase, 35% nanoparticles, 201.2 ± 8.5 nm) 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 (average daily dose of 2 mg/kg bw) with E171 or water (control) which was slowly dripped with a pipette into the mouths of the mice.

54. The animals were weighed at the beginning of the experiment and observed daily. On day 21, the animals were terminated and the lungs, liver, stomach, spleen, kidney, brain, testes and whole intestine were removed. The

concentrations of titanium were determined in 4 animals.

55. No signs of general toxicity were observed. In the brain, kidney and testes, titanium levels were <0.03 µg/g, the limit of quantification (LOQ) 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)

56. In the first round of experiments adult C57BL/6 mice (12 – 18 weeks) (4 animals/group) were treated with a single gavage dose of 40 mg/kg of E171 (>95% anatase, 20 – 340 nm TEM; 44.7% nanoparticles) suspended in water and sonicated or water only as a control. In the second round of experiments, 300 µg/mL of E171 was suspended in buffer and used to fill a closed mid-jejunal loop of 10 cm, pre-treated with inhibitors of tight junctions, micropinocytosis, clathrin-mediated endocytosis or raft-dependent endocytosis.

57. Animals were terminated at 2-, 4-, 8- and 24-hours following treatment. Confocal microscopy and micro x-ray fluorescence imaging were used to analyse the presence of particles in both the first and second round of experiments, whilst inductively coupled plasma mass spectrometry (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.

58. In mice treated with a single dose of E171, the number of titanium dioxide reflective particles in the lumen of the small 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).

59. 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 8-hour timepoint in all three intestinal sections. In the jejunum, the reflective TiO2 particle spots displayed a mean diameter of 700 ± 59 nm (n = 70). From the ICP-MS determined concentrations of TiO2 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-hour timepoint. The authors concluded that TiO2 was absorbed predominantly in the ileum, partly in the jejunum and a small amount was absorbed in the colon. Based on the information on uptake capacity per unit of surface area it was concluded that titanium dioxide is predominantly absorbed by the small intestinal villi and to a lesser extent through Peyer's patches.

Riedle et al., (2020)

60. C57BL/6 mice (6 males and 6 females per treatment group) were fed diets with food grade TiO2 (anatase, with a diameter of 119 nm) at doses of 0, 6.25, 62.5 and 625 mg/kg of diet. These were equal to 0 and approximately 1, 10 and 100 mg food grade TiO2/kg bw per day.

61. The animals were terminated at 6, 12 and 18 weeks. Animals terminated 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 TiO2.

62. Reflectant foci, indicative of TiO2 presence, were found at the base of the Peyer's patches in all dose groups. Scanning electron microscopy (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.

Bettini et al., (2017)

63. The COT noted that there was evidence of significant clearance through organs, including the liver, in the Bettini et al. (2017) study which examined the fate of TiO2 along the gut-liver axis in adult male rats. Firstly, rats (10 per group) were dosed daily by gavage with food-grade TiO2 (E171) or TiO2 NM-105 (10

mg/kg bw/day) for 7 days (the control group was given water). These animals were used for tissue imaging, flow cytometry and cytokine assays and to carry out tissue inflammation and gut permeability measurements.

64. It was found that TiO2 particles did not re-agglomerate in vivo during transit through the gut. Following absorption, light-diffracting TiO2 particles were found along the small intestine, in the colonic (large intestine) mucosa and liver of rats dosed orally with E171 for 7 days, but not in the controls. Using NanoSIMS analyses of subcellular TiO2 distribution in the immune cells of Peyer's patches after 7 days of oral exposure, Ti was detected in the gut lumen and in the colonic mucosa. TiO2 was also found in the liver, with the highest density found close to the portal vein sinus. There was no significant change in epithelial paracellular permeability to 51Cr-ethylenediaminetetraacetic acid (EDTA) observed in the E171 group when compared with control animals. This indicates that oral exposure to E171 did not affect gut permeability in vivo.

Farrell and Magnuson (2017)

65. A Good Laboratory Practice (GLP)-compliant study of food-grade TiO2 (anatase) was carried out in rats and performed in accordance with OECD test guideline (TG) 417 to assess the absorption, distribution and excretion routes in rats after oral exposure to TiO2. The TiO2 form of interest from this study was the anatase form (D50 = 133 – 146 nm) and was incorporated into the feed at a dose equivalent to 30 mg/kg bw per day of male and female SD rats (n = 10 per sex per group). This is based on 200 ppm in feed and assumes daily consumption of 30 g diet per day by a 200 g rat. Rats received either TiO2 or a control diet (no TiO2 added, background concentration 7 – 9 ppm) for 7 days. Following the 7 days treatment, the TiO2 was withdrawn from the diet and 3 rats per sex were terminated at 1, 24 and 72 hours. After the TiO2 was removed from the diet, the animals were housed individually and those allocated to the 72 hours group were housed in metabolic cages and all excreta including the cage washings were kept. The Ti content of excreta, whole blood, liver, kidneys and muscle was measured using inductively coupled plasma – atomic emission spectrometry (ICP-AES).

66. TEM micrographs indicated that the anatase TiO2 test material exists primarily as aggregated particles up to approximately 1 µm in diameter and approximately 36% of the discrete but aggregated particles are <100 nm in diameter. However, it is not anticipated that these will de-aggregate during the manufacture of pharmaceutical or food products or in the gastrointestinal (GI) tract. Aggregation of the anatase TiO2 was also assessed using dynamic light

scattering technique. The median particle size (d50) was determined as 336 nm. There was no measurable quantity of TiO2 that had a particle size <100 nm.

67. No adverse effects were found during clinical observations related to the consumption of TiO2. Ti levels in liver, kidney and muscle samples were below the limit of detection (LOD) (0.1 to 0.2 mg/kg wet weight) in most rats at most time points; of a total of 270 tissue samples (90 each from liver, kidney and muscle), 176 (65%) were below the LOD. Sporadic observations of levels above the LOD were found, in the range of 0.1 to 0.3 mg/kg wet weight and occurred at similar levels and frequency in TiO2-exposed rats as those in the control group.

68. The majority of Ti was excreted in faeces with concentrations in urine below the LOQ (<0.04 mg/L; equivalent to < 2% daily dose/L) for all samples except the 0-to-24-hour urine sample for one rat (0.05 mg/L); collection of urine and faeces began after withdrawal of the test diet and therefore no mass balance recovery was estimated. Whole blood Ti concentrations were also below the LOD in both treatment and control groups. The authors concluded that food-grade TiO2 administered in diet is not appreciably absorbed and distributed in mammalian tissues, and there is no evidence of accumulation in liver, kidney and muscle following repeated oral exposure for 7 days.

EBRC (2022)

69. An unpublished summary report of a GLP-compliant, multi-site toxicokinetics study of 5 different grades of TiO2 carried out in accordance with OECD 417 test guidelines was submitted to the FSA by the TDMA (EBRC, 2022). In this study, male and female CD rats received either a vehicle control or a single dose of 1,000 mg/kg bw of TiO2 administered by oral gavage and the total Ti content of whole blood was measured for up to 96 hours post-dosing. A soluble Ti reference substance (Titanium (IV) bis(ammonium lactato)dihydroxide solution (50 wt. % in H2O)) was administered orally (100 mg/kg bw) or intravenously (10 mg/kg bw) to assess the relative bioavailability of the various grades of TiO2.

70. The test articles included E171-E which had a median particle diameter (SD) of 99.9 ± 2.0 nm and contained approximately 50-51% by number of constituent particles in the nanoscale (LNE, 2020). The other four particles were identified as G6-3 (a rutile TiO2-NP coated with alumina and hydrophobic organic, D50 = 9.2 ± 2.0 nm), G2-5 (uncoated anatase TiO2-NP, D50 = 5.5 ± 2.0 nm), G3-1 (uncoated pigmentary rutile TiO2, D50 = 146.9 ± 5.9 nm) and G4-19 (pigmentary rutile TiO2 coated with alumina and polyol, D50 = 177.5 ± 3.9 nm). (Health Canada, 2022).

71. Blood was collected over a period of up to 96-hours post-dosing and Ti concentrations measured by ICP-MS/MS following microwave-assisted acid digestion of whole-blood samples using H_2SO_4. The use of this analytical method and of whole blood includes the bioavailability of particulate TiO_2 as well as any dissolved Ti.

72. Blood Ti concentrations of vehicle-control treated male rats were variable with several males considered statistical outliers and a decision was made to exclude these from data processing.

73. After oral administration of all five TiO_2 test articles, the mean blood Ti concentrations of male and female rats was below 0.2 µg Ti/g blood. After i.v. and oral administration of the soluble titanium reference standard, substantially higher blood concentrations of 90 µg Ti/g blood and 0.9 µg Ti/g blood, respectively were measured.

74. The group that had received the food-grade TiO_2 test item E171-E had the highest blood Ti concentrations following oral dosing. Areas under the curve (AUC) were plotted for rats that had been dosed with the reference substance orally or by i.v. and that had received E171-E by oral administration. The maximum relative oral bioavailability of E171-E was determined to be 0.0013%.

75. The measured Ti blood levels from rats exposed to the other four forms of TiO_2 were below the LOD after background correction. The authors concluded that the oral bioavailability of all five of the TiO_2 forms tested was very close to the LOD of the analytical system. It was also noted that most reagents used in the process contained low but measurable Ti background concentrations, Therefore the analysis of low levels is made challenging. The background levels of Ti measured in the blood of control animals, especially in the males, shows some variability, which is consistent with time zero levels measured during dietary studies.” (EBRC, 2022).

E171 studies in humans

Pele et al., (2015)

76. Eight healthy volunteers 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.

77. Baseline blood samples were also taken at 9 am. Following that, the subjects received two tablets, each containing 50 mg of pharmaceutical/food grade TiO2 (anatase, d50 of 260nm) (total dose 100 mg). Blood samples were collected at: 30 minutes, 1-, 1.5-, 2-, 3-, 6-, 8- and 10-hours post TiO2 ingestion. Of the 8 volunteers, only 7 completed the study as blood could not be withdrawn from the cannula of 1 subject.

78. 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); and 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 for whom samples could not be collected at 8 hours (2 subjects) and 10 hours (1 subject).

79. 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 5 ng/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.

80. 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 the ileum). This was because 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)

81. The test material used was titanium dioxide (E171) 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.

82. Human placentae were collected at term from normal pregnancies. Transplacental passage of titanium dioxide (E171) (15 µg/mL) was determined using an ex vivo placental transfusion model with a perfusion time of 90 minutes. 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.

83. All term placental samples (n=22) contained titanium with the total content ranging from 0.01 to 0.48 mg/kg of tissue. STEM-EDX confirmed the presence of titanium and oxygen in the particle deposits seen by TEM, as well as aluminium, silicon, 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.

84. Meconium samples were also collected from nappies. 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 silicon, aluminium, iron and zinc. Analysis of all particles indicated a diameter of 5-194 nm, with 26/33 (80%) samples in the nano range.

85. 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.

86. 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.

87. 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.

Studies used to review the toxicokinetics of the nanoparticle form of TiO2

Studies in rats and mice

88. Three studies investigated the kinetics of TiO2 nanoparticles using intravenous injection (Geraets et al., 2014; Disdier et al., 2015; and Kreyling et al., 2017a). In these, just under 100% of the administered dose was biodistributed to the organs. However, when administered by gavage Kreyling et al (2017b) estimated absorption from the GI tract was 0.6% of the administered dose. Therefore Kreyling et al. (2017b) concluded that the kinetic pattern following the intravenous (i.v.) route were very different from those of the oral route and that "i.v. injection appears not to be an adequate surrogate for assessing the biodistribution and potential health effects occurring after oral exposure to TiO2 nanoparticles". The focus of the COT assessment is TiO2 as a food additive, where the relevant route of administration is oral, therefore, studies using i.v. administration have not been considered further in this Statement, but the study details are included in the study summaries in Annex D.

89. Of the remainder of the studies that assessed TiO2-NP kinetics, none involved dietary administration. Seven studies dosed the animals using gavage (Kreyling et al., 2017b; Geraets et al., 2014; Ammendolia et al., 2017; Chen et al., 2020; Warheit, Boatman and Brown, 2015; Tassinari et al., 2014; and Bettini et al., 2017). The study by Hendrickson et al., (2016) used intragastric administration, and the study by Hendrickson et al., (2020) used an isolated intestinal loop technique.

90. The studies that used nanoparticles (not classed as food-grade or E171 by the authors) involved a range of sizes. The majority of the studies measured individual nanoparticles (engineered nanoparticles such as NM-101 at 5-10 nm up to about 60 nm in one dimension) and the size of agglomerates/aggregates (which ranged from about 80 nm up to approximately 2 µm) predominantly using TEM, SEM and dynamic light scattering (DLS) techniques. A few studies also assessed the percentage (by number) of the particles. For example, in Ammendolia et al. (2017) the average diameter of NPs in MilliQ water ranged from 70 – 1,200 nm with a peak at 60 – 90 nm, average diameter of 76 nm. This corresponded to an abundance of 11% of the particles. Only 13% of the NPs had dimensions less than 100 nm. In the study by Tassinari et al (2014) TEM showed individual NPs of 20 – 60 nm and analysis using SEM showed 87% of particles were 30 – 900 nm and 48% of those were 100 – 300 nm, with an average diameter of size distribution of 284 ± 43 nm. Thirteen percent of particles had dimensions <100 nm whereas the size distribution was dominated by agglomerates up to 1.6 µm in diameter.

91. The study by Kreyling et al (2017b) which looked at the distribution of [48V]TiO2NP in rats after 1 hour and up to 7 days following intra-esophageal instillation estimated only approximately 0.6% of the dose was absorbed across the intestinal barrier in the first hour after gavage. After 7 days, only about 0.05% was retained in the body. The rest of the material is excreted in the faeces. Other studies agreed that the absorption of TiO2 across the gut epithelium was very low following dosing via gavage (oral route).

92. Kreyling et al (2017b) and others (Geraets et al., 2014; Ammendolia et al., 2017; Chen et al., 2020; Warheit, Boatman and Brown, 2015; Tassinari et al., 2014; and Bettini et al., 2017) also looked at deposition of TiO2 NPs in various tissues. The tissues in which measurable levels were detected across all studies include small intestine, liver, spleen, kidneys, lungs, heart and brain. However, the studies used different nanomaterials, different dosing duration and evaluation timepoints, and different methods of analysis.

Studies in humans

Heringa et al., (2018)

93. Titanium was measured using high resolution ICP-MS in liver and spleen from 15 deceased human subjects (nine women and six men) who had donated their bodies for research and educational purposes. The donors had died at the age of 56 to 104 years. Samples were fixed in 4% formaldehyde after collection. Two of the subjects had received titanium implants. The LOD of the method was 10 ng/g tissue. TiO2 particles were measured using single particle ICP-high resolution MS (spICP-HRMS). The smallest particle size that could be detected was 85 nm.

94. Total Ti content in the liver ranged from 0.02 to 0.09 mg Ti/kg tissue with an average value of 0.04 ± 0.02 mg Ti/kg tissue. In spleen, total Ti content ranged from 0.02 to 0.4 mg Ti/kg tissue with an average value of 0.08 ± 0.1 mg Ti/kg tissue. Levels in the two subjects who had received titanium implants were comparable to those in the other 13 subjects. TiO2 particles were detected in 7/15 liver and 13/15 spleen samples. The number-based TiO2 particle size distributions in liver and spleen were comparable and had a size range of 85–550nm and 85–720nm, respectively. In the tissues, 24% of the TiO2 particles in the number-based size distribution was <100 nm, but this fraction may be underestimated, given the LODsize was 85 nm.

95. The particle mass concentration in liver ranged from 0.01 to 0.3 mg titanium/kg tissue. In the spleen, the concentration ranged from 0.01 to 0.4 mg

titanium/kg tissue. The average concentration in samples where titanium could be determined was 40 ng/g in the liver and 80 ng/g in the spleen.

96. Small tissue grains of liver and spleen from two subjects were analysed using SEM-EDX to visualize the titanium dioxide particles. The observed particles were composed of titanium and oxygen and were present as an aggregate or agglomerate, consisting of smaller primary particles of 75–150 nm. Presence of titanium was also confirmed semi-quantitatively by EDX analysis in dry-ashed liver and spleen samples.

Peters et al (2020)

97. Post-mortem human liver, spleen, kidney, jejunum and ileum samples were analysed from 15 human subjects, 7 male and 8 female, who died at the age of 64– 98 years. Only 12 kidneys, jejunum and ileum were obtained. From these persons, written informed consent was obtained during life that allowed the use of their entire bodies for educational and research purposes. Samples were fixed in 4% formaldehyde after collection. Titanium was measured by ICP-HRMS, with an LOD of 10 ng/g tissue. TiO2 NPs were quantified using spICP-HRMS. The size limit for detection was 50 nm. The presence of TiO2 NPs was confirmed by SEM-EDX.

98. The total titanium concentration in the organs ranged from 0.01 to 2.0 mg titanium/kg tissue with an average value of 0.17 mg titanium/kg tissue and a standard deviation of 0.33 mg/kg. The authors considered that this was an indication of large differences between subjects and organs. The highest concentrations were detected in the jejunum and ileum (average of 0.34 and 0.43 mg titanium/kg respectively), followed by the kidney, spleen and liver (0.08, 0.06 and 0.03 mg titanium/kg respectively).

99. The particle sizes ranged between 50 and 500 nm in the different tissues and were comparable. 17% of particles were <100 nm. TiO2 particle mass concentrations ranged from 0.01 - 1.8 mg Ti/kg tissue with an average of 0.14 ± 0.30 mg Ti/kg tissue. The titanium dioxide particle concentrations were considered by the authors to represent about 80% of the total titanium present.

EFSA review and conclusions on ADME of TiO2

100. 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 had reviewed the toxicokinetics of E 171 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).

101. In humans, the Panel considered that after oral administration of 100 mg E171 (Pele et al., 2015), blood Ti increased ca. 5- to 10-fold from 6 to 10 h post-dosing, demonstrating some oral systemic availability. A study by Guillard et al., (2020), indicated that TiO2 is systemically available after ingestion and can distribute to the placenta. The Panel noted that the extent of transfer across placental membranes was small. (EFSA, 2021).

102. The Panel noted that materials other than E 171, mainly TiO2 NPs, were also 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. 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 by Hendrickson et al., (2020), the authors provided data indicating the presence of TiO2 NPs either as single particles or as smaller and larger agglomerates in intestine, liver and spleen.

103. “The Panel considered that from the two studies analysing tissues from deceased subjects (Heringa et al., 2018 and Peters et al., 2020), the presence of Ti-containing nanoparticles was observed in liver, spleen and kidney as well as in the intestine. Quantification of the Ti in the organs and comparison with the estimated mean daily intake of E 171, allowed the Panel to conclude that the oral systemic availability of TiO2 NP would be low (less than 1% by mass). (EFSA, 2021).

104. Overall, the FAF 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. The Panel had also considered that rat studies with TiO2 NPs, with primary particle sizes between 7 and 90 nm, showed long half-lives, a potential for accumulation and long time to reach steady state (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 E171 TiO2. (EFSA, 2021).

Health Canada review and conclusions on ADME of TiO2

105. 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. (Health Canada, 2022).

106. There is 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. (Health Canada, 2022).

107. 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). (Health Canada, 2022).

108. 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 what humans are exposed to 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. (Health Canada, 2022).

FSANZ review and conclusions on ADME of TiO2

109. Studies submitted to FSANZ with food grade TiO2 indicated that the relative oral bioavailability in rats is ≤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).

JECFA 2024 conclusion on ADME of TiO2

110. The extent of absorption of TiO2 is difficult to measure because of the variability in background concentrations of Ti in tissue, and concentrations of Ti in blood and tissue that are close to the detection limits. It is also difficult to compare the extent of absorption between studies due to the different vehicles used to suspend the TiO2 (which may affect the size of aggregates), the methods of preparation (e.g. sonication) and the methods of detection.

111. JECFA reviewed and summarised the animal studies (Riedle et al, 2020; Talamini et al, 2019; Farrell and Magnuson, 2017; Leuschner, 2020; Bettini et al, 2017; Vignard et al, 2023) and absorption studies in humans (Bockmann et al, 2000; Balcaen et al, 2014; Jones et al, 2015; and Pele et al, 2015).

112. JECFA concluded that when TiO2 is administered to animals, (oral) absorption is very low (e.g. < 0.00075%). Absorption in humans is also very low.

COT review and conclusions on ADME of TiO2

113. It should be noted that various chemical forms of TiO2 were used in the different toxicokinetic studies. The COT concluded 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 rather than via complex food groups (Bettini et al., 2017; Talamini et al., 2019; and Comera et al., 2019). The COT raised concerns that TiO2 may not fully disperse in water, which could affect absorption, however the effect remains unclear. Some

studies where the TiO2 was prepared suspended in water included the addition of stabilisers to force deagglomeration, while other studies utilised long sonication steps, which can cause structural changes to TiO2.

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

115. The COT noted that TiO2 particles can cross the placenta. However, because gastrointestinal absorption is low, this would be a very small number from dietary exposure. 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 differences in pH and be transferred elsewhere.

116. 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.

117. The COT noted that the Heringa et al. (2018) study, in samples from human subjects after many decades of exposure, would have reflected steady state levels of TiO2. Due to the nature of the study, it was possible to identify the sources of the TiO2 but these would not have included just the diet and hence the values reported are likely to be an overestimation of the quantities that can be accumulated over a lifetime from food.

118. Overall, the COT concluded that the form of TiO2 may affect the likelihood and quantity of the TiO2 material crossing barriers into organs. 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 on the absorption of TiO2, the Committee could not ascribe a specific percentage for the absorption of TiO2. However, the Committee considered that, on balance, absorption is low.