Meeting

Studies on Titanium dioxide other than E171, or with titanium dioxide nanoparticles

This is a paper for discussion.

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

Rats

Disdier *et al.,* (2015)

109. The test material used was: 75% anatase, 25% rutile titanium dioxide nanoparticles 21.5± 5nm.

110. Adult Fischer rats were treated with 1 mg/kg titanium dioxide or saline buffer (control); no dispersion protocol was applied for the *in* vivo experiment. The dose was administered intravenously, and samples were taken at 30 minutes, 1, 2,6 and 24 hours and 7-, 28-, 90- and 365-days post treatment from blood, liver, brain, spleen, kidney and lungs. Blood and brain samples were additionally collected at 5 and15 minutes post injection. Titanium concentrations were determined by ICP-MS.

111. The authors reported that titanium burdens in the liver, spleen and lungs of the treated group were significantly higher for all time points post injection, however the levels declined over time. Levels in the liver were higher than the spleen and lungs. Titanium burden after a year remained high, suggesting biopersistence (approximately 33% of the titanium burden of the early time points). The titanium burden in the kidneys increased significantly from 30 minutes to 24 hours but decreased significantly 7 days after i.v. administration. No statistically significant results were reported for the blood samples. For the time points before 24 hours, there was a statistically significant increase if titanium concentrations in the brain. After 24 hours, titanium content did not

differ from controls. No further details were given in the text, but graph 2 provides more details on the levels of titanium in the different organs. The authors estimated that they recovered approximately 44 % of the administered dose in the liver, 10 % in lungs and 2 % in spleen 6 hours post administration.

Kreyling *et al.,* (2017a)

112. The test item used was: titanium dioxide anatase nanoparticles, 7-10 nm.

113. Female Wistar- Kyoto rats were dosed with 10-20 µg of radiolabelled titanium dioxide given as a single i.v. injection of nanoparticles suspended in water.

114. The animals were sacrificed at 1, 4, 24 hours and 7and 28 days post administration (n=4 per time point). All organs as well as blood and all excretions of the animals were collected. The results of a separate intravenous study performed to investigate the absorption and biodistribution of soluble ionic ^{48V} were used to correct ^{48V} release from $^{[48V]}$ titanium dioxide nanoparticle.

115. The highest $^{[48V]}$ titanium dioxide accumulations were found in liver (95.5%ID on day 1), followed by spleen (2.5%), carcass (1%), skeleton (0.7%) and blood (0.4%). Detectable nanoparticle levels were found in all other organs. The $^{[48V]}$Titanium dioxide NP content in blood decreased rapidly after 24h while the distribution in other organs and tissues remained rather constant until day-28. Particularly, 4 hours post, administration, 99.5% of the radioactive dose was found in the liver and at 28 days 88.9% of the dose was detected in the liver. The spleen and the kidneys contained: spleen between 2.5% and 4%, and kidneys between 0.05% and 0.2%. All other tissues had lower contents. The bones (including the marrow) and the remaining tissues contained 1% and 0.7%. The radiolabelled compound was excreted in urine and within 28 days the excretion amounted to roughly 1%. Highest excretion occurred on day 1. Excretion by the faeces, indicative of biliary excretion, amounted to 3% over 28 days.

Kreylig *et al.,* (2017b)

116. A similar experiment to the one described above was also carried out, with the animals exposed via the oral route (a single dose of an aqueous, radiolabelled-nanoparticle suspension by intra-esophageal instillation) in female Wistar-Kyoto rats.

117. Titanium concentrations were determined at five retention time points 1 h, 4 h, 24 h, 7 d and 28 d after gavage in four rats for each time point. However, after observing in the seven-day experiment that fecal excretion of the test item was complete after 4–5 days, no further animals were sacrificed for a 28-day biodistribution.

118. Blood, all organs, tissues and excreta were collected, and the concentration of radioactive titanium dioxide was measured. Most of the radioactivity was excreted in the faeces. Absorption was calculated as the fraction of the dose that could not be accounted for by the radioactive content of the intestinal tract plus faeces. Approximately 0.6% of the dose was absorbed within an hour post-treatment. Seven days post treatment, roughly 0.05% of the dose administered was still present.

119. The authors noted that the distribution patterns between animals were variable and that several data were below the LOD during the first 4 hours. The spleen, kidneys, heart and uterus contained detectable levels even after 4 hours post treatment. Maximum retention was reached in the spleen, kidneys and heart at 24 hours post-treatment. In the liver, lung and blood, nanoparticle retention declined from 4 hours to 7 days. In the brain, uterus and kidneys, the highest concentrations were observed at day 7. The peak concentration in liver and spleen was 12.5% (4 hours) and 2.6% (24 hours) of the absorbed dose, respectively. According to the authors, due the slow excretion kinetics, accumulation of systemically circulating particles in specific cells and organs is likely to occur in subjects chronically exposed to titanium dioxide nanoparticles. When comparing the biodistribution of the radioactive titanium dioxide nanoparticles retained after oral administration with the results obtained after intravenous injection (Kreyling et al., 2017a), the authors concluded that the kinetics patterns are very different and intravenous injection does not appear an adequate surrogate for assessing the biodistribution occurring after oral exposure to titanium dioxide nanoparticles.

Geraets *et al.*, (2014)

120. In the EFSA 2021 Opinion, only the i.v experiment was discussed; however, this paper also contains the result of an oral administration experiment.

121. The test item used was: NM-100, NM101, NM-102, NM-103 and NM-104.

122. In the oral study, the single dose groups received a gavage dose of 2.3 mg/rat corresponding to 6.8-8.6 mg/kg bw. The repeat dose groups received five consecutive daily administrations of 2.3 mg titanium dioxide in one mL per rat resulting in a cumulative dose range of 34.1- 42.4 mg/kg bw for male rats and 54.5- 59.9 mg/kg bw for female rats.

123. In the i.v. study, the suspensions prepared contained 2.3 mg titanium dioxide/mL. The single i.v. dose treated rats received a dose of 8.4-9.8 mg/kg bw and 12.4- 14.1 mg/kg bw for male and female rats respectively, via the tail vein. The repeated dose treated rats received a cumulative dose that ranged from 42.3-49.4 mg/kg bw and 61.2-71.9 mg/kg bw for male and female rats, respectively. Thus, the actual dose in mg/kg bw depended on the weight of the rats.

124. After repeated oral exposure (overall dose of 11.5 mg Titanium dioxide) titanium levels were near or below the detection limit in liver and spleen, indicating a very low absorption. In two out of 30 liver/spleen samples of exposed animals (for NM-102 and NM-103) titanium levels were above the LOD, whereas all mesenteric lymph nodes (MLN) samples (including controls) contained titanium amounts above LOD. Only a small increase in titanium content was observed, because the background levels in MLN were 2–3 times the LOD. MLN from control rats contained 0.14 µg titanium whereas the highest titanium average was 0.36 µg and was located in MLN from NM-104 exposed rats. This gives an increase of 0.226 µg titanium in MLN or 0.003% of the 6895 µg Titanium exposure in the dose. The total recovery of dosed titanium in all tested organs (expressed as % of the total dose) was estimated to be approximately 0.02%.

125. In the i.v. study, the highest levels were observed in the liver, but redistribution to the spleen was observed over the 90-day post-exposure period Day 2/Day 6 and Day 90). Redistribution to remaining tissues was not identifiable. The authors hypothesised that release of particles from liver and possibly other organs may be responsible for the increase in spleen levels. Titanium was detected in all investigated tissues in the present study, i.e., blood, liver, spleen, kidney, lung, heart, brain, thymus and reproductive organs.

126. Both after single and repeated i.v. exposure, blood titanium levels in blood decreased rapidly during the first minutes after which the titanium levels slowly decreased and approached the limit of detection at 24 hours post exposure.

127. Based on the available data, the authors concluded that elimination of total Titanium dioxide has a long half-life. For the liver, which was considered the main target organ, the estimated half-life was 28–248 days.

128. The authors considered that the data showed that at the long run Titanium dioxide particles will accumulate in the spleen. Finally, they noted that the expected accumulation with daily exposure as a consequence of the negligible elimination might indicate a potential concern for human health risk.

Tassinari *et al.,* (2014)

129. The test material used was titanium dioxide nanoparticles (anatase, primary size 25nm, BET surface area 45–55 m^2 /g, purity 99%).

130. Male and female Sprague-Dawley rats (7 rats/sex/dose) were treated with 0, 1, 2 mg/kg body weight (bw) per day by gavage. The controls received distilled water.

131. Twenty-four hours after the last treatment (day 6), male and female rats were anaesthetised, and blood samples were. Subsequently, the animals were sacrificed, and the uterus, ovary, testes, thyroid and adrenals were excised and weighted. Spleen was sampled both for histopathological examination and for studying tissue deposition of Titanium dioxide nanoparticles.

132. The titanium content in the samples was determined by ICP-MS using an Elan DRC II spectrometer. The highest titanium concentration was detected in the thyroid; however, the levels was no statistical significance between treated animals and controls (higher dose, 2 mg/kg bw per day and at 0.24 ± 0.09 vs. 0.22 ± 0.04 mg/g in controls). A significant increase was observed at the higher dose in the ovary (0.28 ± 0.07 vs. 0.12 ± 0.04 mg/g fresh weight). Levels in uteri were low (0.051 ± 0.006 vs. 0.49 ± 0.04 mg/g fresh weight). A significant increase in the concentration of titanium in the spleen concentration was observed at a dose of 2 mg/kg bw per day.

133. Overall, the authors considered that these results indicated the potential for titanium dioxide bioaccumulation.

Hendrickson *et al*., (2016)

134. The test material used was: NM-101 (5-10nm) and NP-25 (20-25nm).

135. Male Sprague Dawley rats were treated with 250 mg/kg bw/d of either one of the test materials, dispersed in an aqueous starch solution containing 0.1% Tween-80 and sonicated via intragastrical administration for 28 days.

136. Within a day of the last exposure, the animals were sacrificed and blood samples as well as samples from the lungs, liver, spleen, testes, small intestine heart, stomach and kidneys were harvested.

137. For animals treated with NM-101, titanium dioxide nanoparticles were detected in all organs and tissues. The organs with the highest concentrations were the spleen (0.227 µg/g) and liver (0.147 µg/g). In the kidneys, small intestine and testicles similar amounts of nanoparticles were detected (0.092, 0.098 and 0.089 µg/g respectively). Titanium dioxide nanoparticles were detected at 0.028 µg/g in the heart, 0.04 µg/g in the lungs and 0.049 µg/g in the brain.

138. In NP-25 treated animals' accumulation of titanium dioxide nanoparticles were detected in the small intestines and liver (0.29 µg/g in the liver) and at low levels (0.01 µg/g) in the kidneys. In the spleen, it was detected at levels of 0.29 µg/g of the organ. No titanium dioxide nanoparticles were detected in the lungs, brain, testicles, heart or blood.

139. The authors concluded that biodistribution differs between smaller (NM-101) and larger (NP-25) titanium dioxide nanoparticles, with the smaller ones showing a greater distribution spread and accumulation in all organs. The smaller particles exhibited reduced but similar tendency. The main difference was that the larger nanoparticles could not overcome the blood brain barrier and penetrate the brain.

140. Due to the fact that the detected levels accounted for less than 1% of the administered dose, the authors concluded that the data was evidence of the limited bioavailability and efficient excretion of titanium dioxide.

Ammendolia *et al.*,2017

141. The test material used was titanium dioxide nanoparticles (anatase, primary size 25nm BET) surface area 45-55 m^2 /g, purity 99%, suspended; the suspensions were sonicated.

142. Sprague-Dawley rats (10/sex/group) were treated with 1 or 2 mg/kg bw/d titanium dioxide nanoparticles or vehicle only (ultrapure water) via gavage for 5 consecutive days.

143. A day (24 hours) after the last dose, the animals were sacrificed, and the small intestine was excised. A piece of jejunum was used for histological analysis and the remaining part of small intestine was sampled for studying either tissue accumulation of titanium dioxide nanoparticles, determined as titanium by ICP-MS.

144. Titanium was detected at in small intestine tissue at 0.08±0.02lg/g in the control, 0.09 ± 0.02l g/g in the low dose group and at 0.13 ± 0.03lg/g at the high dose group.

Hendrickson *et al.,* 2020

145. The test material used was titanium dioxide nanoparticles, rutile, rod/ needle like shape, 5930 nm.

146. Wistar rats were treated with 50 mg/kg bw titanium dioxide using an isolated intestinal loop technique.

147. Three hours post treatment, the isolated loop was cut out. The liver and spleen were collected.

148. The presence of particles in tissues was studied by TEM and diffraction analysis. Loose agglomerates (100 nm and larger) were detected. Diffraction analysis was used to confirm that the particles were titanium dioxide. Titanium dioxide nanoparticles were detected on the surface and between the microvilli of the mucosal cells of the small intestine and also in the mucosal tissue. Nanoparticles were detected in the Peyer’s patches, both as single nanoparticles and agglomerates of sizes ranging between 20 and 60 nm. In the liver, parenchymal tissue aggregates of titanium dioxide nanoparticles (150–200 nm) and up to 300 nm were seen. In the spleen red pulp, single nanoparticles (20–30 nm), agglomerates (up to 100 nm) and conglomerates (up to 800 nm) were observed.

Chen *et al.,*2020a

149. The test material used was titanium dioxide nanoparticles anatase, 29 nm (SEM).

150. Sprague- Dawley rats were dosed with 0, 2, 10, 50 mg/kg bw by oral gavage for 90 days. The test material was sonicated prior to treatment.

151. The tissue distribution of the titanium dioxide nanoparticles was evaluated by determining the titanium content in blood and tissues including liver, stomach, small intestine, colon, spleen, heart, lung, kidneys and testicles by high resolution ICP-MS.

152. Significantly increased titanium dioxide nanoparticle levels were only detected in the colon of rats exposed to 50 mg/kg test material, compared with the control group. There was no dose-response relationship, however. The authors hypothesised that the significant increase of titanium dioxide in colon tissue was due to the titanium dioxide nanoparticles attaching on the surface of the colonic mucosa tissue and not in mucosa cells. As most of orally ingested test material was excreted through feces, it resulted to long-term retention in large intestine. The titanium dioxide nanoparticles did not enter the colon epithelial cells and were mainly deposited in the intestinal cavity or between villi. The content of Ti in all tissues was very low, which was approximately 0.0001%-0.00001% or 100-1000 ng/g tissue, except for the colon of the high dose group. All spleen and heart tissue samples from rats contained very low titanium levels, which were below the limit of detection (LOD) of 0.032 µg/g. Finally, they concluded that the results indicate that the absorption and distribution of titanium dioxide nanoparticles was very low after low-dose and long-term oral administration.

153. The EFSA evaluation also cites a second study by Chen *et al*¸ (2020b), however it has not been described in the current paper as, following evaluation by the Secretariat it appears to investigate effects on lipid metabolism rather than addressing toxicokinetics/absorption.

Human studies

Heringa *et al* (2018)

154. 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 LOD of the method was 10 ng/g tissue.

155. 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 considering that the smallest

titanium dioxide particle that could be detected with the method used was 85 nm.

156. 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.

157. 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)

158. Post-mortem human liver, spleen, kidney, jejunum and ileum samples were analyzed from 15 human subjects, 7 male and 8 female, who died at the age of 64–98 years. From these persons, written informed consent was obtained during life that allowed the use of their entire bodies for educational and research purposes.

159. 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).

160. The particle sizes were measured by spICP-MS and ranged between 50 and 500 nm in the different tissues (50 nm was the lower size detection limit). The titanium dioxide particle concentrations were considered by the authors to represent about 80% of the total titanium concentration.