Studies on TiO2 Nanoparticles - Review of EFSA Opinion

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235. The additional toxicity studies used to assess the toxicity of titanium dioxide were scored from 1-4 and specified nanoparticle size where available, with studies ranked 1 and 2 and using nanoparticles > 30nm being the optimal studies for consideration, however, some studies ranked 3 and 4 and with nanoparticles < 30nm were used in the absence of studies ranked 1 and 2.

Studies in mice: None available

Studies in rats:

Reproductive Toxicity Studies with TiO2 fraction Nanoparticles

236. Warheit et al. (2015b) (Score: 4 for NSC) - Oral prenatal developmental toxicity study in rats using five different TiO2 materials, TiO2 Nanoparticles or TiO2 containing a fraction of nanoparticles up to a dose of 1,000 mg TiO2 Nanoparticles/kg bw. No maternal and developmental effects were observed up to the highest dose tested, when administered from gestation days (GDs) 6 to 15.

237. In three studies, time-mated pregnant Sprague–Dawley, Crl:CD(SD), rats (n=22/group) were daily exposed to TiO2 (uf-1, uf-3 and pg-1) by gavage on GDs 6–20. In three additional studies, pregnant Wistar rats (n=22–23/group) were daily exposed to TiO2 (uf-2 and pg-2) by gavage from GDs 5 to 19. The dose levels used in the studies were 0, 100, 300 or 1,000 mg/kg bw per day. The dose volume was 5 mL/kg bw per day.

1) anatase/rutile (89/11%)(uf-1), d50 = 43 nm (XSDC), d50 = 23 nm (TEM), irregular;

2) anatase (100% nano) (uf-2), d50=42 nm (XSDC), d50=19 nm (TEM), irregular;

3) rutile (100% nano) (uf-3), d50=47 nm (XSDC),d50=22 nm (TEM), rod-like;

4) anatase (27% nano) (pg-1), d50=153 nm (XSDC), d50=120 nm(TEM), irregular;

5) rutile (11% nano) (pg-2), d50=195 nm (XSDC), d50=165 nm (TEM), irregular.

238. Gross necropsy included gross examination of the dam, counting of the number of corpora lutea, implantation sites, resorptions, live and dead fetuses, fetal sex and weight. Fetal pathological external, visceral and skeletal examinations were performed in order to identify any abnormalities. At 1,000 mg uf-1/kg per day, mean fetal sex ratio and the means for male and female fetuses per litter were statistically significantly different from the control group means. The mean number of male fetuses was 7.2 compared with 5.5 male fetuses for the concurrent control group; the test facility historical control group data ranged at that time from 5.2 to 7.4. The mean number of female fetuses was 4.8

compared with 6.7 for the concurrent control group; the test facility historical control group data ranged at that time from 5.8 to 8.3. Mean fetal sex ratio of the 1,000 mguf-1/kg bw per day group was 60% (males/females) compared with a sex ratio of 46% in the concurrent control group; the test facility historical control group data ranged at that time from 43% to 53%.

239. Apart from some incidental changes in body weight and feed intake, no other changes were observed in the dams or the fetuses in these studies. The authors concluded that there were no significant toxicological or developmental effects in females or fetuses at any of the dose levels or compounds tested and considered the NOAEL for each compound to be 1,000 mg/kg bw per day, the highest dose tested. The Panel agreed with both the author and the ANS Panel conclusions (2016 ANS Panel - Overall, the Panel noted that prenatal developmental studies with three pigment-grade (pg-1, pg-2 and pg-3) and three ultrafine (uf-1, uf-2 and uf-3)/nanoscale (anatase and/or rutile) TiO2 particulates performed according to the OECD guidelines (TG 414) did not give concern for maternal or developmental toxicity up to the highest dose tested (1,000 mg/kg bw per day).

TiO2 Nanoparticles < 30 nm (See Annex B for data summaries)

Studies in mice:

Karimipour et al. 2018 (Score: 2 for NSC)

240. The effects of oral administration of TiO2 Nanoparticles (10–25 nm) were tested at 100 mg/kg bw per day for 5 weeks on the histology of ovaries, oestrogen and malondialdehyde (MDA) serum levels (7 animals/group), fertility (10 animals/group) and IVF rates (10 animals/group) in female mice.

241. A significantly decreased pregnancy rate was observed (70% vs. 100% in the control group), along with a 20% decrease in litter size and increases in circulating oestrogen (20%) as well as MDA (25%). Degeneration and reduction of follicles, cyst formation and impairment of follicular development in the ovaries of the TiO2 Nanoparticles group (no quantitative data). A lower number of oocytes were isolated from the exposed group and a higher percentage of developmental arrest before the blastocyst stage after in vitro fertilisation. It was suggested that the observed effects could be the consequence of an indirect effect of TiO2 Nanoparticles through the generation of increased ROS levels.

242. Impairment of female fertility at the only dose tested was observed, therefore the Panel considered that the study showed an impairment of female fertility at a dose of 100 mg TiO2 Nanoparticles (10–25 nm)/kg bw per day.

Khorsandi et al. 2016 (Score: 2 for NSC)

243. The effects of oral administration of TiO2 Nanoparticles on testicular parameters in young adult male NMRI mice at 4 doses between 0 and 300 mg/kg bw per day (8 animals/group) for 35 days were investigated. Dose-dependent decreases in testis weight occurred from a dose of 100 mg/kg bw per day. At higher doses, additional testicular parameters were affected. While body weight was unaffected by treatment, the authors reported dose-dependent decreases in testis weight from a dose of 100 mg/kg bw per day. Both the mid- and high-dose groups showed decreases in serum and testicular testosterone levels, the diameter and total volume of seminiferous tubules, the height of the spermatogenic epithelium and total Leydig cell numbers. Contrarily, the total volume of the interstitial tissue was found to be increased. The Panel considered that TiO2 Nanoparticles (size unknown) from 100 mg/kg bw per day had an effect on testis weight.

Khorsandi et al. 2017 (Score: 2 for NSC)

244. TiO2 Nanoparticles (20–30 nm) were administered by oral gavage at 300 mg/kg bw per day to eight young adult male NMRI mice for 35 days. The authors reported significant decreases in testis weight, circulating and testicular testosterone, testicular catalase (CAT) and superoxide dismutase (SOD) concentrations, sperm counts and sperm motility.

245. Significant decreases in testis weight, circulating and testicular testosterone, testicular catalase (CAT) and superoxide dismutase (SOD) concentrations, sperm counts, and sperm motility were observed. Significant increases were also found in the percentage of abnormal or degenerative spermatogenic tubules, germ cell apoptosis, testicular MDA concentration and in the percentage of sperm with abnormal morphology. The Panel considered that testicular toxicity was observed with TiO2 Nanoparticles (20–30 nm) at 300 mg/kg bw/d, the only dose tested.

Karimi et al. 2019 (Score: 2 for NSC)

246. Eight 6- to 8-week-old male NMRI mice were treated daily by gavage with 50 mg TiO2 Nanoparticles (<30 nm)/kg bw per day for 35 days.

247. The TiO2 nanoparticles significantly reduced testis weight accompanied by reduced serum testosterone, reduced seminiferous tubule diameter and epithelium height and reduced the maturity of the germinal epithelium. Reduced sperm counts, increased sperm abnormalities and reduced sperm motility. The Panel noted that 50 mg TiO2 Nanoparticles/kg bw per day, the only dose tested, resulted in adverse effects on the testis compared with a control group.

Lu et al. (2020) (Score: 4 for NSC)

248. Four groups of 15 male ICR mice, age 6–8 weeks were treated daily by gavage with TiO2 Nanoparticles (7 nm) at 4 doses between 0 and 100 mg/kg bw per day for 30 days.

249. It was noted that there was tight junction damage in the blood–testis barrier (BTB) at 50 and 100 mg/kg bw. Serum testosterone was 50% decreased at the two highest doses tested.

250. Sperm motility was dose-relatedly reduced, accompanied by increased sperm malformation rates.

251. The Panel noted that the histopathological pictures on BTB were hard to interpret and considered that TiO2 Nanoparticles (7 nm at 50 or 100 mg/kg bw per day) resulted in a dose-related reduction of sperm motility and increased sperm malformations, accompanied by histological observations in the testis, changes in BTB-related protein levels, changes in MAPK-related levels and reduced circulating testosterone concentrations.

Studies in rats:

Lee et al. 2019 (Score: 3 for NSC)

252. Mated female Sprague–Dawley rats (12 females per group) were treated with TiO2 Nanoparticles (21 nm) daily by gavage at dose levels of 0, 100, 300 and 1,000 mg/kg bw per day from GDs 6 to 19.

253. No statistically significant differences were noted in general clinical signs, bodyweight, organ weights (absolute and relative to body weight), macroscopic findings. No significant differences for caesarean section parameters and fetal external and visceral examinations. The Panel considered that no adverse maternal and development al effects were reported with TiO2 Nanoparticles (21 nm) up to 1,000 mg/kg bw per day, the highest dose tested.