Fifth draft statement on the safety of Titanium Dioxide (E171) as a Food Additive- Reproductive and developmental studies using the nanoparticle form of TiO2

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Reproductive and developmental studies using the nanoparticle form of TiO2

Lee et al., (2019)

229. Mated female Sprague–Dawley rats (12 females per group) were treated with TiO2 NPs (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. The physicochemical characterization of TiO2 nanoparticles, included analyses of primary shape, primary size, purity and hydrodynamic size. The majority of the TiO2 nanoparticles had spherical and anatase crystal shapes with a purity of 100%. The mean primary size of the TiO2 NPs was 17.8 ± 5.46 nm. The hydrodynamic size of the TiO2 NPs was 341.5 nm, which indicated that TiO2 NPs were prone to aggregation.

230. In the Lee *et al*., (2019) study, 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 COT considered that no adverse maternal and developmental effects were reported with TiO2 NPs (21 nm) up to 1,000 mg/kg bw per day, the highest dose tested.

Warheit, Boatman and Brown, (2015)

231. Warheit, Boatman and Brown (2015) undertook an 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 NPs/kg bw. See paragraphs 97-99.

232. Gross necropsy included gross examination of the dam, counting of the number of corpora lutea, implantation sites, resorptions, live and dead foetuses, fetal sex and weight. Foetal 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%.

233. 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.

EFSA review and conclusions

234. Male Fertility: The Panel noted that the epidydimal sperm parameters were not evaluated but that this deviation has no effect on the final conclusion of the study. There were no effects on any of the sperm endpoints in the cohort 1A.

235. Female Fertility: The EFSA Panel concluded that there were no indications of effects on general toxicity, thyroid or sex hormone levels, reproductive function and fertility in either male or female rats, no effects on pre- and postnatal development or on neurofunctional endpoints in F1 offspring.

236. Developmental Toxicity: To check whether the significant differences in grip strength and hindlimb splay could be due to systematic bias in group testing order, the testing order was checked The Panel considered that there was no

systematic bias in group testing order and that this was therefore not a plausible explanation for the observed group differences. Grip strength and hindlimb splay belong to the same domain of neurological function, i.e., motor function and/or sensory–motor coordination. However, the effects observed (i.e., increase in hindlimbs play and increase in mean forelimb grip strength) seem to point in opposite directions when it comes to muscle strength. In particular, an increase in hindlimb splay can be interpreted as muscular weakness whereas an increase in mean forelimb grip strength could be indicative of myotonia.

237. The Panel noted that the effects observed were not correlated to any other changes (e.g., alterations in muscle tone, righting reflex, gait, wire manoeuvre, posture). No dose response was observed for any of these endpoints or for the two functional measurements, indicating that the likelihood of an association with test substance is low. No other changes in the functional observation battery measurements or locomotor activity were noted.

238. Furthermore, there were no notable histopathological findings in brain or in peripheral nerve (sciatic). Based on all the above considerations, the Panel considered that the effects on grip strength and hindlimb splay were not treatment related. However, the Panel noted that quantitative information on peripheral nerves was not available. Overall, the Panel considered that E171 had no adverse effects on neurofunctional endpoints in F1 cohort 2A offspring at the doses used.

239. EFSA conclusions on developmental toxicity results of the EOGRT study: No effects of E171 on pre- and postnatal development were observed. Data on the attainment of puberty in males (i.e., an appropriate assessment of the timing of the balanopreputial separation) were missing. The Panel did not consider this to be critical in this case.

240. To check whether the significant differences in grip strength and hindlimb splay could be due to systematic bias in group testing order, the testing order was checked The Panel considered that there was no systematic bias in group testing order and that this was therefore not a plausible explanation for the observed group differences. Grip strength and hindlimb splay belong to the same domain of neurological function, i.e., motor function and/or sensory–motor coordination. However, the effects observed (i.e., increase in hindlimbs play and increase in mean forelimb grip strength) seem to point in opposite directions when it comes to muscle strength. In particular, an increase in hindlimb splay can be interpreted as muscular weakness whereas an increase in mean forelimb grip strength could be indicative of myotonia.

241. The Panel noted that the effects observed were not correlated to any other changes (e.g., alterations in muscle tone, righting reflex, gait, wire manoeuvre, posture). No dose response was observed for any of these endpoints or for the two functional measurements, indicating that the likelihood of an association with test substance is low. No other changes in the functional observation battery measurements or locomotor activity were noted.

242. Furthermore, there were no notable histopathological findings in brain or in peripheral nerve (sciatic). Based on all the above considerations, the Panel considered that the effects on grip strength and hindlimb splay were not treatment related. However, the Panel noted that quantitative information on peripheral nerves was not available. Overall, the Panel considered that E171 had no adverse effects on neurofunctional endpoints in F1 cohort 2A offspring at the doses used.

243. EFSA conclusions on developmental toxicity results of the EOGRT study: No effects of E171 on pre- and postnatal development were observed. Data on the attainment of puberty in males (i.e., an appropriate assessment of the timing of the balanopreputial separation) were missing. The Panel did not consider this to be critical in this case.

244. EFSA concluded that there were no indications of effects on general toxicity, thyroid or sex hormone levels, reproductive function and fertility in either male or female rats, no effects on pre- and postnatal development or on neurofunctional endpoints in F1 offspring.

245. With regards to other endpoints EFSA concluded that no effects on reproductive and developmental toxicity were observed up to a dose of 1,000 mg/kg bw per day, the highest dose tested, of a commercially-available E171, were observed in the EOGRT study. No other reliable studies were found in the literature addressing these effects with E171.

246. EFSA Opinion: The Panel agreed with both the author and Food Additives & Nutrient Sources Added to Food Panel conclusions (2016 ANS Panel - Overall, the Panel noted that prenatal developmental studies with three pigment-grade (pg-1, pg-2and 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)).

Health Canada review and conclusions

247. Health Canada noted that the EORGT study (LPT 2020) was designed to investigate the induction of ACF using a commercially-available food grade E171 at doses from 0-1000 mg/kg bw/day and that no effects were observed up to the highest dose tested. They concluded on the basis of the EORGT study (LPT 2020) that a NOAEL of 1000 mg/kg bw/d could be established for reproductive and developmental effects and that the EGORT study (LPT 2020) was assessed as the most reliable currently-available study that addresses potential reproductive and developmental effects of E171.

FSANZ review and conclusions

248. FSANZ noted the 2016 EFSA request for an additional developmental and reproductive toxicity study in the form of the subsequent EORGT study. FSANZ stated that the EORGT study (LPT 2020) using food-grade TiO2 showed no evidence of developmental or reproductive toxicity with an upper test dosage and NOAEL of 1000 mg/kg bw/day.

249. FSANZ noted an additional study (food-grade TiO2 by oral gavage and in the diet) which described germ cell sloughing in mouse testes (Rodríguez-Escamilla et al., 2019) however these changes were not observed in the follow-up study. Combined with the lack of histopathological changes observed in the testes in three additional studies up to 1,000 mg/kg bw/day (EOGRT (LPT, 2020), Han et al. 2021 and Blevins et al. 2019)

COT review and conclusions

250. The COT considered the EOGRT report to be detailed. No toxicological effects on the reproductive & developmental system were observed. A range of endpoints, including body weight, haematology, urinalysis, sex hormone levels, thyroid hormone levels, circadian rhythm and sperm parameters, which were detailed looking at both the F0 and F1 generations.

251. It was noted that there was a suggestion of focal effects on the testes and epididymides in 1 male in the highest dose group, which was considered to be spontaneous and not test item-related, and abnormalities in sperm. Data from the EOGRT study showed test item-related differences for the examined absolute and relative testis weights between the control and the treatment groups (low = 100 mg, intermediate = 300 mg or high = 1,000 mg TiO2 E171/kg bw per day). These slight differences included a small but statistically significantly reduced absolute right testes weight at the intermediate and the high dose level. These were only

noted for the right testes, not the left testes and it was concluded that changes were spontaneous and not of toxicological relevance. The COT agreed with the authors' conclusions. Overall, no adverse effects on reproductive and developmental toxicity were observed, up to the highest dose tested.

252. The COT considered that the EOGRT study was carried out according to the relevant scientific guidelines with no obvious deficiencies. Based on the evidence reviewed, no reproductive & developmental toxicity was observed. Data from peer reviewed literature were inconclusive.

253. The best overall quality additional studies were determined by the COT to be the studies by Warheit *et al.*, 2015, and & Lee *et al.*, 2019. The COT considered the 1,000 mg/kg bw per day NOAEL established in the EOGRT study may result from low absorption. Other studies indicate NOAELs consistent with that from the EOGRT study. The COT noted variations in method endpoints between several of the studies and as a consequence the physicochemical differences between the test items were unclear.

254. The COT concluded that there was no strong evidence that TiO2 was reprotoxic.

255. No evidence of reproductive or developmental toxicity or gross or histopathological abnormalities were found in male or female reproductive organs in the EOGRT in rats following dietary exposure to food-grade TiO2 at doses up to 1,000 mg/kg bw/day. No other reliable studies with food-grade TiO2 were identified in the literature. In the final summary the authors concluded that "Health Canada Food Directorate's position is that there is no conclusive scientific evidence that the food additive TiO2 is a concern for human health" and the sub-group agrees with these conclusions.