Fifth draft statement on the safety of Titanium Dioxide (E171) as a Food Additive- Neurotoxicity

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Neurotoxicity

Studies using E171 or equivalent form of TiO2

EOGRT Study (LPT, 2020)

256. The EOGRT study is described in detail in the EOGRT summary section (paragraphs 134 - 135).

Studies using the nanoparticle form of TiO2

Sofranko et al., (2021)

257. Sofranko et al., 2021 investigated the effects of silver and TiO2 NPs on behaviour and neuropathology in male and female C57BL/6J mice. Mice were fed pellets with 10 mg/g TiO2, 2 mg/g polyvinylpyrrolidone-coated Ag or control pellets for 28 days with and without a 14-day post-exposure recovery. Inflammation, oxidative stress, and blood-brain barrier integrity were not significantly affected in male or female mice. No consistent significant treatment-related effects on anxiety and cognition were observed for either test material. The authors concluded that there were no substantial neuropathological changes following subacute exposure to foodborne TiO2 in mice.

Canli et al., (2020)

258. Female albino rats were given sublethal doses (0.5, 5.0 and 50 mg/kg bw per day) of sonicated TiO2 NPs for 14 days via oral gavage in 200 µL water (n = 6 per group). Control animals (n = 6) received 200 µL water only. Following dosing, total protein, the activities of ATPases and acetylcholinesterase A(ChE), the levels

of TBARS and the different forms of GSH were measured in tissues from the liver, kidney, brain and intestine.

259. The anatase TiO2 NPs were about 21 nm, >30 m^2/g surface area, >99% purity, and a density of 4.26 g/cm^3. None of the rats died within the 14-day treatment period. TEM images showed that TiO2 NPs seemed to accumulate in the brain, kidney and liver tissues in a dose-dependent manner. There was no TiO2 NP deposition in the same tissues from control animals. Brain AChE activity decreased at all TiO2 NP doses compared to controls, however, brain ATPase activities were generally stimulated. ATPase activities in the intestine and kidney did not change significantly. Levels of the different forms of GSH did not change significantly. There were no significant changes in TBARS levels, except for a decrease at the highest TiO2 NP dose.

Grissa et al., (2016)

260. The effects of oral intake of TiO2 NPs (5–10 nm) at 0, 50, 100, and 200 mg/kg bw for 60 days on the brain of Wistar rats was investigated. The coefficient of the brain, AChE activities, level of IL-6, and the expression of glial fibrillary acidic protein (GFAP) were assessed to quantify the brain damage. The results showed that high-dose TiO2 NPs could induce a downregulated level of AChE activity and an increase in plasma IL-6 compared to the control group. There was a dose-dependent decrease between doses and an associated increase in cerebral IL-6 as a response to a local inflammation in the brain. Elevated levels of immunoreactivity to GFAP were observed in the rat cerebral cortex. The authors concluded that oral intake of TiO2 NPs can induce neuroinflammation and could be neurotoxic and hazardous to health. (Grissa *et al.,* 2016 (Abstract only)).

EFSA review and Conclusions

261. The EFSA conclusions on the neurotoxicity of the EOGRT study considered that the effects on grip strength and hindlimb splay were not treatment-related but that quantitative information on peripheral nerves was not available. Overall, the Panel considered that E171 had no adverse effects on neurofunctional endpoints at the doses used.

262. No neurotoxicity studies performed with E171 were identified from the published literature that were considered sufficiently reliable. Some papers were identified noting effects of TiO2 NP.

Health Canada review and Conclusions

263. Health Canada (2022) summarised the evidence for neurotoxicity of TiO2 as follows: “The concerns pertaining to potential neurotoxicity associated with TiO2-NPs in E171 appear to be based predominantly on studies which used test articles that did not correspond to food-grade TiO2 and/or dosing paradigms that are considered to be of limited relevance to human dietary exposure. In an EOGRT study where developmental neurotoxicity was investigated in rats exposed to E171 at doses up to 1,000 mg/kg bw/d via the diet, no adverse effects on neurodevelopmental or neurofunctional endpoints were observed. Endpoints examined included auditory startle response as well as a functional battery that included grip strength and locomotor activity. No treatment-related changes were observed in any of these endpoints and there were no notable histopathological findings in the brain or peripheral nerves. Similarly, in the only available study in which TiO2-NPs were administered via the diet (Sofranko *et al.*, 2021), no neurotoxicity was observed.”

264. Health Canada’s more detailed analysis of neurotoxicity potential focussed on some of the studies used by EFSA and this is extracted at the end of their document. However, Health Canada did not include the study by Canli *et al.* (2020) which found reduced brain cholinesterase activity and increased brain Na/K-ATPase activity in female rats dosed for 14 days with TiO2 nanoparticles, presumably because of the limited relevance to human dietary exposure. Nevertheless, EFSA had concluded that these adverse effects, which were seen at the lowest of three doses tested (0.5 mg/kg bw/day), were “the most sensitive endpoint”, even though this was not consistent with the findings of Grissa *et al.* (2016) which reported reduced brain cholinesterase activity at 100 but not 50 mg/kg bw/day in male rats dosed for 60 days with TiO2 nanoparticles.

265. Health Canada also noted the study by Sofranko *et al.* (2021), published after the EFSA Opinion, which concluded that sub-acute (28 day) exposure to TiO2-P25 did not cause neurotoxicity (although P25 is referred to as foodborne, this is not considered as a food additive). Other studies which have been published since the 2021 EFSA Opinion are of very limited relevance as they were conducted *in vitro* using rat cortical cells (Gerber *et al.*, 2022) or involved i.v. injection of sonicated TiO2 nanoparticles (Ciu *et al.*, 2021; Naima *et al.*, 2021).

FSANZ review and Conclusions

266. FSANZ only identified one study with neurotoxicological endpoints using food-grade TiO2 administered via the diet, the EORGT Study (LPT 2020). FSANZ concluded that no adverse neurofunctional effects were observed (in auditory startle response and a functional observation battery).

COT review and Conclusions

267. Overall, there is no new evidence on neurotoxicity to justify a change to the COT position on this endpoint as stated in its 2021 interim position paper, namely "The findings of the studies on neurotoxicity were considered inconsistent by the COT. It was noted that the EOGRT study did not report any effects and that most of the other studies on this endpoint were of nanomaterials. In the EFSA evaluation, the issue of the test material in the EOGRT not being dispersed was taken into consideration with regards to the conclusions on this endpoint, as they considered that had it been dispersed and stabilised in the nano form some effects could possibly have been observed. The COT, as previously, questioned the relevance of such dispersion to the real-world use of TiO2. Members noted that the histopathology tests performed for the EOGRT study were standard and were not sensitive enough in comparison to other studies on this endpoint that performed specific neuro-histopathology testing." COT, 2022.

268. It should be recognised that this qualified COT opinion on neurotoxicity is more conservative than that of Health Canada who considered the EOGRT to be sufficiently sensitive and relevant to conclude on the lack of neurotoxicity potential of food grade TiO2.

Derivation of a Health-Based Guidance Value (HBGV)

269. The Committee concluded that on the basis of the available evidence, 1,000 mg/kg bw/day was a reasonably robust Point of Departure (POD). This was based on the EOGRT study findings as well as studies by Warheit *et al.*, 2015b and Lee *et al.*, 2019 that reported no effects up to the same dose. There was variability noted in the other studies, but nothing which would undermine the value of 1,000 mg/kg bw/day to be used as the POD. It was also noted by the COT that this is the highest dose of E171 or equivalent TiO2 tested in a study of this quality.

270. A standard uncertainty factor of 100 (10 for inter species variability and 10 for individual variability) was agreed by Members and applied to the POD which results in a HBGV of 10 mg/kg bw/day. There is likely to be additional conservatism in the application of this uncertainty factor to the NOAEL of E171 due to the low absorption of TiO2 and because there is no metabolism of TiO2 particles.

Exposure Assessment

271. The exposures to TiO2 from medication, personal healthcare products and through dermal, inhalation and intravenous routes are not considered in this assessment. The exposures considered are only those from food.

272. Titanium dioxide can be found in a number of food categories including bakery products, soups, broths, sauces, salads, savoury based sandwich spreads and processed nuts. It is also used in confectionary, chewing gum, food supplements and cake icing (EFSA, 2016).

Occurrence data in food

273. Only food as a source of TiO2 is considered in this exposure assessment. Food consumption data from the National Diet and Nutrition Survey (NDNS) (Bates, 2014; 2016; Roberts 2018; Bates, 2020) and the Diet and Nutrition Survey of Infant and Young Children (DNSIYC) (Department of Health, 2013) were used to estimate exposure to titanium dioxide. Maximum occurrence levels of titanium dioxide for specific food items, reported by EFSA (2021), were also used in the estimation of exposure (Table 6). Food categories were created by the FSA Exposure Assessment Team (EAT) using data from NDNS and DNSIYC to reflect those created by EFSA for the Food Additive Intake Model and as presented in Annex II of Regulation (EC) No 1333/2008, part D. Foods in NDNS and DNSIYC were matched to food categories associated with the regulation on food additives to enable an assessment of exposure based on maximum levels reported by industry for titanium dioxide and those reported in the scientific literature. Assessments were carried out in Crème which is the software used by the FSA EAT to conduct exposure assessments.

274. The occurrence data used were those reported in EFSA, 2021 which were obtained from industry as reported by the Dutch National Institute for Public Health and the Environment (RIVM) along with levels reported in analytical

studies. These levels are presented in Table 1 for sixteen food categories, although titanium dioxide is approved in many other food categories (forty-eight in total) (Table 1, Annex E). For the exposure assessment, only use levels for these sixteen food categories were taken into account, as no data were available for the other categories and it was not possible to use the maximum permitted levels (MPLs) for TiO2 as they were established at quantum satis, rather than a specific value being ascribed. The assessment was based on maximum use levels reported to provide conservative scenarios of exposure for the population groups considered (Table 7).