Neurotoxicity - Statement on the safety of Titanium Dioxide (E171) as a Food Additive

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Studies using E171 or equivalent form of TiO2

EOGRT Study (Leuschner, 2020)

264. Details of the design on the EOGRT study are given in paragraphs 122 – 125. Male and female offspring were tested for auditory startle response between PND 23 and 25, including grip strength evaluation and for quantitative locomotor activity between PND 58 and 64. No differences in the response to an auditory startle stimulus were observed and an increase in hindlimb splay was observed in females, reaching statistical significance at 100 and 1,000 mg/kg bw per day. A statistically significant increase in mean forelimb grip strength was noted at 300 mg/kg bw per day in both males and females.

265. Grip strength and hindlimb splay belong to the same domain of neurological function, however, the increase in hindlimb splay and increase in mean forelimb grip strength are opposed in this case - increases in hindlimb splay indicate muscular weakness but an increase in mean forelimb grip strength may indicate myotonia. No dose response was observed for any of these endpoints or for the two functional measurements, indicating that the likelihood of an effect of the test substance is low. No other changes were observed including histopathological findings in brain or in peripheral nerve tissue.

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268. No reliable studies on neurotoxicity using E171 were identified in the published literature.

Studies using the nanoparticle form of TiO2

Sofranko et al., (2021)

269. 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 containing 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 period. 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)

270. Female albino Wistar rats were given 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 thiobarbituric acid reactive substances (TBARS) and the different forms of glutathione (GSH) (oxidised, reduced and total) were measured in tissues from the liver, kidney, brain and intestine.

271. The anatase TiO2 NPs were about 21 nm, >30 m2/g surface area, >99% purity, and a density of 4.26 g/cm3. 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. None of these changes showed a dose-response relationship. 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)

272. 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 male Wistar rats was investigated. The brain to body weight ratio, 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 mid- and high-dose TiO2 NPs reduced the relative weight of the brain, induced a downregulation of AChE activity and an increase in cerebral IL-6 secretion compared to the control group. Plasma AChE activity decreased and IL-6 levels increased at all dose levels. Elevated levels of immunoreactivity to GFAP were observed in the rat cerebral cortex in mid- and high-dose groups. The authors concluded that oral exposure to TiO2 NPs can induce neuroinflammation and could be neurotoxic. (Grissa et al., 2016).

EFSA review and conclusions on neurotoxicity of TiO2

273. With respect to the EORGT study, the EFSA 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. 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. There were no notable histopathological findings in brain or in peripheral sciatic nerve. 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 at these doses had no adverse effects on neurofunctional endpoints in F1 cohort 2A offspring."

274. 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. EFSA concluded "Overall for neurotoxicity, adverse effects were seen with TiO2 NPs < 30 nm. In mice, Zhou et al. (2017; scoring 3 for NSC, nanoscale considerations), reported adverse effects (i.e. inhibited dendritic outgrowth, increased autophagy and oxidative stress and reduced mitochondrial function) in ex vivo hippocampal neurons of weanling mice after dosing TiO2 NPs (6–7 nm) during gestation and early lactation at a dose of 1 mg/kg bw per day, the lowest dose tested. In adult female rats (Canli et al., 2020; scoring 3 for NSC), adverse effects (reduced brain cholinesterase, and increased brain Na/K-ATPase activity) were observed with TiO2 NPs (21 nm) at 0.5 mg/kg bw per day, the lowest of three doses tested, in a 14-day study." EFSA did note that there was an apparent 200-fold difference in potency in the effects of TiO2 NP on brain cholinesterase activity between Grissa et al. (2016) and Canli et al (2020), which adds to uncertainty.

Health Canada review and conclusions on neurotoxicity of TiO2

275. 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."

276. Health Canada 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 were considered 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 on neurotoxicity of TiO2

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

COT review and conclusions on neurotoxicity of TiO2

278. 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.)

279. 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 TiO_2.