Meeting

Cover Paper and Tables - Third draft statement on the safety of Titanium Dioxide (E171) as a Food Additive

Introduction

1. Titanium dioxide (TiO_2) was an authorised Food Additive (E171) in the EU and currently remains authorised in the UK, under Retained EU Regulation No. 1333/2008 and Retained EU Regulation No. 231/2012. It is used in food as a colour to make food more visually appealing, to give colour to food that would otherwise be colourless, or to restore the original appearance of food. It is commonly used in products such as bakery products, soups, broths, sauces, salad dressings, savoury based sandwich spreads, processed nuts, confectionary, chewing gum, food supplements and cake icing.

2. Titanium dioxide has been the subject of multiple safety evaluations. In 2016, the EFSA ANS (Food Additives and Nutrient Sources) Panel evaluated the safety of E171 TiO_2 and identified several uncertainties in their evaluation included the unspecified identity and characterisation of E171 as it was not determined whether the test material was compliant with the specification of E171 requirements. The EFSA 2016 review determined that E171 TiO_2 consisted mainly of micro-sized TiO_2 particles, with a nano-sized (<100 nm) fraction which was less than 3.2% by mass. Uncertainties around the identity and characterisation of E171 were highlighted, noting that no limits for the particle size of E171 were set. In 2019, the specifications of E171 titanium dioxide were reviewed by the EFSA FAF Panel (Food and Feed). A recommendation for re-assessment of the safety of titanium dioxide was proposed.

3. In the EFSA 2021 Opinion, the EFSA FAF Panel considered that some findings regarding immunotoxicity, inflammation and neurotoxicity with respect to TiO_2 nanoparticles may be indicative of adverse effects. On the basis of the currently available evidence and the uncertainties, in particular a concern regarding genotoxicity which could not be resolved, the EFSA Panel concluded

that E171 can no longer be considered as safe when used as a food additive.

4. In 2021 the COT published an interim position on titanium dioxide (COT 2021) capturing the outcomes of the discussions and outlining the next steps. Members were asked to evaluate the EFSA Opinion and comment on whether they agreed with EFSA's conclusions and further guidance on the next steps that should be taken; producing an opinion paper following a review of the new EFSA opinion and the extended one generation reproductive toxicity (EOGRT) study data by both the COT and COM (Committee on Mutagenicity).

5. This draft statement (Annex A) includes the COT conclusions on the following endpoints: ADME, Aberrant Crypt Foci as a marker for Carcinogenicity, Allergenicity, Reproductive and Developmental Toxicity, potential evidence of Immunotoxicity, Inflammation and Neurotoxicity and the derivation of a Health-Based Guidance Value, and a review of genotoxicity endpoints by the COM. Additionally, this statement also includes the titanium dioxide exposure assessment for the UK population.

Questions for the Committee

6. The Committee are asked to consider the following questions:

i. Are Members content with the layout and structure of the draft statement?

ii. Would Members like to see any additional information on studies already included, or consider that other studies should be included?

iii. Do Members agree with the layout and structure of the table summarising the studies?

iv. Do Members have any other comments?

Secretariat

February 2024

Absorption, Distribution, Metabolism and Excretion (ADME) – E171 animal studies

Reference	TiO2 characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls.	Results
Talamini et al., 2019	E171 (35% nano determined by TEM), 99.3% pure anatase, 201.2 ± 8.5 nm in suspension (NTA). No sonification or deagglomeration to simulate realistic conditions.	This work was reviewed by the Institute for Pharmacological Research Mario Negri IRCCS Animal Care and Used Committee (IACUC) and then approved by the Italian National Institute of Health (code:42/2016-PR).	Treatments were given 3 days per week for 3 weeks for a total of 9 treatments in 21 days. Average daily dose of ~2 mg/kg bw. Treatments were dripped slowly into the mice's mouths, allowing each drop to be swallowed.	NFR male mice (22/group) were administered either water (control) or 5 mg/kg bw E171 suspended in water. Ti concentrations in tissues were determined by single particle ICP-MS analysis.	Ti concentrations in the liver (0.94 ± 0.57 µg/g tissue) and large intestine (1.07 ± 0.38 µg/g tissue) were significantly higher in treated mice compared to controls. Ti concentrations in the brain, kidney, and testes were below the quantificationlimit (0.03 µg/g). Ti concentrations in lungs, spleen, stomach, and small intestine were not statistically significant between treated and control mice.

				No evidence of gross alteration of immune-cell physiology or inflammation at doses up to 100 mg/kg bw/d via the diet.
Riedle et al., 2020	E171, anatase, 119 nm.	Mice were exposed to 0, 1, 10, or 100 mg/kg bw/d E171 via the diet for 6, 12 and 18 weeks.	Mice were divided into 4 groups of 18 and given 0, 6.25, 62.5, or 625 mg/kg diet (equivalent to approximately 0, 1, 10, or 100 mg/kg bw). Then 6 mice per group were euthanized at 6, 12 and 18 weeks.	Authors demonstrate E171 uptake by Peyer’s patches, validating the delivery model. Presence of E171 particles detected by reflectance confocal microscopy (no quantification of particles completed). Weak signals observed at the base of Peyer’s patches at low and mid-doses. Higher signals observed at highest dose, indicating evidence of dose-response.

Allergenicity

Reference	TiO2 characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls,	Results	Notes, comments, other
Phue et al., (2022)	Food grade titanium dioxide nanoparticles and E171.		Used ELISA to study the alterations of the IgG binding, and mast cell degranulation assay to study allergenicity of milk and individual milk proteins (β-lactoglobulin and casein) in the presence of E171.	For ELISA, primary antibody for casein (Anti-casein rabbit antibody-cat # ab166596), primary antibody for β-lactoglobulin (Anti-LGB rabbit antibody-cat # ab112893) and secondary anti-rabbit antibody (cat # 6721) were used. Quebon skimmed milk was used.	Significant enhancement in the allergenicity of milk proteins/ skimmed milk interacted with both E171 and food grade titanium dioxide nanoparticles. The presence of E171 showed the highest level of LAD2 degranulation (a proxy for allergenicity), followed by food grade titanium dioxide nanoparticles.	No information

Inflammation and Immunotoxicity

Reference	TiO2 characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls,	Results

Talamini et al., 2019	E171 (35% nano determined by TEM), 99.3% pure anatase, 201.2 ± 8.5 nm in suspension (NTA). No sonification or deagglomeration to simulate realistic conditions.	This work was reviewed by the Institute for Pharmacological Research Mario Negri IRCCS Animal Care and Used Committee (IACUC) and then approved by the Italian National Institute of Health (code:42/2016-PR).	Treatments were given 3 days per week for 3 weeks for a total of 9 treatments in 21 days. Average daily dose of ~2 mg/kg bw. Treatments were dripped slowly into the mice’s mouths, allowing each drop to be swallowed.	NFR male mice (22/group) were administered either water (control) or 5 mg/kg bw E171 suspended in water. Ti concentrations in tissues were determined by single particle ICP-MS analysis.	Ti concentrati in the liver (0.94 ± 0.5 µg/g tissue) and large intestine (1 ± 0.38 µg/g tissue) were significantly higher in treated mic compared t controls. Ti concentrati in the brain kidney, and testes were below the quantificati limit (0.03 µg/g). Ti concentrati in lungs, spleen, stomach, ar small intest were not statistically significant between treated and control mic

Pinget et al., 2019	E171, anatase, 30-300 nm. E171 was	No information.	Mice were exposure to E171 via drinking water for 4 weeks at doses of 0, 2, 10, 50 mg/kg bw/d. Dose is calculated based on water intake measured per cage. Microbiota populations in	Male C67BL/6JAusb mice were exposed to E171 via drinking water at doses of either 0, 2, 10, or 50 mg TiO2/kg BW/day for 3 weeks to determine impact on colonic microbiota composition and on gut bacterial metabolites (10 mice/group). Incubated commensal bacteria derived from mouse colons anaerobically for 5 days with dose of 0, 2, 10, 50 µg/ml of TiO2 biofilm formation (6 mice/group). Impact of TiO2 on colonic epithelial function was determined by comparison of gene expression of key markers Muc2, Tjp1, Defb3, and Gzmb in colonic	At the highe dose tested TiO2 had minimal impact on t composition the gut microbiota. Alterations bacterial metabolites were obser from 10 mg bw/d. Doses of 10 and 50 µg/m TiO2 significantly promoted biofilm formation b commensal bacteria. There was reduced expression the colonic mucin 2 ge a key component the intestin

Riedle et al., 2020	E171, anatase, 119 nm.	No information.	Mice were exposed to 0, 1, 10, or 100 mg/kg bw/d E171 via the diet for 6, 12 and 18 weeks. E171 was formulated into diet.	6-week-old male and female C57BL/6 mice (6/sex/group) were exposed to E171 daily via diet for 6, 12 and 18 weeks. Mice were divided into 4 groups of 18 and given 0, 6.25, 62.5, or 625 mg/kg diet (equivalent to approximately 0, 1, 10, or 100 mg/kg bw). Then 6 mice per group were euthanized at 6, 12 and 18 weeks.	No evidence of gross alteration o immune-cel physiology inflammatio at doses up 100 mg/kg bw/d via the diet. Authors demonstrat E171 uptak by Peyer's patches, validating t delivery model. Presence of E171 partic detected by reflectance confocal microscopy (no quantificati of particles completed) Weak signa observed at the base of Peyer's patches at l and mid-doses. High signals observed at highest dos indicating evidence of dose-

Liu et al., 2020	This is a review, and is only mentioned once in the TiO2 statement in a quote from the Health Canada report.	No information.	No information.	No information.	No information

Han et al., 2020	E 171, anatase, 150 nm, 99.5% purity	Study conducted according to	E171 suspended in distilled water, sonicated for at least 10 minutes. E171 administered by oral gavage at doses of 0, 10, 100 or 1,000	Sprague–Dawley rats (10/sex/group) were administered E171 by oral gavage at doses of 0, 10, 100 or 1,000 mg/kg bw/d for 90 days.	Statistically significant decreases i GM-CSF plasma leve (~30% in females) an plasma IgM (~12% in females and 9% in males were observ at the highe dose compared t controls. E171 accumulatio in the stomach wa of several r administere 1,000 mg/k E171 for 90 days. Ti concentrati increased in the colons o both sexes administere 1,000 mg/k E171 compared with the control, whi colonic, superoxide dismutases (SOD)-1 (m and female and SOD-2

Studies used to review the toxicokinetic and absorption of the nanoparticle form of TiO2

Reference	TiO2 characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls,

Tassinari et al., 2014	TiO2 nanoparticles (anatase, primary size 25 nm, BET surface area 45-55 m^2/g, purity 99%).	All experiments on animals were performed according to the European Community Council Directive 86/609/EEC (EEC 1986).	TiO2 nanoparticles were administered by oral gavage over 5 consecutive days at a dose of 0, 1, 2 mg/kg body weight per day.	Sprague-Dawley rats were divided into 3 treatment groups (7 rats/sex/group). Treatment groups were high dose (2 mg/kg bw), low dose, (1 mg/kg bw), and controls (CTRL) (vehicle only (distilled water).

Warheit et al., 2015	1) anatase/rutile (89/11%) (uf-1), d50=43 nm d50=23 nm Methods: XSDC and TEM respectively. Shape: Irregular. 2) anatase (100% nano) (uf-2) d50= 42 nm d50=19 nm. Methods: XSDC and TEM respectively. Shape: Irregular. 3) rutile (100% nano) (uf-3), d50=47 nm d50=22 nm Methods: XSDC and TEM respectively. Shape: rod-like. 4) anatase (27% nano) (pg-1), d50=153 nm d50=120 nm Methods: XSDC and TEM respectively. Shape:	OECD Guideline 414	Sterile water-based TiO2 sample formulations were administered by oral gavage to time-mated rats from the time of approximate implantation until the day prior to expected parturition. Dose levels: 0, 100, 300 or 1,000 mg/kg bw per day. Dosage volume: 5 mL/kg bw per day.	Three studies (Group size n=22): Time-mated pregnant Sprague–Dawley rats, (Crl:CD(SD)) exposed to TiO2 (uf-1, uf-3 and pg-1) by gavage on Gestational Days 6–20. Three additional studies (Group size n=22-23) pregnant Wistar rats exposed to TiO2 (uf-2 and pg-2) by gavage from Gestational Days 5 to 19. Necropsy: • Gross examination of the dam. • Counting of corpora lutea. • Implantation sites. • Resorption live and dead fetuses. • Fetal sex. • Fetal weight. • Fetal

				Preliminary work: TiO2 NP suspensions at different concentrations (2.5, 5, and 10 mg/kg of body weight [BW]) administered to mice by intragastric administration for 90 consecutive days. Treatment with 10 mg/kg BW TiO2 NPs resulted in the most severe organ damage and used as the highest concentration for further experiments.
	The mean diameter of TiO2 NP ranged		Nanoparticulated anatase TiO2 was prepared via controlled hydrolysis of titanium tetrabutoxide.	
Gao et al.,	from208 to		The particle sizes of both the powder and the nanoparticles	90-Day Study: Two

Gao et al., 2013	The mean diameter of TiO2 NPs was 294 nm (range, 208–330 nm).	Anatase TiO2 NPs were prepared via controlled hydrolysis of titanium tetrabutoxide and powdered TiO2 NPs were suspended in 0.5% (w/v) hydroxypropylmethylcellulose (HPMC). Prior to dosing, the mice were acclimated to this environment for 5 days. The control group was treated with 0.5%, w/v HPMC and three experimental groups were treated with 2.5, 5, and 10 mg/kg body weight (BW) of TiO2 NPs respectively.	One hundred and fifty CD-1 (Imprinting Control Region) male mice, aged 5 weeks with a mean body mass of 22 ±2 g. Four mice groups (n = 30 each): one control group (treated with 0.5%, w/v HPMC) and three experimental groups [2.5, 5, and 10 mg/kg body weight (BW) of TiO2 NPs]. TiO2 NPs suspensions were administered by intragastric administration daily for 90 days and effects recorded daily.	

Bettini et al., 2017	1) E 171, anatase, 20–340 nm (118 nm) (TEM); 44.7% particles 100 nm; 2) TiO2 NPs (NM-105), anatase/rutile, 15–24 nm.	**OECD?**	Series One Dosage: 200 µ L with TiO2 NM-105, E171 (10 mg/kg of BW/day) or water for 7 days by gavage. Series Two Dosage: E-171 at 200 µ g or 10 mg/kg of BW/day via drinking water for 100 day (with or without DMH treatment). Series Three Dosage: No treatment followed by a single dose of 10 mg/kg E-171.	Series One: rats (n = 10 rats/group) dosed daily by intragastric gavage (200 µ L) with TiO2 NM-105, E171 (10 mg/kg of BW/day) or water for 7 days. Tissue imaging, flow cytometry and cytokine assays, tissue inflammation and gut permeability measurements were conducted. Series Two: rats (n = 11 to 12 per group) were treated or not with 1,2-dimethylhydrazine (DMH) to induce colon carcinogenesis and exposed to E-171 at 200 µ g or 10 mg/kg of BW/day via drinking water for 100 days. Control animals (n = 12) received water only. Flow cytometry and cytokine assays were assessed for gut inflammation and ACF.	

Karimpour *et al*., 2018	TiO2 NPs, anatase, 10–25 nm.	One dose of TiO2NP (100 mg/kg per day) or the test vehicle (control group) daily for 5 weeks. NMRI = Naval Medical Research Institute.	54 ten week old (25±2 g) adult female NMRI mice were divided into a control group which received vehicle (saline solution) orally and TiO2NP group which received 100 mg/kg per day TiO2NP solution orally.	Pregnancy and in vitro fertilization rates, histological changes in ovaries, malondyaldehyde and estrogen hormone levels in the blood serum were assessed after five weeks. 24 hours post last administration of test item: 3 control or test female mice were housed with 3 male mice for 11 days. The percentage of pregnancy and numbers of newborns were evaluated.

Khorsandi *et al.*, 2016	TiO2 NPs 30 nm.	**T**est item: NTiO2 nanopowder (TNP, Sigma) made with 100 ml BSA (bovine serum albumin) solution dissolve din Milli-Q water. Oral Dosage Groups: TNP-1: 75 mg/kg TNP, TNP-2: 100 mg/kg TNP, TNP-3: 300 mg/kg TNP. Control: saline solution.	32 adult 6–8 weeks old male NMRI mice (25–30 g). Four groups of 8 mice with a dosage of 75, 100 and 300 mg/kg TNP for 35 consecutive days respectively for each of the test groups and the control group received saline orally for 35 consecutive days. Testicular testosterone levels, testis weight, total volumes of testis, seminiferous tubules, interstitial tissue and total Leydig cell numbers were measured.	

Lee *et al*., 2019.	TiO2 NPs P25 (15–24 nm).	OECD Guideline 414 (Pre-natal Toxicity Study).	Test item: Nanoparticles in deionised water. 80/20 anatase/rutile. Mean diameter of approximately 21 nm (minimum of 100 particle sizes averaged) administered daily by oral gavage. Dosage: Test item was administered from Gestational Days 6 to 19 at dose levels of 0, 100, 300 and 1000 mg/kg with a dose volume of 10 mL/kg.	Sprague–Dawley rats (12 females per group). Quantitative analysis in blood/tissues. Four groups of twelve females per group in the toxicology group (total test animals: 48) and four groups of four females in the tissue distribution group (total test animals: 16).

Aberrant Crypt Foci (ACF) as a marker for carcinogenicity

Reference	TiO2 characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls.	Results

				E-171 consumption did not alter T-cell-mediated mechanisms of immune control. Dietary E-171 did not induce inflammation peripherally or in the GI tract.
			Six-week-old male Wistar Han IGS (Crl:WI (Han)) rats. Test material: Food grade sample E-171. DIfferent grades of commercially-available E-171 were averaged to produce the test material supplied. Test material was added to feed. Two feed batches: batch one was fed throughout the 7-day study and through week 10 of the 100-day study. Batch two was fed post-week 10 of the 100-day study.	An increase was observed in the relative spleen weight in 22.4 mg E-171/kg bw per day + DMH compared to not initiated animals and an increase in IL-17A in colon (22.4 mg E 171/kg bw per day + DMH) and IL-12p70 in plasma (3.5 mg E 171/kg bw per day + DMH), with no dose-related effects. No changes were observed in spleen cellularity.
		E-171	7-day study: 4 groups of 5 animals (randomised based on weight),	No changes were observed in the percentage of CD103+ DC, CD4+ T helper cells or total or

Akagi et al., 2023 – 28 Day Study	6 nm TiO2 nanoparticles.	No information.	5 female and 5 male F344/DuCrlCrlj rats.	TiO2 NPs with a crystallite size of 6 nm were examined in male and female F344/DuCrlCrlj rats by repeated oral administration of 10, 100, and 1000 mg/kg bw/day (5/sex/group) for 28 days.	No mortality was observed in any group, and no treatment-related adverse effects were observed in body weight, urinalysis, haematology, serum biochemistry, or organ weight. Histopathological examination revealed TiO2 particles as depositions of yellowish-brown material. The particles observed in the gastrointestinal lumen were also found in the nasal cavity, epithelium, and stromal tissue in the 28-day study. Overall, No effects were observed after repeated oral administration of TiO2 with a crystallite size of 6 nm at up to 1000 mg/kg bw/day regarding general toxicity,

Reproductive toxicity

Reference	TiO2 characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls.	Results	N c

Results: F0 - Dose-dependent marginal increase in TiO2 blood and urine concentration in rats dosed with 1000 mg/kg bw/day.

No test item-related effects on sexual function or fertility in males or females. No test item-related pre- or postnatal loss observed.

No test item-related thyroid hormone or haematological effects.

No test item-related differences in splenic lymphocyte subpopulation distribution.

No test item-related changes related to histopathology examinations including the testis and epididymides and intestinal examinations for

						No test item-related effects in behaviour or external appearance. No test item-related thyroid hormone effects.
TDMA, 2020 – Satellite study	Test substance: Anatase E-171, 51% of particles 100 nm. Dietary particle size: 31-43% of particles 100 nm.	OECD Test Guideline 443.	F0 satellite group: 0, 100, 300, and 1000 mg/kg bw/day over 10 weeks (prior to mating and up to the end of weaning periods).	CD® (Sprague Dawley) IGS Rat (Crl:CD(SD). F0 satellite group – 30 male, 30 female per group **+ additional 40 (20 male, 20 female) for use as an F1 generation of satellite animals to be used as the positive control group in the KLH-assay (?)** Endpoint: AGD	No test item-related effects on body weight, food consumption and water consumption. No test item-related effects on haematology and biochemical parameters or urinalysis. No test item-related effects on thyroid and sexual hormones or sperm. No test item-related changes in bone marrow or organ weights. No test item-related histopathological effects in the high dose group. No test item-related induction	N

Immunotoxicity

Reference	TiO2 characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls.	Results	N co ot

					Statistically significant decreases in GM-CSF plasma levels (~30% in females) and plasma IgM (~12% in females and 9% in males) were observed at the highest dose compared to controls. E171 accumulation in the stomach wall of several rats administered 1,000 mg/kg E171 for 90 days.	
Han et al., 2020	E171, anatase, 150 nm, 99.5% purity.	Study conducted according to OECD TG 408.	E171 suspended in distilled water, sonicated for at least 10 minutes. E171 administered by oral gavage at doses of 0, 10, 100 or 1,000 mg/kg bw/d for 90 days. Quantitative analysis in Sprague-	Sprague–Dawley rats (10/sex/group) were administered E171 by oral gavage at doses of 0, 10, 100 or 1,000 mg/kg bw/d for 90 days. Ti concentrations were measured in the colons, kidneys, and spleens harvested from	Ti concentration increased in the colons of both sexes administered 1,000 mg/kg E171 compared with the control, while colonic, superoxide dismutases (SOD)-1 (male and female) and SOD-2 (female) protein levels were down-regulated. When exposed to AGS cells	N ir

NCI, 1979 – see link ->	TR-097: Titanium Dioxide (CASRN 13463-67-7) (nih.gov) Titanium dioxide anatase. Purity: 98%.	No information.	Groups of 50 rats of each sex and 50 mice of each sex were administered titanium dioxide in the diet at one of two doses, either 25,000 or 50,000 ppm, for 103 weeks and then observed for 1 additional week. Matched controls consisted of 50 untreated rats of each sex and 50 untreated mice of each sex. All surviving rats and mice were killed at 104 weeks.	Administration of the titanium dioxide had no appreciable effect on the mean body weights of rats or mice of either sex. With the exception of white feces, there was no other clinical sign that was judged to be related to the administration of titanium dioxide. Survival of the rats and the male mice at the end of the bioassay was not affected by the test chemical; mortality in female mice was dose related. Sufficient numbers of dosed and control rats and mice of each sex were at risk for development of late-appearing tumors.	In the male and female mice, no tumours occurred in dosed groups at incidences that were significantly higher than those for corresponding control groups. It is concluded that under the conditions of this bioassay, titanium dioxide was not carcinogenic by the oral route for Fischer 344 rats or B6C3F1 mice.	N ir

Akagi et al., 2023 – 28 Day Study	6 nm TiO2 nanoparticles.	No information.	5 female and 5 male F344/DuCrlCrlj rats.	TiO2 NPs with a crystallite size of 6 nm were examined in male and female F344/DuCrlCrlj rats by repeated oral administration of 10, 100, and 1000 mg/kg bw/day (5/sex/group) for 28 days.	No mortality was observed in any group, and no treatment-related adverse effects were observed in body weight, urinalysis, haematology, serum biochemistry, or organ weight. Histopathological examination revealed TiO2 particles as depositions of yellowish-brown material. The particles observed in the gastrointestinal lumen were also found in the nasal cavity, epithelium, and stromal tissue in the 28-day study. Overall, no effects were observed after repeated oral administration of TiO2 with a crystallite size of 6 nm at up to 1000 mg/kg bw/day regarding general toxicity,	N in

Akagi et al., 2023 – 90 Day Study	6 nm TiO2 nanoparticles.	No information.	10 female and 10 male F344/DuCrlCrlj rats.	No information.	TiO2 NPs with a crystallite size of 6 nm were examined in male and female F344/DuCrlCrlj rats by repeated oral administration of 100, 300, and 1000 mg/kg bw/day (10/sex/group) for 90 days.	N w in a tr re a e o b u h s b o w a w o P p il c ly m ly b a ly ti tr 9 O e o a o a c o t

Neurotoxicity

Reference	TiO2 characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls.	Results

Sofranko et al., 2021	10 mg/g TiO2, 2 mg/g polyvinylpyrrolidone-coated Ag.	OECD 424 Neurotoxicity study in the rodents.	No information.	10 female and 10 male C57BL/6J mice.	The mice ad libitu pellets d 10 mg/g mg/g polyviny coated A pellets fo

Grissa et al. (2016)	TiO2 NPs, anatase, 5–12 nm (TEM, XRD).	No information.	Internal exposure: quantitative in male Wistar rat tissues; methodology with important flaws.	No information.	There wa statistica significa related i the level 100 and bw per d groups o and a sta significa related i brain TN mg/kg b TiO2 NPs

Gerber et al., 2022	TiO2 NPs, average primary particle size of 26.2 ± 10.7 nm.	No information.	No information.	The aim of the study was to investigate the effects of two common types of NP, titanium dioxide NP (TiO2NP) and silver NP (AgNP), on neuronal function following acute (0.5 h), sub-chronic (24 h and 48 h) and chronic (14 days) exposure in vitro rat cortical cells. Acute and sub-chronic exposure to TiO2NP is without effects, whereas chronic exposure only modestly reduces neuronal function without affecting morphology.	No inform

Ciu et al., 2021	No information.	No information.	36 male Sprague Dawley rats aged postnatal day 21 (PND 21) were injected intraperitoneally with TiO2 NPs (20 mg/kg) and/or BEO (200 mg/kg).	TiO2 NPs exposure during the adolescent period induced anxiety-like behaviour, cognitive impairment, neuroinflammation and oxidative damage in hippocampus, and BEO treatment could significantly ameliorate the neurotoxicity induced by TiO2 NPs exposure.	No inform
Naima et al., 2021	No information.	No information.	Rats were injected intravenously with a single dose of TiO2-NPs (20 mg/kg body weight) and were subjected to cognitive and emotional tests using Morris water maze and elevated plus maze.	Acute intravenous injection of TiO2-NPs impaired behaviour performances through brain biochemical and structural changes and precautions should be taken to their usage in food additive and medical applications.	No inform