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

Introduction

1. Titanium dioxide (TiO2) 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 TiO2 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 TiO2 consisted mainly of micro-sized TiO2 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 reassessment 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 TiO2 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 \pm 0.57 µg/g tissue) and large intestine (1.07 \pm 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.

Riedle

et al., 2020 E171, anatase, 119 nm.

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

No evidence of

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.	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,	Results
				controls,	

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	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.	tissue) v significa higher in treated compare controls Ti concent in the bi kidney, testes w below th quantific limit (0. μg/g). Ti concent
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.	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).	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	(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.	compa control Ti concer in the l kidney testes below f quantif limit (0 µg/g). Ti concer

Ti concentrati in the liver (0.94 ± 0.5) µg/g tissue) and large intestine (1 ± 0.38 μg/g were antly

mic

red t s. trati brain

and were the ficati .03

ntrati in lungs, spleen, stomach, a small intest were not statistically significant between treated and control mic

et al., Defb3, and the intestiv	Pinget	E171, anatase, 30-300 nm.	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	At the high 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/n TiO2 significantly promoted biofilm formation to commensa bacteria. There was reduced expression the colonic mucin 2 ge a key component
---------------------------------	--------	------------------------------	-----------------	--	---	---

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 inflammatic 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 quantificatio of particles completed) Weak signal 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 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.	ormation
---	----------

Statistically significant decreases i **GM-CSF** plasma leve (~30% in females) ar plasma IgM (~12% in females and 9% in male were observ at the highe dose compared t controls. E171 accumulation in the stomach wa of several r administere 1,000 mg/k E171 for 90 days. Ti concentrati increased in the colons of both sexes administere Sprague-Dawley 1,000 mg/k E171 (10/sex/group) compared with the administered control, whi E171 by oral colonic, gavage at doses superoxide of 0, 10, 100 or dismutases 1,000 mg/kg (SOD)-1 (m and female and SOD-2

E171 suspended rats

water, sonicated were

bw/d for 90

days.

in distilled

minutes.

E171

for at least 10

administered by

oral gavage at

doses of 0, 10,

100 or 1,000

Study Han et E 171, anatase, conducted al., 150 nm, 99.5% according to 2020 nurity

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

TiO2 Quality of Reference characterisation study e.g., OECD/GLP

Method and duration of dosing to include species,

Study methodology numbers, controls,

All

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

Sprague-Dawley

1) anatase/rutile (89/11%) (uf-1), d50=43 nm d50=23 nm Methods: Three studies XSDC and TEM (Group size n=22): respectively. Time-mated Shape: pregnant Irregular. Sprague-Dawley rats, (Crl:CD(SD)) 2) anatase exposed to TiO2 (100% nano) (uf-1, uf-3 and pg-(uf-2) d50 = 421) by gavage on nm d50=19 **Gestational Days** nm. 6-20. Methods: Three additional XSDC and TEM studies (Group size respectively. n=22-23) pregnant Shape: Wistar rats Irregular. exposed to TiO2 Sterile water-based TiO2 (uf-2 and pg-2) by sample formulations were rutile 3) gavage from administered by oral gavage (100% nano) Gestational Days 5 to time-mated rats from the (uf-3), d50=47 to 19. time of approximate nm d50=22 Warheit OECD implantation until the day nm Methods: Necropsy: et al., Guideline prior to expected parturition. XSDC and TEM 414 2015 Gross respectively. Dose levels: 0, 100, 300 or examination 1,000 mg/kg bw per day. Shape: rodof the dam. like. • Counting of Dosage volume: 5 mL/kg bw corpora lutea. per day. 4) anatase Implantation (27% nano) sites. (pg-1), Resorption d50=153 nm live and dead d50=120 nm fetuses. Methods: • Fetal sex. XSDC and TEM Fetal respectively. weight.

Shane

• 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 Gao et al., from 208 to Nanoparticulated anatase TiO2 was prepared via controlled hydrolysis of titanium tetrabutoxide.

The particle sizes of both the powder and the nanoparticles

90-Day Study: Two

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

suspensions were administered by intragastric administration daily for 90 days and effects recorded daily.

One hundred and

				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.
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 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.

с · т

Karimpour TiO2 NPs, et al., anatase, 2018 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.

			32 adult 6-8 weeks old male NMRI mice (25-30 g).
Khorsandi <i>et al.,</i> 2016	TiO2 NPs 30 nm.	Test 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.	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.

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

Aberrant Crypt Foci (ACF) as a marker for carcinogenicity

Reference TiO2 St characterisation

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

E-171

consumption dic not alter T-cellmediated mechanisms of immune control.

Dietary E-171 did not induce inflammation peripherally or in the GI tract.

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 colo (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 doserelated effects.

No changes wer observed in spleen cellularity.

No changes wer observed in the percentage of CD103+ DC, CD4+ T helper cells or total or

Six-week-old male Wistar Han IGS (Crl:Wl (Han)) rats.

Test material: Food grade sample E-171. DIfferent grades of commerciallyavailable 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 7day study and through week 10 of the 100-day study. Batch two was fed postweek 10 of the 100-day study.

7-day study: 4 groups of 5 animals (randomised based on weight).

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 bod weight, urinalysis, haematology, serum biochemistry, or organ weight. Histopathologica 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
					TiO2 with a crystallite size o 6 nm at up to 1000 mg/kg bw/day regarding general toxicity

Reproductive toxicity

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

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

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

No test itemrelated thyroid hormone or haematological effects.

No test itemrelated differences in splenic lymphocyte subpopulation distribution.

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

No test item-

assay (?)

Immunotoxicity

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

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.

E171

Han et E171 al., 150 2020 purit	L, anatase, nm, 99.5% zy.	Study conducted according to OECD TG 408.	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-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	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.	N ir
			Sprague-	harvested from	to AGS cells	

NCI, See link -> TR-097: Titanium Dioxide Groups of 50 rats 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. for 1 additional Week. CASRN 13463- pm, for 103 Weeks and then observed for 1 additional Week. Consisted of S0 untreated mice 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 there was no tumours other clinical sign that was dosed groups at judged to be incidences that related to the administration dioxide. Survival those for of titanium dioxide. Survival those for of the rats and control affected the bioassay chemical; titanium dioxide mortality in sufficient sufficient sex were at risk for development to flate- appearing tumors.	N ii
---	--	------

Akagi et al., 6 nm TiO2 information. 5 male 2023 - nanoparticles. F344/DuCrlC 28 Day 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	Nir
---	--	---	-----

Akagi et al., 2023 6 nm TiO2 - 90 Day nanoparticles. Study	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	NwiratinaeobuhsbowawoPpilclynlybalytiti9 Ceoa
Study		Tats.		100, 300, and 1000 mg/kg bw/day (10/sex/group)	9 0 e ⁻ 0
				for 90 days.	a o a
					01 01
					0
					to

Neurotoxicity

Reference TiO2 characterisationQuality of
study e.g.,
OECD/GLPMethod and
duration of
dosingStudy methodologynumbers, controls.

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

The mice

pellets for

Grissa et TiO2 NPs, anatase, No al. 5-12 nm (TEM, informatior (2016) XRD).	Internal exposure: quantitative in male Wistar rat tissues; methodology with important flaws.	statistica significa related i the level 100 and bw per d groups d and a sta significa related i brain TN mg/kg by 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	No infor
				modestly reduces neuronal function without affecting morphology.	

No information. al., 2021	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 infor
Naima et al., 2021	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 infori