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

In this guide

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Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment.

Third draft statement on the safety of Titanium Dioxide (E171) as a Food Additive - Annex D

Summary table of studies

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).	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.	NFR male mice (22/group) were administered either water (control) or 5 mg/kg bw E171 suspended in water.	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 quantification limit (0.03 µg/g).
	No sonification or deagglomeration to simulate realistic conditions.		Treatments were dripped slowly into the mice’s mouths, allowing each drop to be swallowed.	Ti concentrations in tissues were determined by single particle ICP-MS analysis.	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

Allergenicity

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.

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 concentra in the live (0.94 ± 0 µg/g tissu and large intestine ± 0.38 µg tissue) we significan higher in treated m compared controls. Ti concentra in the bra kidney, ar testes we below the quantifica limit (0.03 µg/g). Ti concentra in lungs, spleen, stomach, small inte were not statistical significan between treated ar control m

			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).	At the hig dose teste TiO2 had minimal impact on compositi the gut microbiot Alteration bacterial metabolit were obse from 10 n bw/d.
			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).	Doses of and 50 µg TiO2 significan promoted biofilm formation commens bacteria.
		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.	Impact of TiO2 on colonic epithelial function was determined by comparison of gene expression	There was reduced expressio
E171, anatase,				

					No evider of gross alteration immune-c physiolog inflamma at doses u 100 mg/k bw/d via t 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. 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.	Authors demonstr E171 upta by Peyer's patches, validating delivery model. Presence E171 part detected reflectanc confocal microscop (no quantifica of particle complete Weak sign observed the base Peyer's patches a and mid-doses. Hig signals

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.

		Statistical significan decreases GM-CSF plasma le (~30% in females) a plasma Ig (~12% in females a 9% in ma were obse at the hig dose compared controls.
		E171 accumula in the stomach w of several administe 1,000 mg E171 for 9 days.
E171 suspended in distilled water, sonicated for at least 10 minutes.	Sprague–Dawley rats (10/sex/group) were administered E171 by oral gavage at doses of 0, 10, 100 or 1,000 mg/kg	Ti concentra increased the colons both sexe administe 1,000 mg E171 compared with the control, w colonic, superoxid
E171 administered by		

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,**	**Results**

					In the high do treatment gr significant increases in t Ti tissue leve were found in spleen (0.036 0.009 vs. 0.0 0.008 µg/g fr weight; p ≤ 0 and ovaries (± 0.07 vs. 0. 0.04 µg/g fre weight; p ≤ 0
Tassinari et al., 2014	TiO2 nanoparticles (anatase, primary size 25 nm, BET surface area 45-55 m2/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)).	Sex-related histological alterations w observed at h dose levels in thyroid, adre medulla, adre cortex (fema and ovarian granulosa, wi general toxic Altered thyro function was indicated by reduced T3 (males). Testosterone levels increas high-dose ma and decrease females.

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:	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	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 • resorptions • live and dead fetuses	At 1,000 mg 1/kg per day, mean fetal se ratio and the means for ma and female fetuses per li were statistic significantly different fron control group means. Mean male fetuses: 7.2 Mean male fetuses contr group: 5.5 Test facility historical con group data ra 5.2 to 7.4. Mean female fetuses: 4.8 Mean female fetuses contr group: 6.7 Test facility historical con group data ra 5.8 to 8.3. Mean fetal se ratio of the 1 mguf-1/kg bw day group: 6

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 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.	Titanium was detected in th immune cells Peyer's patch Dendritic cell percentage w increased, observed day after exposur no effect at 1 days. No effects in spleen. Regulatory T and T-helper were significa decreased da after exposur and at 100 da for rats expos to E 171. Stimulation in vitro of immu cells isolated Peyer's patch had a decrea T-helper (Th) IFN-γ secretio and splenic Th1/Th17 inflammatory responses increased

				54 ten week old (25±2 g) adult female NMRI mice were divided into a control group which received vehicle (saline solution) orally and TiO2 NP group which received 100 mg/kg per day TiO2 NP solution orally.	There was a significantly decreased pregnancy ra (70% vs. 100 the control gr a 20% decrea litter size and increases in circulating oestrogen (2 and MDA (25
Karimpour *et al*., 2018	TiO2 NPs, anatase, 10–25 nm	One dose of TiO2 NP (100 mg/kg per day) or the test vehicle (control group) daily for 5 weeks NMRI = Naval Medical Research Institute	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	Degeneration reduction of follicles, cyst formation and impairment o follicular development the ovaries w observed in t TiO2 NPs gro but no quantitative d was provided Additionally a lower numbe oocytes was isolated from TiO2 NP grou well as a high percentage o development arrest before blastocyst sta after in vitro fertilisation. T authors prop that this coul have been an indirect effec TiO2 NPs thro

Khorsandi *et al.*, 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	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.	Body weight unaffected by treatment. Dose-depend decreases in weight were observed fro dose of 100 m bw per day. Mid- and high dose groups showed decr in serum and testicular testosterone levels, the diameter and volume of seminiferous tubules, the height of the spermatogen epithelium ar total Leydig c numbers how the total volu of the interst tissue increas

			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	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)	No statistical significant differences in general clinic signs, body weight, organ weights (abs and relative t body weight) macroscopic findings exce statistically significant decrease in f intake but no correlated decreased bo weight or boo weight gain d the study per of the female the high-dose group. No statistical significant differences in caesarean se parameters a fetal externa visceral examination. A small but statistically significant increase (4% observed in t number of ossification
Lee *et al*., 2019	TiO2 NPs P25 (15–24 nm)	OECD Guideline 414 (Pre-natal Toxicity Study)			

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 d not alter T-cel mediated mechanisms o immune contr
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.		Dietary E-171 did not induce inflammation peripherally o the GI tract. An increase w observed in th relative splee weight in 22.4 mg E-171/kg b per day + DM compared to n initiated anim and an increas in IL-17A in co (22.4 mg E 171/kg bw per day + DMH) a IL-12p70 in plasma (3.5 m E 171/kg bw p day + DMH), with no dose-related effects No changes w observed in spleen cellularity.

Akagi et al., 2023 – 28 Day Study	6 nm TiO2 nanoparticles	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 w observed in an group, and no treatment-related advers effects were observed in bo weight, urinalysis, haematology, serum biochemistry, organ weight. Histopathologi examination revealed TiO2 particles as depositions of yellowish-brow material. The particles observed in th gastrointestin lumen were al found in the nasal cavity, epithelium, an stromal tissue the 28-day study. Overall, No effects were observed afte repeated oral administration TiO2 with a crystallite size 6 nm at up to 1000 mg/kg bw/day regarding general toxicit

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

Results:

F0 - Dose-dependent marginal increase in TiO blood and urin concentration rats dosed wit 1000 mg/kg bw/day

No test item-related effects on sexual function or fertility in mal or females. No test item-relat pre- or postna loss observed

No test item-related thyroi hormone or haematologica effects..

No test item-related differences in splenic lymphocyte subpopulation distribution

					No test item-related effects behaviour or external appearance.
					No test item-related thyroid hormone effects.
					No test item-related effects on body weigh food consumption a water consumption.
					No test item-related effects on haematolo and biochemical parameters or urinalysis.
LPT, 2020	Test substance: Anatase E-171, 51% of particles 100 nm.	OECD Test Guideline	F0 satellite group: 0, 100, 300, and 1000 mg/kg bw/day over 10 weeks (prior to	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	No test item-related effects on thyroid and sexual hormo or sperm. No test item-related chang in bone marro

Immunotoxicity

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

					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 wa 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.	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,	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-

NCI, 1979 – see link ->	TR-097: Titanium Dioxide (CASRN 13463-67-7) (nih.gov) Titanium dioxide anatase Purity: 98%	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. is concluded tha under the conditions of th bioassay, titanium dioxide was not carcinogenic by the oral route fo Fischer 344 rats or B6C3F1 mice

Akagi et al., 2023 – 28 Day Study	6 nm TiO2 nanoparticles	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 wa observed in any group, and no treatment-related adverse effects were observed in bod weight, urinalysis, haematology, serum biochemistry, o organ weight. Histopathologic 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 i the 28-day study.	Overall, No effects were observed after repeated oral administration o TiO2 with a crystallite size o 6 nm at up to 1000 mg/kg bw/day regarding general toxicity

Akagi et al., 2023 – 90 Day Study	6 nm TiO2 nanoparticles	10 female and 10 male F344/DuCrlCrlj rats	TiO2 NPs with a crystallite size o 6 nm were examined in male and femal F344/DuCrlCrlj rats by repeate oral administration o 100, 300, and 1000 mg/kg bw/day (10/sex/group) for 90 days

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	10 female and 10 male C57BL/6J mice	The mic ad libitu pellets c 10 mg/g mg/g polyviny coated A pellets f

Grissa et al. (2016)	TiO2 NPs, anatase, 5–12 nm (TEM, XRD).	Internal exposure: quantitative in male Wistar rat tissues; methodology with important flaws.	There w statistic significa related the leve 100 and bw per groups and a st significa related brain TN mg/kg b TiO2 NP

Gerber et al., 2022	TiO2 NPs, average primary particle size of 26.2 ± 10.7 nm	The aim of the study was to investigate the effects of two common types of NP, titanium dioxide NP (TiO2 NP) 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 TiO2 NP is without effects, whereas chronic exposure only modestly reduces neuronal function without affecting morphology.

Ciu et al., 2021	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.
Naima et al., 2021	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.