Annex D

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This is a draft statement for discussion. This does not represent the views of the Committee and should not be cited.

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). 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 quantification limit (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-

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 concentr in the liv (0.94 ± µg/g tiss and larg intestine ± 0.38 µ tissue) w significa higher in treated r compare controls. Ti concentr in the br kidney, a testes w below th quantific limit (0.0 µg/g). Ti concentr in lungs, spleen, stomach small int were not statistica significa between treated a control r

				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 hi dose tes TiO2 had minimal impact o composi the gut microbio Alteratio bacteria metabol were obs from 10 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 µ TiO2 significa promote biofilm formatio commen 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 of key markers Muc2, Tjp1, Defb3, and Gzmb in colonic	There wa reduced expressi the colo mucin 2 a key compon the intes
Pinget et	E171, anatase, 30-300 nm. E171 was		Microbiota populations in		

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.	No evide of gross alteratio immune physiolo inflamm at doses 100 mg/ bw/d via diet. Authors demonst E171 up by Peyer patches, validatin delivery model. Presence E171 pa detected reflectar confocal microsc (no quantific of partic complet Weak sig observed the base Peyer's patches and mid- doses. H signals observed highest indicatin evidence 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.

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.	Statistica significa decrease GM-CSF plasma l (~30% i females) plasma l (~12% i females 9% in m were obs at the hi dose compare controls. E171 accumul in the stomach of severa administ 1,000 m E171 for days. Ti concentr increase the colo both sex administ 1,000 m E171 compare with the control, colonic, superoxi dismutas (SOD)-1 and fem and SOD

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

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)).	In the high treatment g significant increases ir Ti tissue lev were found spleen (0.0 0.009 vs. 0. 0.008 µg/g weight; p ≤ and ovaries ± 0.07 vs. 0 0.04 µg/g fr weight; p ≤ Sex-related histological alterations observed at dose levels thyroid, adr medulla, ac cortex (fem and ovarian granulosa, w general tox Altered thyr function wa indicated by reduced T3 (males). Testosteron levels incre high-dose m and decreas females. In the splee treated ani TiO2 aggreg and increas white pulp

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.	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. • resorptions. • live and dead fetuses. • fetal sex. • fetal weight. • fetal pathological external,	At 1,000 mg 1/kg per da mean fetal ratio and th means for m and female fetuses per were statist significantly different fro control grou means. Mean male fetuses: 7.2 Mean male fetuses con group: 5.5. Test facility historical co group data 5.2 to 7.4. Mean femal fetuses: 4.8 Mean femal fetuses con group: 6.7. Test facility historical co group data 5.8 to 8.3. Mean fetal s ratio of the mguf-1/kg b day group: (males/fem Mean contr group fetal ratio: 46%.

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	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.	Titanium wa detected in immune cel Peyer's pat Dendritic ce percentage increased, observed da after expos no effect at days. No effects i spleen. Regulatory and T-helpe were signifi decreased after expos and at 100 for rats exp to E 171. Stimulation vitro of imm cells isolate Peyer's pat had a decre T-helper (Th IFN-γ secret and splenic Th1/Th17 inflammato responses increased. With exposu TiO2 NP the an observed increase in percentage dendritic ce

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.	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. 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.	There was a significantly decreased pregnancy (70% vs. 10 the control a 20% decr litter size ar increases in circulating oestrogen (and MDA (2 Degeneratio reduction o follicles, cys formation a impairment follicular developmen the ovaries observed in TiO2 NPs gr but no quantitative was provide Additionally lower numb oocytes was isolated fro TiO2 NP gro well as a hi percentage developmen arrest befor blastocyst s after in vitr fertilisation authors pro that this co have been a indirect effe TiO2 NPs th

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 weigh unaffected treatment. Dose-deper decreases i weight were observed fr dose of 100 bw per day. Mid- and hi dose groups showed dec in serum ar testicular testosteron levels, the diameter ar volume of seminiferou tubules, the height of th spermatoge epithelium total Leydig numbers ho the total vo of the inter tissue incre

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) .	No statistica significant differences general clin signs, body weight, org weights (ab and relative body weigh macroscopi findings exc statistically significant decrease in intake but n correlated decreased l weight or b weight gain the study p of the fema the high-do group. No statistica significant differences caesarean s parameters fetal extern visceral examinatio A small but statistically significant increase (4% observed in number of ossification centres in t metatarsals both hindlir the fetuses

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 Dietary E-171 did not induce inflammation peripherally o 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 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.
	E-171 concentrations: 0 mg/kg* dose	7-day study: 4 groups of 5 animals (randomised based on weight),	No changes w observed in th percentage of CD103+ DC, CD4+ T helpe cells or total o

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. Histopatholog 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 T blood and ur concentratio rats dosed w 1000 mg/kg bw/day.

No test item-related effec on sexual function or fertility in ma or females. N test item-rela pre- or postn loss observe

No test item-related thyro hormone or haematologi effects.

No test item-related differences i splenic lymphocyte subpopulatio distribution.

No test item-related chan related to histopatholo examinations including the testis and epididymides and intestina

LPT, 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 (?).	No test item-related effec behaviour or external appearance. No test item-related thyro hormone effects. No test item-related effec on body weig food consumption water consumption No test item-related effec on haematol and biochem parameters o urinalysis. No test item-related effec on thyroid ar sexual hormo or sperm. No test item-related chan in bone marr or organ weights. No test item-related histopatholo effects in the high dose gr No test item-

Immunotoxicity

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

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	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. 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

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, nc tumours occurred in dosed groups a 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 boc 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 TiO_2, 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.