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

Reference	TiO_2 characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls.	Results	Notes, comments, other
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	**This study aimed to investigate whether TiO2 is deposited in the digestive system and internal organs and whether there are any molecular and cellular alterations associated with an inflammatory response.**

					small intestine were not statistically significant between treated and control mice.	
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.	No evidence of gross alteration of immune-cell physiology or inflammation at doses up to 100 mg/kg bw/d via the diet. 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).	**Author notes that '(OECD) guidelines for repeat dose oral toxicity testing (e.g., TG 408) do not mandate gavage but also allow for a test material to be incorporated in the diet or dissolved in drinking water, so any dietary approach could still be adopted within the OECD framework.'**

					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	**TiO_2 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	For ELISA, primary antibody for casein (Anti-casein rabbit antibody-cat # ab166596), primary antibody for β-lactoglobulin (Anti-LGB rabbit antibody-cat #	Significant enhancement in the allergenicity of milk proteins/ skimmed milk interacted with both E171 and food grade titanium dioxide nanoparticles.	No information

			and casein) in the presence of E171.	ab112893) and secondary anti-rabbit antibody (cat # 6721) were used. Quebon skimmed milk was used.	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	**TiO_2 characterisation**	**Quality of study e.g., OECD/GLP**	**Method and duration of dosing**	**Study methodology to include species, numbers, controls.**	**Results**	**Notes, comments, other**
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	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	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	NFR male mice (22/group) were administered either water (control) or 5 mg/kg bw E171 suspended in water. Ti concentrations in tissues were	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	**This study aimed to investigate whether TiO2 is deposited in the digestive system and internal organs and whether there are any molecular and cellular**

	realistic conditions.	National Institute of Health (code:42/2016-PR).	slowly into the mice's mouths, allowing each drop to be swallowed.	determined by single particle ICP-MS analysis.	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.	**alterations associated with an inflammatory response.**
Pinget et al., 2019	E171, anatase, 30-300 nm. E171 was dispersed in drinking water using sonication.	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	Male C67BL/6JAusb mice were exposed to E171 via drinking water at doses of either 0, 2, 10,	At the highest dose tested, TiO2 had minimal impact on the composition of the gut microbiota.	No information.

			calculated based on water intake measured per cage. Microbiota populations in fecal samples or the small intestine were examined through 16S rRNA sequencing. Canonical correspondence analysis (CCA) constrained to the 4 distinct TiO2 concentrations used.	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	Alterations in bacterial metabolites were observed from 10 mg/kg bw/d. Doses of 10 and 50 µg/ml TiO2 significantly promoted biofilm formation by commensal bacteria. There was reduced expression of the colonic mucin 2 gene, a key component of the intestinal mucus layer, and increased expression of the beta defensin gene, indicating that TiO2 significantly impacts gut	

				expression of key markers Muc2, Tjp1, Defb3, and Gzmb in colonic tissue of mice administered 0, 2, 10, or 50 mg TiO2/kg BW/day in drinking water (n = 5–8 mice per group). To investigate impact of TiO2 on colonic inflammation, flow cytometric analysis, gene expression determined by qPCR and tissue staining were used on mice administered 0, 2, 10, or 50 mg TiO2/kg BW/day in drinking water (n = 8–10 mice per group).	homeostasis. These changes were associated with colonic inflammation, as shown by decreased crypt length, infiltration of CD8+ T cells, increased macrophages as well as increased expression of inflammatory cytokines.	

				TiO2 impact on adaptive immune cell infiltration into the colon was investigated using gene expression via qPCR and flow cytometric analysis on mice treated with 0, 2, 10, or 50 mg TiO2/kg BW/day in drinking water (5-8 mice/group).		
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,	No evidence of gross alteration of immune-cell physiology or inflammation at doses up to 100 mg/kg bw/d via the diet. Authors demonstrate E171 uptake by Peyer's patches,	**Author notes that '(OECD) guidelines for repeat dose oral toxicity testing (e.g., TG 408) do not mandate gavage but also allow for a test material to be incorporated in the diet or**

				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.	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.	**dissolved in drinking water, so any dietary approach could still be adopted within the OECD framework.'**
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.	No information.

Han et al., 2020	E 171, 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–Dawley rat's tissues.	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 all rats at necropsy.	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. Ti concentration increased in the colons of both sexes administered 1,000 mg/kg	Liu et al., 2020

					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 (human stomach epithelial cell line) for 24 h, E171 (40 µg/mL) was located in the perinuclear region. The E171 treatment affected expression of ER stress-related proteins but did not induce cell death up to the used maximum	

					concentration (40 µg/mL). A gene profile analysis also showed that immune response-related microRNAs were most strongly affected by E171 exposure.	

Studies used to review the toxicokinetic and absorption of the nanoparticle form of TiO_2

Reference	TiO_2 characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls,	Results	Notes, comments, other
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	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)	In the high dose treatment group, significant increases in total Ti tissue levels were found in spleen (0.036 ± 0.009 vs. 0.046 ± 0.008 µg/g fresh weight; $p \leq 0.05$) and ovaries (0.28	No information.

		86/609/EEC (EEC 1986).		(vehicle only (distilled water).	± 0.07 vs. 0.12 ± 0.04 µg/g fresh weight; p ≤ 0.01). Sex-related histological alterations were observed at both dose levels in thyroid, adrenal medulla, adrenal cortex (females) and ovarian granulosa, without general toxicity. Altered thyroid function was indicated by reduced T3 (males). Testosterone levels increased in high-dose males and decreased in females. In the spleen of treated animals TiO2 aggregates	

					and increased white pulp (high-dose females) were detected, even though Ti tissue levels remained low reflecting the low doses and the short exposure time.	
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	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.	At 1,000 mg uf-1/kg per day, mean fetal sex ratio and the means for male and female fetuses per litter were statistically significantly different from the control group means. Mean male fetuses: 7.2 Mean male fetuses control group: 5.5 Test facility historical control	No information.

	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: irregular. 5) Rutile (11% nano) (pg-2), d50=195 nm d50=165 nm Methods: XSDC and			Necropsy: • Gross examination of the dam. • Counting of corpora lutea. • Implantation sites. • Resorptions • live and dead fetuses. • Fetal sex. • Fetal weight. • Fetal pathological external, visceral and skeletal examinations for abnormalities.	group data range: 5.2 to 7.4. Mean female fetuses: 4.8 Mean female fetuses control group: 6.7 Test facility historical control group data range: 5.8 to 8.3. Mean fetal sex ratio of the 1,000 mguf-1/kg bw per day group: 60% (males/females) Mean control group fetal sex ratio: 46% Test facility historical control group data range: 43% to 53%. A few incidental changes in body weight and feed intake were noted, no other changes were	

	TEM respectively. Shape: irregular.				observed in the dams or the fetuses.	
Gao et al., 2012	The mean diameter of TiO2 NP ranged from208 to 330 nm (mainly 294 nm).		Nanoparticulated anatase TiO2 was prepared via controlled hydrolysis of titanium tetrabutoxide. The particle sizes of both the powder and the nanoparticles suspended in 0.5% (w/v) HPMC solution were measured using TEM and mean particle size was determined by measuring more than 100 randomly-sampled particles.	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. 90-Day Study: Two groups (N = 30) including one group of control animals. Treatment: 0.5% (w/v) HPMC) 10 mg/kg BW TiO2	Titanium accumulated in the ovaries, while no titanium was detected in non-exposed mice. TiO2 NPs exposure resulted in increased content of Ca, Na, K, and Zn, and decreased content of Mg, Cu, and Fe in the ovary ($P < 0.05$). TiO2 NPs exposure resulted in significant decreases in the mating rate, pregnancy rate, number giving birth, and number of fetuses ($P < 0.05$).	No information.

				NPs administered by intragastric administration daily for 90 days. After 90 days, fertile females were tested for fertility by caging with males of known fertility (10 males and 10 females). Blood samples from the eye, serum and ovaries were analysed.	Twenty-eight days after birth, the survival rate and body weight of young mice were significantly decreased compared with control mice ($P < 0.05$). TiO_2 NPs exposure caused significant increases in E2 and FSH, and reduction of P4, LH, and T ($P < 0.05$); however, the levels of PRL and SHBG had no significant changes ($P > 0.05$). Significant histopathologic changes were observed in the ovarian tissue compared with the control;	

					specifically, the ovarian tissue had abnormal pathologic changes, including ovarian atrophy, disturbance of primary and second follicle development, irregular arrangement of cells, and a shapeless follicular antrum. Significant ROS production and an increase in 8-OHdg in the ovary caused by TiO2 NPs were observed ($P < 0.05$ or 0.01). TiO2 NPs conglomerates in the cytoplasm and nuclei of ovarian cells were observed.	

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.	Results identified that TiO2 NPs can cross the blood–testis barrier and accumulate in the testes, resulting in the reduction of relative testicular mass in addition to pathological changes in the testis and significantly decreased sperm concentrations, sperm motility, and increased abnormal sperm concentrations and sperm lesions in the cauda epididymis. Gene expression in 142 genes of known functions were significantly changed by long-term exposure to TiO2 NPs, affecting steroid and hormone	

					metabolism, spermiogenesis and hormone levels.	
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	Titanium was detected in the immune cells of Peyer's patches. Dendritic cell percentage were increased, observed days after exposure but no effect at 100 days. No effects in the spleen. Regulatory T cells and T-helper cells were significantly decreased days after exposure and at 100 days for rats exposed to E 171. Stimulation in vitro of immune cells isolated from Peyer's patches	Peyer’s patches = clusters of subepithelial, lymphoid follicles found in the intestine. Th = T-helper cells.

				drinking water for 100 days. Control animals (n = 12) received water only. Flow cytometry and cytokine assays were assessed for gut inflammation and ACF. Series Three: untreated rats (n = 4) were evaluated for ex-vivo cytotoxicity and proliferative assays on isolated immune cells. E-171 particle agglomeration was assessed in the luminal contents of the jejunum and colon collected from 4 rats 4 h after a single dose of 10 mg/kg was delivered.	had a decrease in T-helper (Th)-1 IFN-γ secretion and splenic Th1/Th17 inflammatory responses increased. With exposure to TiO2 NP there was an observed increase in the percentage of dendritic cells in Peyer's patches with no decrease in the percentage of Tregs. E 171 exposure (1 week) did not initiate intestinal inflammation, but E 171 treatment (100-day) showed colon microinflammation - significantly increased IL-1β, IL-8 and TNF-α expression in the	

					colon and increased IL-10.	
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	There was a significantly decreased pregnancy rate (70% vs. 100% in the control group), a 20% decrease in litter size and increases in circulating oestrogen (20%) and MDA (25%). Degeneration and reduction of follicles, cyst formation and impairment of follicular development in the ovaries was observed in the TiO2 NPs group but no quantitative data was provided. Additionally a lower number of oocytes was isolated from the	

				were housed with 3 male mice for 11 days. The percentage of pregnancy and numbers of newborns were evaluated.	TiO2 NP group as well as a higher percentage of developmental arrest before the blastocyst stage after in vitro fertilisation. The authors proposed that this could have been an indirect effect of TiO2 NPs through the generation of increased ROS levels.	
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.	Body weight was unaffected by treatment. Dose-dependent decreases in testis weight were observed from a dose of 100 mg/kg bw per day. Mid- and high-dose groups showed decreases in serum and	No information.

				Testicular testosterone levels, testis weight, total volumes of testis, seminiferous tubules, interstitial tissue and total Leydig cell numbers were measured.	testicular testosterone levels, the diameter and total volume of seminiferous tubules, the height of the spermatogenic epithelium and total Leydig cell numbers however the total volume of the interstitial tissue increased.	
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,	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	No statistically significant differences in general clinical signs, body weight, organ weights (absolute and relative to body weight), macroscopic findings except a statistically significant decrease in food intake but no correlated decreased body	No information.

			300 and 1000 mg/kg with a dose volume of 10 mL/kg.	(total test animals: 16).	weight or body weight gain during the study period of the females of the high-dose group. No statistically significant differences in caesarean section parameters and fetal external and visceral examination. A small but statistically significant increase (4%) was observed in the number of ossification centres in the metatarsals of both hindlimbs of the fetuses of 100 mg/kg bw per day group which may have been incidental.	

Aberrant Crypt Foci (ACF) as a marker for carcinogenicity

Reference	TiO_2 characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls.	Results	Notes, comments, other
Blevins et al., 2019	E 171, anatase, 110–115 nm (SEM), 36% particles < 100 nm.	No information.	E-171 concentrations: 0 mg/kg* dose (22.3 ± 1.2, 23.7±1.8 ppm) 40 ppm dose (59.6 ±1.1, 61.0±2.6 ppm) 400 ppm dose (384 ± 8, 387±13 ppm) 5000 ppm dose (4310 ± 132, 4610±160 ppm).	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-	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. 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	No information.

				week 10 of the 100-day study. 7-day study: 4 groups of 5 animals (randomised based on weight). Total food and water consumption were calculated at the end of the study. Body weights measured at the start and end of the 7-day exposure period. 100-day study: 8 groups of 16 animals. Groups 1-4 were dosed with 180 mg/kg BW dimethylhydrazine dihydrochloride (DMH · 2HCl) in 1.5% EDTA-0.9% NaCl, pH 6.5 by intraperitoneal	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. No changes were observed in the percentage of CD103+ DC, CD4+ T helper cells or total or activated Treg in peripheral blood, spleen or Peyer's patches in animals exposed to E-171 + DMH compared to animals treated with only DMH. No treatment related histopathological	

				injection (the highest dose that showed no signs of toxicity in pilot studies). Animals in groups 5-8 were given a dose of 1.5% EDTA-0.9% NaCl, pH 6.5. by intraperitoneal injection. Seven days post-injection, feeding of the test material was commenced at 0, 40, 400, or 5000 ppm E-171 in groups 1-4 and 5-8 for 100 days.	changes were observed in the duodenum, jejunum, ileum, spleen, liver, lung and testes in animals exposed only to E-171. Rats treated with DMH only and those which received E-171 in the diet after the initiation displayed several histopathological abnormalities. A significant surface area of the epithelial surface of the sampled colon (proximal, middle and distal) was obscured when observed by light microscopy therefore the	

					entire surface of the colon samples for ACF and ABC could not be examined. No change in the number of ACF and ABC were observed due to E-171 exposure alone.	
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	No information.

					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, accumulation of titanium in the liver, kidneys, and spleen, abnormality of colonic crypts, and induction of DNA strand	

					breaks and chromosomal aberrations.	

Reproductive toxicity

Reference	TiO_2 characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls.	Results	Notes, comments, other
TDMA, 2020 – Main Study.	Test substance: Anatase E-171, median particle diameter 99.9 (± 2.0 nm), 51% of particles < 100 nm. Dietary particle size: 109.2 (± 1.4) to 113.7 (± 4.9 nm), 31-43% of particles < 100 nm.	OECD Test Guideline 443.	Dosages (by oral diet, dependent on endpoint for each cohort): F0, F1-1A and 1B - 0, 100, 300, and 1000 mg/kg bw/day over 10 weeks (prior to mating and up to the end of weaning periods). F1-2A and 2B: indirectly exposed to test item via milk of feed-dosed rats. Additional background levels of TiO2 in feed were estimated to be approximately 1.4 mg/kg bw/day.	96 male and 96 female CD® (Sprague Dawley) IGS Rat (Crl:CD(SD). Test Grouping Sizes: F0 – 4 groups, 20 male, 20 female per group. F1-1A and 1B – 20 male, 20 female F1-2A, 2B and 3– 4 groups, 10 male, 10 female.	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.	**KLH (Keyhole Limpet Haemocyanin)**

			Diet mixtures were prepared weekly. Food intake was monitored and volumes of dosed feed was adjusted according to previous consumption weekly. Administration of KLH and cyclophosphamide KLH via intravenous bolus injection in F3 cohort.	Endpoint Groupings: F1-1A and 1B - reproductive & developmental toxicity. F1-2A and 2B - developmental neurotoxicity. F3 - developmental immunotoxicity Test Item Exposure: F0 - Exposure to test item was from 10 wks prior to mating until F1 generation weaning. F1 – Exposure to test item was from weaning to PND 4/PND 8 of F2 generation.	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 ACF. **Reproductive & developmental toxicity** **F1-1A and 1B**	

				F2 – Exposure from birth until PND 4 or PND 8 (via milk).	No test item-related effects on sexual function or fertility in males (F1-1A – sexual hormones and maturation and sperm) or females (F1-!B - sexual maturation and hormones, number and length of estrous cycles, follicle number or corporea lutea). No treatment-related pre- or postnatal loss. No external or internal abnormalities detected in pups. No test item-related effects on growth or sexual development. No test item-related differences in	

					body weight or food consumption. No test item-related thyroid hormone or haematological effects reported at any dose for F1-1A cohort. No test item-related differences in weight or histopathology (spleen, thymus, lymph nodes, bone marrow, white blood cell count, and splenic lymphocyte subpopulation distribution in F1-1A. No test item-related effects on pathology or histopathology.	

					There was a statistically significant decrease in the antigen specific IgM level was measured at the highest dose tested (1,000 mg/kg bw/d) in males only (–9%) and without an apparent dose-response. No dose-dependent increase in TiO2 blood concentration in rats up to 1000 mg/kg bw/day. Observation of pale faeces not of toxicological relevance. **Developmental neurotoxicity F1-2A and 2B**	

					No test item-related pre- or postnatal loss observed. No external or internal abnormalities detected in pups. No test item-related effects on growth or sexual development. No test item-related differences in body weight or food consumption. No test item-related effects on neurofunctional endpo**ints (F1-2A).** Dose-dependent elevations of blood TiO2 levels were found	

					exceeding 300 mg/kg. No test item-related effects on pathology or histopathology. Observation of pale faeces not of toxicological relevance. **Developmental immunotoxicity F3** No test item-related differences in body weight or food consumption. No mortality and no test item-related effects reported at any dose for any generation. No ACF were found in the	

					colons of animals in any dose group. Observation of pale faeces not of toxicological relevance.	
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: ACF.	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. No test item-related effects on haematology and biochemical parameters or urinalysis.	No information.

					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 of aberrant crypt foci (ACF) in rat colons. Satellite animals of F1 used as the positive control for the KLH assay (at a different time and ages from F1-3 cohort). No additional	

					controls were tested. No test animals died prior to sacrifice. Observation of pale faeces not of toxicological relevance.	

Immunotoxicity

Reference	TiO_2 characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls.	Results	Notes, comments, other
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.	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	No information.

			Quantitative analysis in Sprague-Dawley rat's tissues.	Ti concentrations were measured in the colons, kidneys, and spleens harvested from all rats at necropsy.	compared to controls. E171 accumulation in the stomach wall 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 (human stomach epithelial cell line) for 24 h, E171 (40 µg/mL) was located in the perinuclear region. The E171 treatment affected expression of ER stress-related proteins but did not induce cell death up to the used maximum concentration (40 µg/mL). A gene profile analysis also showed that immune response-related microRNAs were most strongly affected by E171 exposure.	

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	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.	No information.

				control rats and mice of each sex were at risk for development of late-appearing tumors.		
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	No information.

					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, accumulation of titanium in the liver, kidneys, and spleen, abnormality of colonic crypts, and induction of DNA strand breaks and chromosomal aberrations.	
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	No mortality was observed in any group, and no treatment-related adverse effects were observed in body weight,

					oral administration of 100, 300, and 1000 mg/kg bw/day (10/sex/group) for 90 days.	urinalysis, haematology, serum biochemistry, or organ weight. In addition, they were observed in Peyer's patches in the ileum, cervical lymph nodes, mediastinal lymph nodes, bronchus-associated lymphoid tissue, and trachea in the 90-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,

						accumulation of titanium in the liver, kidneys, and spleen, abnormality of colonic crypts, and induction of DNA strand breaks and chromosomal aberrations.

Neurotoxicity

Reference	**TiO_2 characterisation**	**Quality of study e.g., OECD/GLP**	**Method and duration of dosing**	**Study methodology to include species, numbers, controls.**	**Results**	**Notes, comments, other**
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 were fed ad libitum with food pellets dosed with 10 mg/g TiO2, 2 mg/g polyvinylpyrrolidone-coated Ag or control pellets for 28 days.	The neurotoxicity of TiO2 and Ag NMs, applied in food pellets, in male and female C57BL/6 J mice in a 28-day oral exposure study with or without a 14-day post-exposure recovery period. No major neuropathological changes regarding

						neuroinflammation in biochemical and immunohistochemical analyses could be observed and behavioural changes in anxiety and cognition were absent. However, in the Ag NM exposed mice motor performance effects were observed by the rotarod test that differed between sexes. The female mice that were exposed to Ag NM for 28 days, showed a consistent diminished motor coordination and increased cortical activity of specific tyrosine kinases. Female mice that were exclusively investigated in a subsequent toxicokinetic study also revealed whole

						brain levels of Ag that steadily increased during the 28 days of exposure and persisted up to 4 weeks post-exposure. Our study demonstrates that subacute exposure to foodborne TiO2 and Ag NMs does not cause marked neurotoxicity in mice. However, our toxicokinetic and specific toxicodynamic findings with Ag NMs indicate that long-term oral exposures to this nanomaterial may cause adverse effects on the central nervous system in a sex dependent manner.
Grissa et al. (2016)	TiO2 NPs, anatase, 5–12 nm (TEM, XRD).	No information.	Internal exposure: quantitative in male Wistar rat tissues; methodology	No information.	There was a statistically significant dose-related increase in the level of NO in 100 and 200 mg/kg	No information.

			with important flaws.		bw per day TiO2 NPs groups observed and a statistically significant dose-related increase in brain TNF-α in 200 mg/kg bw per day TiO2 NPs group.	
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	No information.	No information.

				only modestly reduces neuronal function without affecting morphology.		
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 information.	No information.
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	Acute intravenous injection of TiO2-NPs impaired behaviour performances through brain biochemical and structural changes and precautions	No information.	No information.

			cognitive and emotional tests using Morris water maze and elevated plus maze.	should be taken to their usage in food additive and medical applications.		