Annex B - Statement on the safety of Titanium Dioxide (E171) as a Food Additive

In this guide

In this guide

- 1. <u>Executive Summary Statement on the safety of Titanium Dioxide (E171) as</u> <u>a Food Additive</u>
- 2. <u>Introduction Statement on the safety of Titanium Dioxide (E171) as a Food</u> <u>Additive</u>
- 3. <u>Titanium Dioxide Statement on the safety of Titanium Dioxide (E171) as a</u> <u>Food Additive</u>
- 4. Absorption, Distribution, Metabolism and Excretion (ADME)
- 5. <u>Review of toxicity for endpoints identified by the COT</u>
- 6. <u>Reproductive and Developmental Toxicity Statement on the safety of</u> <u>Titanium Dioxide (E171) as a Food Additive</u>
- 7. Aberrant Crypt Foci (ACF) as a potential biomarker for carcinogenicity
- 8. <u>Genotoxicity Statement on the safety of Titanium Dioxide (E171) as a Food</u> <u>Additive</u>
- 9. Inflammation and Immunotoxicity Statement on the safety of Titanium Dioxide (E171) as a Food Additive
- 10. <u>Neurotoxicity Statement on the safety of Titanium Dioxide (E171) as a Food</u> <u>Additive</u>
- 11. Establishment of a Health-Based Guidance Value (HBGV) Statement on the safety of Titanium Dioxide (E171) as a Food Additive
- 12. Exposure Assessment Statement on the safety of Titanium Dioxide (E171) as a Food Additive
- 13. <u>Assumptions and uncertainties Statement on the safety of Titanium Dioxide</u> (E171) as a Food Additive
- 14. <u>Risk characterisation Statement on the safety of Titanium Dioxide (E171) as</u> <u>a Food Additive</u>

- 15. <u>Conclusions Statement on the safety of Titanium Dioxide (E171) as a Food</u> Additive
- 16. <u>Abbreviations Table Statement on the safety of Titanium Dioxide (E171) as</u> <u>a Food Additive</u>
- 17. <u>References Statement on the safety of Titanium Dioxide (E171) as a Food</u> <u>Additive</u>
- 18. <u>Annex A Statement on the safety of Titanium Dioxide (E171) as a Food</u> <u>Additive</u>
- 19. Annex B Summary table of studies
- 20. <u>Annex C Statement on the safety of Titanium Dioxide (E171) as a Food</u> <u>Additive</u>
- 21. <u>Annex D Statement on the safety of Titanium Dioxide (E171) as a Food</u> <u>Additive</u>

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

Deference	TiO2	Qualit	y of
Reference	characterisation	study	e.g.,
		OECD/	GLP

Mothad	Study	
method	methodology to	
and	include species,	Results
duration of	numbers,	
aosing	controls,	

			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	Titanium detected immune o Peyer's pa Dendritic percentag increased observed after expo
			and cytokine assays, tissue	no effect a days.
			and gut	No effects spleen.
		Series One Dosage: 200 µ L with TiO2	permeability measurements were conducted.	Regulator and T-hel were sign
		NM-105, E171 (10 mg/kg of BW/day) or water for 7	Series Two: rats (n = 11 to 12 per group) were treated or not with 1,2-	decreased after expo at 100 da rats expos 171.
1) F 171 enstace		days by gavage.	dimethylhydrazine (DMH) to induce	Stimulation of immun
1) E 171, anatase, 20–340 nm (118 nm) (TEM); 44.7% particles 100 nm;	OECD	Series Two Dosage: E- 171 at 200 µ g or 10 mg/kg of	colon carcinogenesis and exposed to E- 171 at 200 µ g or 10 mg/kg of BW/day via	isolated fr Peyer's pa had a dec T-helper (γ secretio
2) TiO2 NPs (NM- 105), anatase/rutile, 15-24 nm.		BW/day via drinking water for 100 days (with or	drinking water for 100 days. Control animals (n = 12) received water	inflammat responses increased
		without DMH treatment).	only. Flow cytometry	With expo TiO2 NP th an observ
		Series Three Dosage: No	and cytokine assays were	increase i percentag

Bettini et

al., 2017

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.	in the live 0.57 μg/g and large (1.07 ± 0 tissue) we significan in treated compared controls. Ti concen in the bra kidney, ar were belo quantifica (0.03 μg/g Ti concen in lungs, s stomach, intestine statistical significan treated ar mice
-----------------------------	-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

					No eviden gross alte immune-c physiolog inflammat doses up mg/kg bw diet.
Riedle et al., 2020	E171, anatase, 119 nm.	N/A	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	Authors demonstra uptake by patches, w the delive Presence particles of by reflecta confocal microscop quantifica

particles completed Weak sign observed base of Pe patches a mid-doses

weeks.

signals of highest do indicating of dose-re

			Mice (4 per group). Dosage: single dose (200 µl) of either		Small inter TiO2 absorpeaked at jejunal an villi and re to basal le h and und at 4 h but present at the jejuna patches. Colon: Lov absorption
Comera et al. 2020	Food grade TiO2 (E171) 95% anatase.	European legislation (Council Directive 2010/63/UE) and French Decree 2013-118- compliant.	E171 at 40 mg/kg of body weight (BW) or 200 µl of vehicle (water) by intragastric gavage. In addition, in some experiments, the gavage solution from sonicated E171 particles was equilibrated in 30% corm oil and vortexed before oral delivery.	Adult C57BL/6 mice (12-18 weeks). Animals were terminated at 2, 4, 8, and 24 hours to recover the intestine.	Blood: TiC particles v detected a 8-hours per treatment 30 minute exposure the prese absence of pharmaco inhibitors paracellul junction (° permeabi absorption jejunal vil decreased (p 0.001 v control) in presence triaminop Other inhi had no sig effect.

Absorptio goblet cel

Dosage: 100–250 ppm.	Method: A defined model intestinal bacterial community.	impact or productio only a min on fatty a profiles w observed
	Dosage: 100–250 ppm.	Dosage: 100–250 ppm. Method: A defined model intestinal bacterial community.

	E171 in	
Proquin	combination with	
et al.	azoxymethane	
2018	(AOM)/dextran	N/A
	sodium sulphate	
	(DSS) vs E171	
	only.	

Dosage: 5 mg/kg bw per day of E171 by BALB/c mice. gavage for 2, 7, 14, and 21 days. productio only a min on fatty a profiles w observed limited ef bacterial communit E171 indu downregu genes inv the immu

At these l concentra

system will indicative impairme Additional signalling involved i variety of cancer ind colorectal were mod and effect observed indicated potential

associatic oxidative

TiO2-only

Decreased expressio protein TJ observed rats only of to E171 (S mg/kg/we shorter lu telomeres

This study no oxidati damage in or lung ar changes i DNA repa of oxidati damage in lung.

Jensen et al. 2019 Vegetable carbon (E153) and foodgrade titanium dioxide (E171), N/A mean TiO2 particle size of 270 nm.

Dosage: 10 weeks by oral gavage once a Rats. week.

Dosage: Four grades of TiO2 (200 ppm) or control (0 ppm) via the diet for 7 days followed by a control diet for 1, 24, or 72	Male and female Sprague-Dawley rats were given TiO2 by the diet equivalent to 30 mg/kg bw/day for 7 days. Animals were then terminated post-feeding at 1, 24 and 72 hours	0.1-0.3 m Ti in blood 0.04 mg/L samples. Ti in urine equal to 2 dose/L an LOQ. Ti in faece found to b
	Dosage: Four grades of TiO2 (200 ppm) or control (0 ppm) via the diet for 7 days followed by a control diet for 1, 24, or 72	Dosage:Four gradesMale and femaleof TiO2 (200Sprague-Dawleyppm) orrats were givencontrol (0TiO2 by the dietppm) via theequivalent to 30diet for 7mg/kg bw/day fordays7 days.followed byAnimals werea controlpost-feeding at 1,24, or 7224 and 72 hours.

hours.

Ti in kidne and musc below LOI mg/kg ww

main rout

excretion

No differe absorption found bet different

TiO2.

Absorption, Distribution, Metabolism and Excretion (ADME) - non-E171/Nanoparticle animal studies

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

	7)				At 1,000 1/kg pe mean fe ratio an means t and fem fetuses were
	1) anatase/rutile				statistic
	(89/11%) (uf-1),				significa
	d50=43 nm			Three studies	differen
	d50=23 nm.			(Group size n=22):	group n
	Methods: XSDC and TEM			pregnant Sprague-Dawley	Mean m fetuses:
	Shape: Irregular.			rats, (Cri:CD(SD)) exposed to TiO2 (uf-	Mean m
				1, uf-3 and pg-1) by	fetuses
	2) anatase (100% nano) (uf-		Sterile water- based TiO2	gavage on Gestational Days	group: 5
	2) d50= 42 nm d50=19 nm.		sample formulations	6-20.	Test fac historica
	Methods: XSDC and TEM respectively. Shape: Irregular.		were administered by oral gavage to time-mated	Three additional studies (Group size n=22-23) pregnant Wistar rats exposed to TiO2 (uf-2 and	group d range: 5 7.4. Mean fe
Warheit, Boatman	3) rutile (100% nano) (uf-3), d50=47 nm	OECD	rats from the time of approximate implantation	pg-2) by gavage from Gestational Days 5 to 19.	Mean fe fetuses
and Brown, 2015	d50=22 nm Methods: XSDC and TEM respectively.	Guideline 414.	until the day prior to expected parturition.	 Gross examination of the dam. 	Test fac historica group d
	Shape: rod-like.		Dose levels:	Counting of	range: 5 8.3.
	 4) anatase (27% nano) (pg- 1), d50=153 nm d50=120 nm Methods: XSDC and TEM 		0, 100, 300 or 1,000 mg/kg bw per day. Dosage volume: 5	 Implantation sites, Resorptions, Live and dead fetuses, Fetal sex 	Mean fe ratio of 1,000 m bw per group: 6

In the h treatme group, significa increase total Ti levels w found in (0.036 : vs. 0.04 0.008 μ weight; 0.05) ar ovaries 0.07 vs 0.04 µg weight; 0.01).

Sex-rela histolog alteratio observe both do in thyro adrenal medulla adrenal (female ovarian granulo without toxicity

Altered function indicate reduced (males) Testoste levels in in highmales a decreas

Tassinari et al., 2014	TiO2 nanoparticles (anatase, primary size 25 nm, BET surface area 45- 55 m ² /g, purity 99%).	All experiments on animals were performed according to the European Community Council Directive 86/609/EEC	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)).
	99%).	Council Directive 86/609/EEC (EEC 1986).	dose of 0, 1, 2 mg/kg body weight per day.	dose, (1 mg/kg bw), and controls (CTRL) (vehicle only (distilled water)).

Ammendolia et al. 2017 Nano-sized titanium dioxide (anatase, primary size 25 nm, BET N/A surface area 45–55 m2/g, purity 99%).

TiO2 NPs at 2 mg/kg bw per day for five days in male and female rats.

Nanopa deposit intestin and inc serum testoste levels. was no of oxida stress o alteratio concent of TiO2 howeve treatme associa testoste Insulin-Growth showed increase prolifera

Followir exposul levels ir liver an were or occasio above t and was detecte lymph r low leve Followir

intraver exposul distribu observe all tissu kidney, spleen, brain, tl and reprodu organs liver ide as the r target.

Recove hours p exposul 64-95% 108% respect male ar female

The ma relative decreas TiO2 up days po exposul 26%.

	TiO2 NPs (sizes	
	NM-100, NM-101,	
Geraets et	NM-102, NM-103,	
al. 2014	and NM-104) with	N/
	differing particle	
	sizes and	

Ά structure.

Dosage: Oral and intravenous administration dosing in rats. of a single or five repeated doses.

TiO2 nanoparticle kinetics were investigated using intravenous injection and oral

For orally dosed rats, liver, spleen and lymph nodes were targeted for analysis.

GIT and seconda organ transloc were siz depend

Larger nanopa exposui

showed deposit

liver, ki

Hendrickson 2 test items TiO2 et al. 2016 NPs (5-10 nm and 20-25 nm respectively). Dosage: Intragastric administration of TiO2 NPs (1 of 2 test items) for 28 Male rats. days at a dose of 250 mg/kg of body weight per day.

spleen, small in (0.01– 0 of organ Smaller nanopa exposur resulted deposite brain, lu heart, li kidneys small in testicles

(0.004µg/g of

blood

TiO2 NF found in small in mucosa and splo

TiO2 NF

Hendrickson

et al. 2020 TiO2 NPs

N/A

Dosage: AModel: Asingle dosePhysiological modelsuspension ofdesigned to mimicTiO2 NPs (250the intestinal lumenmg/kg of bodyof an experimentalweight).animal.

resulted differen change cellular ultrastru in the endopla reticulu mitocho extension the peri spaces caused like stru to appe

The mo sensitiv was not the sple

	Highest
	accumu
	occurre
	liver aft
	day (95
	then the
	spleen (
	carcass
	skeletor
	and blo
	(0.4%).
	NPs we
	detecte
	other or
0	levels lo
	than the

TiO2 NF blood d quickly after ex

Organs tissue N were st day-28.

Kreyling et	TiO2 anatase NPs.	
al. 2017a	Median	N/A
	agglomerate size:	
	70 nm.	

Dosage: 40-400 µg/kg bw single intravenous dose in aqueous suspension.

Female Wistar rats. Clearance and biokinetics were observed from 1hour post-dosage to 4 weeks.

Kreyling et al. 2017b	TiO2 NPs.	N/A	Dosage: Oral dosage of a single dose of an aqueous TiO2 NP suspension at 30–80 µg/kg bw.	Female Wistar-Kyoto rats. Assessed 1 h, 4 h, 24 h and 7 days post-oral exposure.	0.6% of adminis dose pa gastro- intestin after 1 0.05% of dose we distribut the bod days dis across the followin organs: liver (0. lungs (0 ng/g), k (0.29 ng brain
					brain (0.36 ng spleen (ng/g), u (0.55 ng bone de

(0.98 ng

Faecal e was cor after 4-

				6–7-week-old male B6C3F1 mice.	
Sadiq et al. 2012	TiO2 NPs.	N/A	Dosage: 1) Intravenous 0.5, 5.0, and 50 mg/kg TiO2 NPs. 2) Intravenous three daily doses of 50 mg/kg TiO2 NPs Ti levels in bone marrow measured after 4, 24, and 48 hours of the last treatment.	In vivo micronucleus and Pig-a (phosphatidylinositol glycan, class A gene) mutation assays using TiO2 NPs to evaluate genotoxicity. Blood: Samples taken one day before the treatment and on Day 4, and Weeks 1, 2, 4, and 6 after the beginning of the treatment. Pig-a mutant frequencies were determined at Day -1 and Weeks 1, 2, 4 and 6, percent micronucleated- reticulocyte frequencies were measured only on	Blood re No incre Pig-a m frequen %MN-RI Tissue r Ti NPs p at 4 hou exposur %RETs reduced treated on Day depend which s cytotox TiO2-NF bone m No evid genotox

Day 4.

Absorption, Distribution, Metabolism and Excretion (ADME) - E171 and non-E171/Nanoparticle Human Studies

Reference	TiO2 characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls,	Results
Pele et al. 2015	Pharmaceutical/food grade TiO2, anatase, 50-250nm.	This study was conducted based on ethical approval under EC01/037.	Dosage: A single dose of 100 mg TiO2.	Test subjects: Humans with normal intestinal permeability. Blood samples were collected at between 0.5 to 10 h post-oral exposure. Blood samples were analysed for visual TiO2 reflective particles using dark field microscopy.	Early absorption occurred by 2 hours with a peak maximum at 6 hours. Following oral dosing. This mirrors the results of a previous study by Bockmann et al (2000) which the authors were attempting to replicate.

Guillard et al. 2020	Basal Ti level in human placenta study.	N/A
	TiO2 with 55% NPs,	
	20 to 440 nm.	

Samples		
were		All placenta
taken of		contained
plancenta		TiO2 at 0.01
and		to 0.48 mg/kg
meconium	TiO2 in human	of tissue with
from	placentas was	the majority
human	analysed by	below 100nm
babies	ICP-MS and	in size (over
(n=22)	STEM coupled	50%).
and	to EDX	Meconium
tested for	spectroscopy.	samples also
TiO2 and		contained
other		TiO2 between
metals		0 02-1 5
and trace		ma/ka
elements.		

					7 of the 15 livers sampled contained TiO2 and 13 of 15 of spleen samples contained the same.
					Particle sizes respectively for liver and spleen:
Heringa et al.	Post-mortem analysis of human			High resolution ICP- MS was used to detect TiO2 in the liver	85–550nm and 85–720nm with 24% 100 nm in size.
2018	liver and spleen TiO2 analysis.	N/A	N/A	and spleen in 15 deceased humans (9 female and 6 male).	Particle mass concentration for liver and spleen respectively:
					To 0.3 mg t itanium/kg tissue and 0.01 to 0.4 mg titanium/kg tissue.
					Average concentration in liver and spleen samples:
					40 ng/g and 80 ng/g.

		Findings of between 0.01 to 2.0 mg Total Ti/kg with median values (mg Ti/kg):
		Liver = 0.02,
		Spleen = 0.04,
		Kidney = 0.05,
	15 humans	Jejunum = 0.13,
Postmortem tissue	sampled, 8 female and 7	lleum = 0.26.
deceased persons	male aged 64-	Particulate
for the presence of TiO2. Detected particle sizes were in the	Postmortem liver, spleen, kidney,	TiO2 were observed from 0.01 to 1.8 mg Ti/kg with median
range of 50–500 nm, with a mode of 100–160 nm.	ileum were sampled.	values (mg Ti/kg):
200 200		Liver = 0.02,
		Spleen = 0.02,
		Kidney = 0.03,
		Jejunum = 0.08,
		lleum = 0.25.
		Particulate TiO2 accounted for

80% of the

Peters et al. 2020

Aberrant Crypt Foci (ACF) as a marker for carcinogenicity

Quality ofStudyTiO2studyMethod andmethodology toReference characterisation e.g.,duration of dosing include species, ResultsOECD/GLPnumbers,controls,

E-171 consum not alte mediate mechar immun Dietary did not inflamn periphe the GI t Six-week-old male Wistar Han IGS An incr (Crl:WI (Han)) observe relative weight Test material: mg E-1 Food grade per day sample E-171. compar Dlfferent grades initiated of commerciallyand an available E-171 in IL-17 were averaged to (22.4 m produce the test 171/kg material supplied. day + [Test material was IL-12p7 added to feed. plasma E 171/k Two feed batches: day + [batch one was fed with no throughout the 7related day study and through week 10 No chai of the 100-day observe study. Batch two spleen was fed postcellular week 10 of the 100-day study. No chai observe 7-day study: 4 percent groups of 5 CD103animals CD4+7 (randomised

cells or

based on weight)

rats.

F-171

Akagi et al., 2023 6 nm TiO2 N/A 5 female and 5 male 3 tudy

	One of three pigment-grade or one of three		Dosage: Single oral gavage doses of 500. 1000 or 2000	Male and female rats.
Donner et	ultrafine	OECD 474	mg/kg body weight	Blood samples
al. 2016	/nanoscale anatase and/or	Guidelines.	(water) and positive	were collected 48 and 72 h post-
	rutile TiO2 test materials.		controls (cyclophosphamide).	exposure.

There w relevan increas micronu RET fre any TiO exposed at eithe point at dose le

All tests negativ for in vi genotox effects, significa or liver increas followin exposu highest

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 - Dosedependent marginal increase ir blood and concentrat rats dosed 1000 mg/k bw/day.

No test ite related eff on sexual function or fertility in or females test item-r pre- or pos loss observ

No test ite related thy hormone c haematolo effects.

No test ite related differences splenic lymphocyt subpopula distribution

No test ite related cha related to histopatho examinatio including t testis and epididymic and intesti

					No test ite related eff behaviour external appearanc
					No test ite related thy hormone e
					No test ite related eff on body w food consumpti water consumpti
					No test ite related eff on haemat and
				CD® (Sprague Dawley) IGS Rat (Crl:CD(SD)).	biochemica parameter urinalysis.
	Test substance: Anatase E-171,		F0 satellite group: 0, 100, 300, and	F0 satellite group – 30 male, 30 female per group + additional 40	No test ite related eff on thyroid sexual hor or sperm.
Leuschner, 2020 - Satellite study	51% of particles 100 nm. Dietary particle size: 31-43% of particles 100 nm.	OECD Test Guideline	bw/day over 10	(20 male, 20 female) for use	No test ite related cha
		443.	mating and up to the end of weaning periods).	as an F1 generation of satellite animals to be used as the positive control group in the KLH-assay (?)	in bone ma or organ weights.
					No test ite related histopatho effects in t high dose

Endnoint: ACE

					No statistic significant differences general cli signs, body weight, org weights (absolute a relative to weight), macroscop
			Test item:		findings ex statistically
			Nanoparticles in deionised water.	Sprague-Dawley	significant decrease in intake but
			80/20 anatase/rutile.	rats (12 females per group).	correlated decreased
		OECD	Mean diameter of approximately 21 nm (minimum of 100 particle sizes	Quantitative analysis in blood/tissues. Four groups of	weight or k weight gai during the period of t females of
Lee et al., 2019	TiO2 NPs P25 (15-24 nm).	414 (Pre- natal	administered daily by oral gavage.	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).	high-dose No statistic
		Toxicity Study).	Dosage:		significant differences
			Test item was administered from Gestational Days 6 to 19 at dose levels of 0, 100, 300 and 1000		caesarean section parameter fetal exter and viscer examinatio
			mg/kg with a dose volume of 10 mL/kg.		A small but statistically significant increase (4 was observe the number ossification

centres in metatarsal

Immunotoxicity

TiO2 Reference 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 CSF plasma levels (~30% females) and plasma IgM (~12% in females and in males) we observed at highest dose compared to controls.

E171

accumulation the stomach of several rat administered 1,000 mg/kg E171 for 90 days.

⊏171

			E171 suspended in distilled water, sonicated for at least 10 minutes.	Sprague-Dawley rats (10/sex/group) were administered E171 by oral	Ti concentra increased in colons of bot sexes administered 1,000 mg/kg
Han et al. , 2020	E171, anatase, 150 nm, 99.5% purity.	Study conducted according to OECD TG 408.	E171 administered by oral gavage at doses of 0, 10, 100 or 1,000 mg/kg bw/d for 90 days. Ouantitative	gavage at doses of 0, 10, 100 or 1,000 mg/kg bw/d for 90 days. Ti concentrations were measured	with the con- while colonic superoxide dismutases (SOD)-1 (ma and female) SOD-2 (fema protein level were down-
			analysis in Sprague- Dawley rat's	kidneys, and spleens harvested from	regulated. When exposito AGS cells

NCI, 1979

N/A

Titanium dioxide anatase.

(nih.gov)

TR-097: Titanium

Dioxide (CASRN

13463-67-7)

Purity: 98%.

Groups of 50 rats of each sex or mice of either 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 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 those for of the rats and the male mice at the end of the bioassay was not affected conditions of 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 a female mice, tumours occurred in dosed group incidences th were significantly higher than correspondir control group is concluded under the bioassay, titanium dio> was not carcinogenic the oral rout Fischer 344 ı or B6C3F1 m

Akagi et al., 2023 - 28 Day Study	6 nm TiO2 nanoparticles.	N/A	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 observed in a group, and n treatment- related adve effects were observed in h weight, urinalysis, haematology serum biochemistry organ weight Histopatholo examination revealed TiO particles as depositions of yellowish-bro material. The particles observed in t gastrointesti lumen were a found in the nasal cavity, epithelium, a stromal tissu the 28-day study. Overall, no effects were observed aft repeated ora administratio
--------------------------------------------	-----------------------------	-----	---------------------------------------------------	--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

crystallite siz 6 nm at up to 1000 mg/kg

bw/day

regarding general toxic

Akagi et al., 2023 6 nm TiO2 N/A - 90 Day nanoparticles. Study	10 female and 10 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 100, 300, and 1000 mg/kg bw/day (10/sex/group) for 90 days.	No mortality observed in a group, and n treatment- related adve effects were observed in 1 weight, urinalysis, haematology serum biochemistry organ weight addition, the were observe Peyer's patch in the ileum, cervical lymp nodes, mediastinal lymph nodes bronchus- associated lymphoid tise and trachea the 90-day study. Overall, no effects were observed aft repeated ora administratio TiO2 with a crystallite siz 6 nm at up ta 1000 mg/kg bw/day regarding general toxio
-------------------------------------------------------------------------	-----------------------------------------------------	----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

	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	At the highes
	composition and	dose tested,
	on gut bacterial	TiO2 had
	metabolites (10	minimal imp
	mice/group).	on the
		composition
	Incubated	the gut
	commensal	microbiota.
	bacteria derived	Alterations ir
	from mouse	bacterial
	colons	metabolites
	anaerobically	were observe
	for 5 days with	from 10 mg/l
Mice were	dose of 0, 2, 10,	bw/d.
exposure to	50 µg/ml of TiO2	Deces of 10
F171 via	biofilm	
drinking water	formation (6	50 μg/mi HO
for 4 weeks at	mice/group).	significantly
doses of 0.2	Impact of TiO2	formation by
10, 50 mg/kg		
hw/d Dose is		commensal
calculated	function was	bacteria.
based on water	determined by	There was
intake	comparison of	reduced
measured ner		expression o
	of kov markers	colonic muci
		gene, a kev
Microbiota	Mucz, IJPI,	component c
populations in	Czmb in colonia	the intestina

N/A

E171, anatase, 30-300 nm.

Pinget et E171 was

Neurotoxicity

TiO2 Reference characterisation

Quality of Method and study e.g., duration of OECD/GLP dosing

Study methodology to include species, Res numbers, controls,

The TiO2 appl pelle fema mice expo or w post reco No n neur char neur bioc imm anal obse beha in ar cogr abse the A mice perf were the r diffe sexe mice 10 female and 10 expo male C57BL/6J mice. 28 d The mice were fed cons ad libitum with food moto pellets dosed with and 10 mg/g TiO2, 2 corti spec polyvinylpyrrolidone-kina: coated Ag or control Fem pellets for 28 days. were

inve

mg/g

10 mg/g TiO2, 2 **OECD 424** Sofranko Neurotoxicity _{N/A} mg/g et al., polyvinylpyrrolidone- study in the 2021 coated Ag. rodents.

Grissa et al. (2016)	TiO2 NPs, anatase, 5–12 nm (TEM, XRD).	N/A	Internal exposure: quantitative in male Wistar rat tissues; methodology with important flaws.	N/A	Ther stati signi relat the l and per c grou a sta signi relat brair mg/k TiO2
Gerber et al., 2022	TiO2 NPs, average primary particle size of 26.2 ± 10.7 nm.	N/A	N/A	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)	Acut chro TiO2 effec chro only redu func affec morp

exposure in vitro rat

cortical cells.

Ciu et al., 2021	TiO2 NPs.	N/A	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).	, N/A	durir peric anxie beha impa neur and dam hipp BEO signi ame neur by T expo
Naima et al., 2021	TiO2 NPs.	N/A	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.	N/A	Acut injec impa perfo throo biocl struc and shou their addi appl

TiO2

						treat ATPa incre
						Intes ATPa shov char
Canli et al., 2020	TiO2 NPs	N/J	4	Oral administration of TiO2 for 14 days (0, 0.5, 5, and 50 mg/kg bw/day).	Female rats.	Leve show chan TBAF high show decr
						TiO2

agai

The to be sens

accu (dos in tis

Resu brain decr

TiO2