

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

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

Reference	TiO₂ characterisation study e.g., OECD/GLP	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, Results numbers, controls,
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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 days (with or without DMH treatment).

Series Three
Dosage: No

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

Titanium was detected immune cells in Peyer's patches. Dendritic cell percentages increased.

observed after exposure. No effect was seen after 7 days.

No effects were observed in spleen.

Regulatory T cells and T-helper 1 cells were significantly decreased after exposure at 100 days. Rats exposed to E-171.

Stimulation of immune cells isolated from Peyer's patches had a decrease in T-helper 1 cell cytokine secretion. Splenic Th1 inflammatory responses increased.

With exposure to TiO₂ NP there was an observed increase in dendritic cell percentages.

**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 concentration in the liver was $0.57 \mu\text{g/g}$ and large (1.07 \pm 0.1 $\mu\text{g/g}$ tissue) were significantly in treated compared controls.

Ti concentration in the brain, kidney, and were below quantification (0.03 $\mu\text{g/g}$).

Ti concentration in lungs, stomach, intestine were not statistically significant treated and mice.

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 weeks.

No evidence of gross alterations in immune-competence, physiological parameters, or inflammatory responses at doses up to 625 mg/kg bw/d diet.

Authors demonstrated uptake by patches, via the delivery

Presence of particles confirmed by reflectance confocal microscopy quantification of particles completely

Weak signals observed at the base of patches at mid-doses. Signals observed at highest dose indicating dose-response

**Comera
et al.
2020**

Food grade TiO₂
(E171) 95%
anatase.

European
legislation
(Council
Directive
2010/63/UE)
and French
Decree
2013-118-
compliant.

Mice (4 per
group).

Dosage:
single dose
(200 µl) of
either

E171 at 40
mg/kg of
body weight
(BW) or 200
µl of vehicle
(water) by
intra-gastric
gavage.

In addition,
in some
experiments,
the gavage
solution
from
sonicated
E171
particles was
equilibrated
in 30% corn
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.

Small intestine:
TiO₂ absorption
peaked at 4 h in the
jejunal and ileal
villi and returned
to basal level by 24
h and undetectable
at 4 h but present at
the jejunal mucosal
patches.

Colon: Low
absorption.

Blood: TiO₂
particles were
detected in the
8-hours post-
treatment.

30 minute
exposure to
the presence of
absence of
pharmacological
inhibitors of
paracellular
junction (tight
permeability)
absorption in
jejunal villi
decreased
(p 0.001 vs
control) in
presence of
triaminopyrimidine

Other inhibitors
had no significant
effect.

Absorption of
goblet cells
associated

Dudefoi 2017b	Food-grade TiO ₂ (E171-1, 17% NPs and 100% anatase and E171-6a).	N/A	Dosage: 100-250 ppm.	Method: A defined model intestinal bacterial community.	At these low concentrations, the impact on bacterial production was only a minor change in fatty acid profiles was observed. Limited effects on bacterial community structure.
Proquin et al. 2018	E171 in combination with azoxymethane (AOM)/dextran sodium sulphate (DSS) vs E171 only.	N/A	Dosage: 5 mg/kg bw per day of E171 by gavage for 2, 7, 14, and 21 days.	BALB/c mice.	E171 induced downregulation of genes involved in the immune system with indicative of impairment. Additional signalling pathways involved in a variety of cancer incidence. Colorectal tumors were modulated and effects observed. Indicated potential association with oxidative stress.

Jensen et al. 2019

Vegetable carbon (E153) and food-grade titanium dioxide (E171), mean TiO₂ particle size of 270 nm. N/A

Dosage: 10 weeks by oral gavage once a week.

Rats.

TiO₂-only

Decreased expression of protein TJ observed in rats only compared to E171 (5 mg/kg/week) shorter lung telomeres

This study found no oxidative damage in liver or lung and no changes in DNA repair of oxidative damage in lung.

4 different food
Farrell TP grade TiO₂ test
and items containing GLP-compliant,
Magnuson a range of particle OECD TG 41.
B. (2017). sizes and
morphologies.

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 hours.

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.

Ti in kidney and muscle below LOD mg/kg ww

Ti in tissue above LOD 0.1-0.3 mg/kg ww

Ti in blood 0.04 mg/L samples.

Ti in urine equal to 2 dose/L and LOQ.

Ti in faeces found to be main route of excretion.

No difference in absorption found between different grades of TiO₂.

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

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

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

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

Three studies
(Group size n=22):
Time-mated
pregnant
Sprague-Dawley
rats, (CrI: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,

At 1,000
1/kg per
mean fe
ratio an
means f
and fem
fetuses

were
statistic
significa
differen
the con
group m

Mean m
fetuses:

Mean m
fetuses
group: 5

Test fac
historica
group d
range: 5
7.4.

Mean fe
fetuses:

Mean fe
fetuses
group: 6

Test fac
historica
group d
range: 5
8.3.

Mean fe
ratio of
1,000 m
bw per
group: 6
(male)

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 h
treatme
group,
significa
increas
total Ti
levels w
found in
(0.036 :
vs. 0.04
0.008 μ
weight;
0.05) an
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 high-
males a
decrea

**Ammendolia
et al. 2017**

Nano-sized titanium dioxide (anatase, primary size 25 nm, BET surface area 45-55 m²/g, purity 99%).

N/A

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

N/A

Nanopa
depositi
intestin
and incr
serum
testoste
levels. T
was no
of oxida
stress o
alteratio
concent
of TiO₂
howeve
treatme
associat
testoste
Insulin-I
Growth
showed
increas
prolifera

Geraets et al. 2014

TiO₂ NPs (sizes NM-100, NM-101, NM-102, NM-103, and NM-104) with N/A differing particle sizes and structure.

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

TiO₂ nanoparticle kinetics were investigated using intravenous injection and oral dosing in rats. For orally dosed rats, liver, spleen and lymph nodes were targeted for analysis.

Following exposure, TiO₂ levels in liver and spleen were on occasion above the detection limit and were detected in lymph nodes at low levels.

Following intravenous exposure, TiO₂ distribution was observed in all tissues including kidney, spleen, brain, thymus and reproductive organs. The liver identified as the primary target.

Recovery of TiO₂ after 24 hours post-exposure was 64-95% in males and 108% in females.

The major route of elimination was relative to the dose. TiO₂ levels decreased over 14 days post-exposure to 26%.

Hendrickson et al. 2016 2 test items TiO₂ NPs (5-10 nm and 20-25 nm respectively). N/A

Dosage:
Intragastric administration of TiO₂ NPs (1 of 2 test items) for 28 days at a dose of 250 mg/kg of body weight per day. Male rats.

GIT and secondary organ translocation were size dependent

Larger nanoparticles exposure showed deposition in liver, kidney, spleen, small intestine (0.01- 0.05) of organ

Smaller nanoparticles exposure resulted in deposition in brain, lung, heart, liver, kidneys, small intestine, testicles, blood (0.004- 0.01) µg/g of

**Hendrickson
et al. 2020**

TiO₂ NPs

N/A

Dosage: A
single dose
suspension of
TiO₂ NPs (250
mg/kg of body
weight).
Model: A
Physiological model
designed to mimic
the intestinal lumen
of an experimental
animal.

TiO₂ NP
found in
small in
mucosa
and sple

TiO₂ NP
resulted
differen
changes
cellular
ultrastru
in the
endopla
reticulu
mitocho
extensio
the peri
spaces
caused
like stru
to appe

The mo
sensitiv
was not
the sple

Kreyling et al. 2017a

TiO₂ anatase NPs.
Median agglomerate size: 70 nm. N/A

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

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

Highest accumulation occurred in liver after 1 day (95%) then the spleen (10%) and carcass (0.4%). NPs were detected in other organs at levels lower than the

TiO₂ NP blood levels quickly after ex

Organs tissue N were sta day-28.

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 administered dose passed gastro-intestinal tract after 1 h

0.05% of dose was distributed to the body days distributed across tissues following exposure to organs:

liver (0.29 ng/g), lungs (0.29 ng/g), kidney (0.29 ng/g), brain

(0.36 ng/g), spleen (0.36 ng/g), uterus (0.55 ng/g), bone density (0.98 ng/g)

Faecal excretion was confirmed after 4-7 days

				6-7-week-old male B6C3F1 mice.
				In vivo micronucleus and Pig-a (phosphatidylinositol glycan, class A gene) mutation assays using TiO ₂ NPs to evaluate genotoxicity.
			Dosage: 1) Intravenous 0.5, 5.0, and 50 mg/kg TiO ₂ NPs.	Blood re No incre Pig-a m frequen %MN-R
Sadiq et al. 2012	TiO ₂ NPs.	N/A	2) Intravenous three daily doses of 50 mg/kg TiO ₂ NPs Ti levels in bone marrow measured after 4, 24, and 48 hours of the last treatment.	Tissue r Ti NPs p at 4 hou exposur %RETs v reduced treated on Day depend which s cytotox TiO ₂ -NP bone m No evid genotox
				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 Day 4.

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

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

**Guillard
et al.
2020**

Basal Ti level in
human placenta
study.

N/A

TiO₂ with 55% NPs,
20 to 440 nm.

Samples
were
taken of
placenta
and
meconium
from
human
babies
(n=22)
and
tested for
TiO₂ and
other
metals
and trace
elements.

TiO₂ in human
placentas was
analysed by
ICP-MS and
STEM coupled
to EDX
spectroscopy.

All placenta
contained
TiO₂ at 0.01
to 0.48 mg/kg
of tissue with
the majority
below 100nm
in size (over
50%).
Meconium
samples also
contained
TiO₂ between
0.02-1.5
mg/kg.

**Heringa
et al.
2018**

Post-mortem
analysis of human
liver and spleen
TiO₂ analysis.

N/A

N/A

High
resolution ICP-
MS was used
to detect TiO₂
in the liver
and spleen in
15 deceased
humans (9
female and 6
male).

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:

85-550nm
and
85-720nm
with 24% 100
nm in size.

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.

Peters et al. 2020

Postmortem tissue analysis of deceased persons for the presence of TiO₂.

Detected particle sizes were in the range of 50–500 nm, with a mode of 100–160 nm.

15 humans sampled, 8 female and 7 male aged 64–98 years.

Postmortem liver, spleen, kidney, jejunum and ileum were sampled.

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,

Jejunum = 0.13,

Ileum = 0.26.

Particulate TiO₂ were observed from 0.01 to 1.8 mg Ti/kg with median values (mg Ti/kg):

Liver = 0.02,

Spleen = 0.02,

Kidney = 0.03,

Jejunum = 0.08,

Ileum = 0.25.

Particulate TiO₂ accounted for 80% of the

Aberrant Crypt Foci (ACF) as a marker for carcinogenicity

TiO2 Reference characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls,	Results
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consumption
not altered
mediated
mechanism
immune

Dietary
did not
inflammation
peripheral
the GI tract

Six-week-old male
Wistar Han IGS
(CrI:WI (Han))
rats.

An increase
observed
relative

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.

weight
mg E-171
per day
comparing
initiated
and an
in IL-17
(22.4 mg
171/kg
day + D
IL-12p70
plasma

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.

E 171/kg
day + D
with no
related

No change
observed
spleen
cellular

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

No change
observed
percent
CD103-
CD4+ T
cells or

Akagi et al., 2023 - 28 Day Study

6 nm TiO₂ nanoparticles.

N/A

5 female and 5 male F344/DuCrIcrIj rats.

TiO₂ NPs with a crystallite size of 6 nm were examined in male and female F344/DuCrIcrIj rats by repeated oral administration of 10, 100, and 1000 mg/kg bw/day (5/sex/group) for 28 days.

No mor
observe
group, a
treatme
related
effects
observe
weight,
urinalys
haemat
serum
biochem
organ w
Histopa
examin
reveale
particle
deposit
yellowis
material
particle
observe
gastroin
lumen v
found in
nasal ca
epitheli
stroma
the 28-
study.
Overall,
effects
observe
repeate
adminis
TiO₂ wi
crystall
6 nm at
1000 m
bw/day
regardi
genera

Donner et al. 2016	One of three pigment-grade or one of three ultrafine /nanoscale anatase and/or rutile TiO ₂ test materials.	OECD 474 Guidelines.	Dosage: Single oral gavage doses of 500, 1000 or 2000 mg/kg body weight with negative (water) and positive controls (cyclophosphamide).	Male and female rats. Blood samples were collected 48 and 72 h post-exposure.	There was a relevant increase in micronucleated RET frequency in any TiO ₂ exposed rats at either point at the dose level. All tests were negative for in vivo genotoxic effects, significant or liver increases following exposure to the highest
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Reproductive toxicity

Reference	TiO ₂ characterisation	Quality of study e.g., OECD/GLP dosing	Method and duration of dosing	Study methodology to include species, numbers, controls,	Results
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Results:

F0 - Dose-dependent marginal increase in blood and concentration in rats dosed 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 post-loss observations.

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 histopathological examination including testis and epididymis and intestines.

Leuschner, 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 (CrI: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: ACE

No test item related effects on behaviour or external appearance.

No test item related thyroid hormone effects.

No test item related effects on body weight, food consumption or water consumption.

No test item related effects on haematology and

biochemical parameters and urinalysis.

No test item related effects on thyroid or sexual hormones or sperm.

No test item related changes in bone mass or organ weights.

No test item related histopathological effects in the high dose group.

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 statistically significant differences in general clinical signs, body weight, organ weights (absolute and relative to body weight), macroscopic findings except statistically significant decrease in food intake but correlated decreased weight or body weight gain during the period of the females of high-dose. No statistically significant differences in caesarean section parameter fetal external and visceral examination. A small but statistically significant increase (4%) was observed in the number of ossification centres in the metatarsals.

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
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Statistically significant decreases in CSF plasma levels (~30% in females) and plasma IgM (~12% in females and in males) were observed at the highest dose compared to controls.

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

Ti concentrations increased in 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

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

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

Han et al. , 2020

E171, anatase, 150 nm, 99.5% purity.

Study conducted according to OECD TG 408.

**NCI,
1979**

[TR-097: Titanium Dioxide \(CASRN 13463-67-7\) \(nih.gov\)](#)

N/A

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, tumours occurred in dosed groups. Incidences were significantly higher than those for corresponding control groups. It is concluded under the conditions of the bioassay, titanium dioxide was not carcinogenic by the oral route. Fischer 344 rats or B6C3F1 mice.

Akagi et al., 2023 - 28 Day Study

6 nm TiO₂ nanoparticles.

N/A

5 female and 5 male F344/DuCrIjCrIj rats.

TiO₂ NPs with a crystallite size of 6 nm were examined in male and female F344/DuCrIjCrIj rats by repeated oral administration of 10, 100, and 1000 mg/kg bw/day (5/sex/group) for 28 days.

No mortality observed in any group, and no treatment-related adverse effects were observed in body weight, urinalysis, haematology, serum biochemistry, organ weights, and histopathology examination. Histopathology examination revealed TiO₂ particles as yellowish-brown material. The particles observed in the gastrointestinal lumen were also found in the nasal cavity, epithelium, and stromal tissue. 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 toxicology.

Akagi et al., 2023 - 90 Day Study

6 nm TiO₂ nanoparticles.

N/A

10 female and 10 male F344/DuCrIj rats.

TiO₂ NPs with a crystallite size of 6 nm were examined in male and female F344/DuCrIj rats by repeated oral administration of 100, 300, and 1000 mg/kg bw/day (10/sex/group) for 90 days.

No mortality observed in any group, and no treatment-related adverse effects were observed in body weight, urinalysis, haematology, serum biochemistry, organ weights. In addition, the following effects were observed: Peyer's patches in the ileum, cervical lymph nodes, mediastinal lymph nodes, bronchus-associated lymphoid tissue, and trachea. 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 toxicology, accumulation of titanium in the liver, kidneys,

	<p data-bbox="327 2072 614 2161">E171, anatase, 30-300 nm.</p> <p data-bbox="327 2184 486 2228">E171 was</p>	<p data-bbox="646 2004 710 2049">N/A</p>	<p data-bbox="853 1467 1077 2094">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.</p> <p data-bbox="853 2139 1077 2228">Microbiota populations in</p>	<p data-bbox="1109 145 1364 1064">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).</p> <p data-bbox="1109 1108 1364 1691">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).</p> <p data-bbox="1109 1736 1364 2228">Impact of TiO₂ on colonic epithelial function was determined by comparison of gene expression of key markers Muc2, Tjp1, Defb3, and Gzmb in colonic</p>	<p data-bbox="1396 817 1596 1489">At the highest dose tested, TiO₂ had minimal impact on the composition of the gut microbiota. Alterations in bacterial metabolites were observed from 10 mg/kg bw/d.</p> <p data-bbox="1396 1534 1596 1870">Doses of 10 and 50 µg/ml TiO₂ significantly promoted biofilm formation by commensal bacteria.</p> <p data-bbox="1396 1915 1596 2228">There was reduced expression of colonic mucin gene, a key component of the intestinal</p>
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Neurotoxicity

Reference	TiO ₂ characterisation	Quality of study e.g., OECD/GLP	Method and duration of dosing	Study methodology to include species, numbers, controls,	Res
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**Sofranko
et al.,
2021**

10 mg/g TiO₂, 2
mg/g
polyvinylpyrrolidone-
coated Ag.

OECD 424
Neurotoxicity
study in the
rodents. N/A

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
TiO₂
appl
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fema
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expo
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No m
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expo
28 d
cons
moto
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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

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signi
relat
the l
and
per o
grou
a sta
signi
relat
brain
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) exposure in vitro rat cortical cells.

Acut
chro
TiO₂
effec
chro
only
redu
func
affec
morp

**Ciu et al.,
2021**

TiO₂ NPs.

N/A

36 male
Sprague Dawley
rats aged
postnatal day
21 (PND 21)
were injected N/A
intraperitoneally
with TiO₂ NPs
(20 mg/kg)
and/or BEO (200
mg/kg).

TiO₂
durin
perio
anxi
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 N/A
subjected to
cognitive and
emotional tests
using Morris
water maze and
elevated plus
maze.

Acut
injec
impa
perf
thro
biocl
struc
and
shou
their
addi
appl

Canli et al., 2020

TiO₂ NPs

N/A

Oral administration of TiO₂ for 14 days (0, 0.5, 5, and 50 mg/kg bw/day).

Female rats.

Resu
brain
decr
treat
ATPa
incre

Intes
ATPa
show
chan

Level
show
chan
TBAF
high
show
decr

TiO₂
accu
(dos
in tis

The
to be
sens
again
TiO₂