

Cover Paper and Tables - Third draft statement on the safety of Titanium Dioxide (E171) as a Food Additive

Introduction

1. Titanium dioxide (TiO₂) was an authorised Food Additive (E171) in the EU and currently remains authorised in the UK, under Retained EU Regulation No. 1333/2008 and Retained EU Regulation No. 231/2012. It is used in food as a colour to make food more visually appealing, to give colour to food that would otherwise be colourless, or to restore the original appearance of food. It is commonly used in products such as bakery products, soups, broths, sauces, salad dressings, savoury based sandwich spreads, processed nuts, confectionary, chewing gum, food supplements and cake icing.

2. Titanium dioxide has been the subject of multiple safety evaluations. In 2016, the EFSA ANS (Food Additives and Nutrient Sources) Panel evaluated the safety of E171 TiO₂ and identified several uncertainties in their evaluation included the unspecified identity and characterisation of E171 as it was not determined whether the test material was compliant with the specification of E171 requirements. The EFSA 2016 review determined that E171 TiO₂ consisted mainly of micro-sized TiO₂ particles, with a nano-sized (<100 nm) fraction which was less than 3.2% by mass. Uncertainties around the identity and characterisation of E171 were highlighted, noting that no limits for the particle size of E171 were set. In 2019, the specifications of E171 titanium dioxide were reviewed by the EFSA FAF Panel (Food and Feed). A recommendation for re-assessment of the safety of titanium dioxide was proposed.

3. In the EFSA 2021 Opinion, the EFSA FAF Panel considered that some findings regarding immunotoxicity, inflammation and neurotoxicity with respect to TiO₂ nanoparticles may be indicative of adverse effects. On the basis of the currently available evidence and the uncertainties, in particular a concern regarding genotoxicity which could not be resolved, the EFSA Panel concluded

that E171 can no longer be considered as safe when used as a food additive.

4. In 2021 the COT published an interim position on titanium dioxide ([COT 2021](#)) capturing the outcomes of the discussions and outlining the next steps. Members were asked to evaluate the EFSA Opinion and comment on whether they agreed with EFSA's conclusions and further guidance on the next steps that should be taken; producing an opinion paper following a review of the new EFSA opinion and the extended one generation reproductive toxicity (EOGRT) study data by both the COT and COM (Committee on Mutagenicity).

5. This draft statement (Annex A) includes the COT conclusions on the following endpoints: ADME, Aberrant Crypt Foci as a marker for Carcinogenicity, Allergenicity, Reproductive and Developmental Toxicity, potential evidence of Immunotoxicity, Inflammation and Neurotoxicity and the derivation of a Health-Based Guidance Value, and a review of genotoxicity endpoints by the COM. Additionally, this statement also includes the titanium dioxide exposure assessment for the UK population.

Questions for the Committee

6. The Committee are asked to consider the following questions:

- i. Are Members content with the layout and structure of the draft statement?
- ii. Would Members like to see any additional information on studies already included, or consider that other studies should be included?
- iii. Do Members agree with the layout and structure of the table summarising the studies?
- iv. Do Members have any other comments?

Secretariat

February 2024

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
Talamini et al., 2019	E171 (35% nano determined by TEM), 99.3% pure anatase, 201.2 ± 8.5 nm in suspension (NTA). No sonification or deagglomeration to simulate realistic conditions.	This work was reviewed by the Institute for Pharmacological Research Mario Negri IRCCS Animal Care and Used Committee (IACUC) and then approved by the Italian National Institute of Health (code:42/2016-PR).	Treatments were given 3 days per week for 3 weeks for a total of 9 treatments in 21 days. Average daily dose of ~2 mg/kg bw. Treatments were dripped slowly into the mice's mouths, allowing each drop to be swallowed.	NFR male mice (22/group) were administered either water (control) or 5 mg/kg bw E171 suspended in water. Ti concentrations in tissues were determined by single particle ICP-MS analysis.	Ti concentrations in the liver (0.94 ± 0.57 µg/g tissue) and large intestine (1.07 ± 0.38 µg/g tissue) were significantly higher in treated mice compared to controls. Ti concentrations in the brain, kidney, and testes were below the quantification limit (0.03 µg/g). Ti concentrations in lungs, spleen, stomach, and small intestine were not statistically significant between treated and control mice.

**Riedle
et al.,
2020**

E171, anatase,
119 nm.

Mice were divided into 4 groups of 18 and given 0, 6.25, 62.5, or 625 mg/kg diet 0, 1, 10, or 100 mg/kg (equivalent to bw/d E171 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).

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	TiO2 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 and casein) in the presence of E171.	For ELISA, primary antibody for casein (Anti-casein rabbit antibody-cat # ab166596), primary antibody for β -lactoglobulin (Anti-LGB rabbit antibody-cat # ab112893) and secondary anti-rabbit antibody (cat # 6721) were used. Quebon skimmed milk was used.	Significant enhancement in the allergenicity of milk proteins/skimmed milk interacted with both E171 and food grade titanium dioxide nanoparticles. The presence of E171 showed the highest level of LAD2 degranulation (a proxy for allergenicity), followed by food grade titanium dioxide nanoparticles.	No information

Inflammation and Immunotoxicity

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

**Talamini
et al.,
2019**

E171 (35% nano determined by TEM), 99.3% pure anatase, 201.2 ± 8.5 nm in suspension (NTA).

No sonification or deagglomeration to simulate realistic conditions.

This work was reviewed by the Institute for Pharmacological Research Mario Negri IRCCS Animal Care and Used Committee (IACUC) and then approved by the Italian National Institute of Health (code:42/2016-PR).

Treatments were given 3 days per week for 3 weeks for a total of 9 treatments in 21 days. Average daily dose of ~2 mg/kg bw.

Treatments were dripped slowly into the mice's mouths, allowing each drop to be swallowed.

NFR male mice (22/group) were administered either water (control) or 5 mg/kg bw E171 suspended in water.

Ti concentrations in tissues were determined by single particle ICP-MS analysis.

Ti concentrations in the liver (0.94 ± 0.5 µg/g tissue) and large intestine (1 ± 0.38 µg/g tissue) were significantly higher in treated mice compared to controls.

Ti concentrations in the brain, kidney, and testes were below the quantification limit (0.03 µg/g).

Ti concentrations in lungs, spleen, stomach, and small intestine were not statistically significant between treated and control mice.

<p>Pinget et al., 2019</p>	<p>E171, anatase, 30-300 nm. E171 was</p>	<p>No information.</p>	<p>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. Microbiota populations in</p>	<p>Male C67BL/6J Aush 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). 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 expression of key markers Muc2, Tjp1, Defb3, and Gzmb in colonic</p>	<p>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 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</p>
-----------------------------------	---	------------------------	---	--	---

**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,
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,
inflammation
at doses up to
100 mg/kg
bw/d via the
diet.

Authors
demonstrated
E171 uptake
by Peyer's
patches,
validating the
delivery
model.

Presence of
E171 particles
detected by
reflectance
confocal
microscopy
(no
quantification
of particles
completed)

Weak signals
observed at
the base of
Peyer's
patches at low
and mid-
doses. High
signals
observed at
highest doses
indicating
evidence of
dose-

Liu et al., 2020

This is a review, and is only mentioned once in the TiO₂ statement in a quote from the Health Canada report.

No information. No information. No information.

No information

Han et al., 2020

E 171, anatase, 150 nm, 99.5% purity

Study conducted according to

E171 suspended in distilled water, sonicated for at least 10 minutes.

E171 administered by oral gavage at doses of 0, 10, 100 or 1,000

Sprague-Dawley rats (10/sex/group) were administered E171 by oral gavage at doses of 0, 10, 100 or 1,000 mg/kg bw/d for 90 days.

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 was of several hours administered 1,000 mg/kg E171 for 90 days.

Ti concentrations increased in the colons of both sexes administered 1,000 mg/kg E171 compared with the control, which colonic, superoxide dismutases (SOD)-1 (male and female) and SOD-2

Studies used to review the toxicokinetic and absorption of the nanoparticle form of TiO₂

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

**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)).

**Warheit
et al.,
2015**

1)
anatase/rutile
(89/11%) (uf-
1), d50=43 nm
d50=23 nm
Methods:
XSDC and TEM
respectively.
Shape:
Irregular.

2) anatase
(100% nano)
(uf-2) d50= 42
nm d50=19
nm.

Methods:
XSDC and TEM
respectively.

Shape:
Irregular.

3) rutile
(100% nano)
(uf-3), d50=47
nm d50=22
nm Methods:
XSDC and TEM
respectively.

OECD
Guideline
414

Shape: rod-
like.

4) anatase
(27% nano)
(pg-1),
d50=153 nm
d50=120 nm
Methods:
XSDC and TEM
respectively.

Shape:

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, (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.
- Resorption live and dead fetuses.
- Fetal sex.
- Fetal weight.
- Fetal

The mean diameter of TiO₂ NP ranged from 208 to

Nanoparticulated anatase TiO₂ was prepared via controlled hydrolysis of titanium tetrabutoxide.

The particle sizes of both the powder and the nanoparticles

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

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.

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 drinking water for 100 days.

Control animals (n = 12) received water only.

Flow cytometry and cytokine assays were assessed for gut inflammation and ACF.

Karimpour TiO₂ NPs,
et al., anatase,
2018 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 were housed with 3 male mice for 11 days. The percentage of pregnancy and numbers of newborns were evaluated.

Khorsandi
et al.,
2016

TiO₂ NPs 30
nm.

Test item: NTiO₂ nanopowder (TNP, Sigma) made with 100 ml BSA (bovine serum albumin) solution dissolve in Milli-Q water.

Oral Dosage Groups:

TNP-1: 75 mg/kg TNP,

TNP-2: 100 mg/kg TNP,

TNP-3: 300 mg/kg TNP.

Control: saline solution.

32 adult 6–8 weeks old male NMRI mice (25–30 g).

Four groups of 8 mice with a dosage of 75, 100 and 300 mg/kg TNP for 35 consecutive days respectively for each of the test groups and the control group received saline orally for 35 consecutive days.

Testicular testosterone levels, testis weight, total volumes of testis, seminiferous tubules, interstitial tissue and total Leydig cell numbers were measured.

Lee <i>et al.</i> , TiO ₂ NPs P25 2019. (15-24 nm).	OECD Guideline 414 (Pre-natal Toxicity Study).	<p>Test item: Nanoparticles in deionised water.</p> <p>80/20 anatase/rutile.</p> <p>Mean diameter of approximately 21 nm (minimum of 100 particle sizes averaged) administered daily by oral gavage.</p> <p>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.</p>	<p>Sprague-Dawley rats (12 females per group).</p> <p>Quantitative analysis in blood/tissues.</p> <p>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).</p>
--	--	---	--

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
-----------	-----------------------------------	---------------------------------	-------------------------------	--	---------

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.

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

Test material:
Food grade
sample E-171.
Different grades
of commercially-
available E-171
were averaged to
produce the test
material supplied.
Test material was
added to feed.

Two feed batches:
batch one was fed
throughout the 7-
day study and
through week 10
of the 100-day
study. Batch two
was fed post-
week 10 of the
100-day study.

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

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
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

<p>Akagi et al., 2023 - 28 Day Study</p>	<p>6 nm TiO₂ nanoparticles.</p>	<p>No information.</p>	<p>5 female and 5 male F344/DuCrI rats.</p>	<p>TiO₂ NPs with a crystallite size of 6 nm were examined in male and female F344/DuCrI rats by repeated oral administration of 10, 100, and 1000 mg/kg bw/day (5/sex/group) for 28 days.</p>	<p>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.</p> <p>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 stromal tissue in the 28-day study.</p> <p>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.</p>
--	--	----------------------------	---	--	--

Reproductive toxicity

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

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.

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
ACE

					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.
				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 (?)	No test item-related effects on thyroid and sexual hormones or sperm.
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).		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

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	N c o
-----------	-----------------------------------	---------------------------------	-------------------------------	--	---------	-------------

					<p>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.</p>
					<p>E171 accumulation in the stomach wall of several rats administered 1,000 mg/kg E171 for 90 days.</p>
<p>Han et al., 2020</p>	<p>E171, anatase, 150 nm, 99.5% purity.</p>	<p>Study conducted according to OECD TG 408.</p>	<p>E171 suspended in distilled water, sonicated for at least 10 minutes.</p> <p>E171 administered by oral gavage at doses of 0, 10, 100 or 1,000 mg/kg bw/d for 90 days.</p> <p>Quantitative analysis in Sprague-</p>	<p>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.</p> <p>Ti concentrations were measured in the colons, kidneys, and spleens harvested from</p>	<p>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.</p> <p>When exposed to AGS cells</p>

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 control rats and mice of each sex were at risk for development of late-appearing tumors.

In the male and female mice, no tumours occurred in dosed groups at incidences that were significantly higher than those for corresponding control groups. 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.

<p>Akagi et al., 2023 - 28 Day Study</p>	<p>6 nm TiO₂ nanoparticles.</p>	<p>No information.</p>	<p>5 female and 5 male F344/DuCrI rats.</p>	<p>TiO₂ NPs with a crystallite size of 6 nm were examined in male and female F344/DuCrI rats by repeated oral administration of 10, 100, and 1000 mg/kg bw/day (5/sex/group) for 28 days.</p>	<p>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 stromal tissue in the 28-day study.</p> <p>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,</p>
--	--	----------------------------	---	--	---

Akagi et al., 2023
- 90 Day Study
6 nm TiO2 nanoparticles.

No information.

10 female and 10 male F344/DuCrI CrIj rats.

No information.

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

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
---	---------------------------------	-------------------------------	--	---------

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
ad libitum
pellets d
10 mg/g
mg/g
polyviny
coated A
pellets fo

Grissa et al. (2016) TiO₂ NPs, anatase, 5-12 nm (TEM, XRD).

No information.

Internal exposure: quantitative in male Wistar rat tissues; methodology with important flaws.

No information.

There was no statistical significance related to the level of TiO₂ NPs (100 and 200 mg/kg bw per day) in the groups of male and female rats and a statistically significant increase in the level of TiO₂ NPs in the brain TNM was observed at 100 mg/kg bw per day TiO₂ NPs.

Gerber et al., 2022	TiO ₂ NPs, average primary particle size of 26.2 ± 10.7 nm.	No information.	No information.	<p>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.</p> <p>Acute and sub-chronic exposure to TiO₂NP is without effects, whereas chronic exposure only modestly reduces neuronal function without affecting morphology.</p>	No inform
---------------------	--	-----------------	-----------------	---	-----------

Ciu et al., 2021	No information.	No information.	36 male Sprague Dawley rats aged postnatal day 21 (PND 21) were injected intraperitoneally with TiO ₂ NPs (20 mg/kg) and/or BEO (200 mg/kg).	TiO ₂ 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 TiO ₂ NPs exposure.	No information.
Naima et al., 2021	No information.	No information.	Rats were injected intravenously with a single dose of TiO ₂ -NPs (20 mg/kg body weight) and were subjected to cognitive and emotional tests using Morris water maze and elevated plus maze.	Acute intravenous injection of TiO ₂ -NPs impaired behaviour performances through brain biochemical and structural changes and precautions should be taken to their usage in food additive and medical applications.	No information.