Particle and Fibre Toxicology

REVIEW

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Organ and tissue accumulation of titanium dioxide after acute, subacute, subchronic, and chronic oral exposure in mice and rats: a systematic review

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Abstract

Background Titanium dioxide (TiO_2) is a compound that is often used as a white pigment. Commercial TiO_2, such as the food additive E171, contains a mix of particle sizes, including a fraction in the nanoscale range (< 100 nm). It is an ingredient in everyday products such as toothpaste, dietary supplements, and pharmaceuticals. Although the oral and gastrointestinal (GIT) tracts are the initial sites of exposure, in vivo studies have shown that TiO_2 can cross the intestinal epithelium, enter systemic circulation, and accumulate in vital organs, where elimination is slow. This accumulation has been associated with oxidative stress, inflammation, cytotoxicity, and altered cellular function.

Main body This systematic review assesses titanium (Ti) accumulation in vital organs of rats and mice following oral TiO_2 exposure, focusing on dose- and time-dependent patterns across acute, subacute, subchronic, and chronic durations. Following PRISMA guidelines, 3,012 records were identified and screened by title and abstract, with 54 studies meeting predefined inclusion criteria. The findings reveal that acute oral exposure to TiO_2 consistently results in minimal titanium accumulation across all major organs, indicating limited gastrointestinal absorption and rapid excretion. In contrast, subacute and subchronic exposures lead to significant, dose-dependent titanium accumulation, especially in the liver, spleen, kidneys, gastrointestinal tract, and brain. Chronic exposure studies, though fewer, indicate persistent Ti presence, especially in the liver, kidneys, and colon. Ti was also found in the brain, pancreas, and reproductive tissues, with histopathological changes indicating broader systemic effects. A few studies reported negligible accumulation even at high doses.

Conclusion This review highlights the organ-specific and exposure-dependent biodistribution of titanium following oral TiO_2 intake in rodents. The evidence emphasizes the need for standardized reporting and experimental methodologies to improve data comparability across studies. Importantly, it underscores significant gaps in our understanding of chronic and low-dose exposures, conditions more reflective of real-world human scenarios, warranting further investigation to better assess long-term health risks.

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Keywords Titanium dioxide, Oral exposure, Organ accumulation, Rodent model

Introduction

Titanium dioxide (TiO_2) is a naturally occurring oxide of titanium and can be found in different crystalline forms, including rutile, anatase, and brookite [1]. Rutile is the most prevalent naturally occurring form of TiO_2, typically containing about 98% titanium dioxide in the ore, whereas anatase and brookite are less common [2, 3]. TiO_2 ranks among the top five nanoparticles used in consumer products and represents 70% of global pigment production [4]. TiO_2 improves brightness, whiteness, opacity, and resistance to decolourisation in various products, which has resulted in its extensive use across multiple industries, including the food sector [5].

The United States Food and Drug Administration (FDA) approved the use of TiO_2 in food in 1966 by allowing levels up to 1% in food (FDA, 2015). TiO_2 is commonly present in food products in both microparticle and nanoparticle forms, with primary particle diameters ranging from 30 to 400 nm [6]. In food products containing the additive E171 (titanium dioxide), nano-sized TiO_2 primary particles (< 100 nm) are estimated to constitute anywhere from 10 to 55% of the total amount [7].

TiO_2 is frequently consumed due to its ubiquitous presence in a variety of foods, dietary supplements, and the accidental swallowing of toothpaste, especially by young children [8]. TiO_2 exposure through ingestion can also result from its role as an excipient in pharmaceutical products [9]. In the United States, children under 10 years have an estimated average exposure of 1–2 mg TiO_2/kg bw/day, while those above 10 years old have an exposure of 0.2–0.7 mg TiO_2/kg bw/day [10]. Estimates from the UK are slightly higher (under 10, 2–3 mg/kg bw/day; over 10, 1 mg/kg bw/day) [10]. Over several studies, human subjects following a Western European diet and using toothpaste have an estimated mean oral intake of TiO_2 ranging from 0.06 to 5.5 mg TiO_2/ kg bw/day [8, 10–12].

An acceptable daily intake for oral ingestion of TiO_2 has not been established due to the absence of observed toxic effects in early chronic rodent studies, the widely accepted belief in negligible uptake following ingestion [13], and the assumption that the material is both insoluble and inert [14]. The results of human volunteer studies, on the other hand, showed higher blood Ti levels 6 h after consuming food-grade TiO_2 [15], which aligns with previous findings of increased blood Ti levels after ingesting 160 nm and 380 nm TiO_2 particles [16]. Additionally, the European Food Safety Authority (EFSA) has acknowledged that, while food-grade TiO_2 is only absorbed to a limited degree after oral ingestion, it does enter the bloodstream and is distributed to different organs [11].

The ability of TiO_2 to cross the intestinal epithelial barrier is supported by in vitro and ex vivo models, while its subsequent entry into the bloodstream has been directly demonstrated in in vivo studies following oral exposure [17–19]. Nanomaterials generally distribute rapidly from the bloodstream into tissues, with those containing a highly perfused reticuloendothelial system (RES), such as the liver and spleen, being primary targets [20–24]. They have also been detected, albeit in smaller quantities, across protective membranes in the brain, foetus, and testis [25]. Moreover, the elimination process is generally slow, and TiO_2 does not appear to undergo metabolic degradation. With limited elimination and only a small fraction becoming systemically available, repeated exposure will result in tissue accumulation [24]. In vivo studies have revealed that TiO_2 NPs can cause detrimental effects, including DNA damage, genotoxicity, and inflammation [26, 27]. They also contribute to oxidative stress, the formation of DNA adducts, apoptosis, and necrosis [28–36] along with toxic effects on the liver, kidneys, spleen, myocardium, and disruptions in glucose and lipid homeostasis in experimental animals [36–39]. In biological matrices, the concentration of TiO_2 is generally assessed by measuring the Ti level. According to Heringa et al. [40] and Peters et al. [41], it is plausible that the Ti present in human tissues primarily comes from oral exposure to TiO_2 particles. This assumption is supported by the observation that the size range of TiO_2 particles found in human livers and spleens (86–421 nm and 88–445 nm, respectively) closely matches the size range of TiO_2 particles present in food products (30–600 nm in diameter).

To date, a growing number of studies have demonstrated that titanium can be absorbed and distributed across various vital organs and tissues in rodents. However, no systematic review has specifically addressed the quantitative distribution of titanium in their organs following oral exposure. Rodents were selected as the focus of this review due to their widespread use in toxicological studies and their physiological similarities to humans, particularly in gastrointestinal and metabolic processes, making them suitable models for assessing the biodistribution of Ti. This systematic review aims to quantify Ti accumulation in the vital organs of rats and mice following oral exposure and assess its dose- and time-dependent patterns across acute, subacute, subchronic, and chronic exposure scenarios. To achieve this, the review will evaluate current knowledge on the distribution, accumulation, and potential health effects of TiO_2, identify patterns of accumulation, and highlight sensitive target organs.

Method

Search strategy

We conducted a systematic search adhering to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to gather existing data on oral exposure to TiO_2 and its impact on quantitative distribution in the vital organs of rats and mice. Scientific databases PubMed, Scopus, and Web of Science were searched from their inception to January 2025. A broad search string was used to ensure comprehensive coverage: ("titanium dioxide" OR "titanium dioxide nanoparticle*" OR "nano titanium" OR " TiO_2 nanomaterial" OR " TiO_2 nanoparticle*" OR " TiO_2 food grade" OR E171) AND ("oral administration" OR "administrations, oral" OR "oral ingestion" OR "oral intake" OR "oral*" OR "ingested") (title/abstract/keywords/MeSH).

Eligibility criteria and study selection

After removing duplicates, two researchers screened articles by title and abstract. Inclusion criteria were: (1) Oral administration as an intervention (not combined with other chemicals or treatments, but acceptable if oral exposure was used separately alongside other interventions); (2) animal experiments using rats or mice; (3) English language; (4) studies measuring Ti levels in vital organs/tissues as primary or secondary outcomes. Studies were included regardless of TiO_2 particle size, encompassing both nano-sized (< 100 nm) and micro-sized (> 100 nm) particles to reflect the diversity in food-grade TiO_2, but were limited to oral exposure routes to align with dietary intake focus. Exclusion criteria included: (1) absence of full-text access; (2) non-original studies such as reviews, conference abstracts, notes, letters to the editor, or protocols; (3) absence of relevant data on Ti accumulation; (4) studies involving species other than rats or mice; (5) use of non-oral exposure routes (e.g., intravenous, inhalation, or dermal). References were managed, deduplicated, and screened using EndNote and Microsoft Excel.

Eligible studies underwent full-text review. Two independent researchers identified eligible studies, with discrepancies resolved by a third researcher. If full texts were unavailable from the databases, corresponding authors were contacted by email, followed by a reminder if there was no response after five business days.

Data extraction

Data extraction was done using a spreadsheet in Microsoft Excel, covering: (i) bibliographic details (author, title, year, journal); (ii) animal characteristics (species, strain, age, sex); (iii) study design; (iv) TiO_2 NP characteristics (crystalline phase, size, hydrodynamic size, specific surface area, surface charge, dispersion method, aggregation, and shape); (v) intervention details (dose(s), duration, exposure frequency, route of administration); (vi) vital organs examined for Ti content, tissue amount used, methods used for Ti quantification, detection limits, and time points of analysis; (vii) Outcome measures included the mean Ti concentrations, the statistical significance of accumulation as reported by the authors, and qualitative observations related to distribution and accumulation patterns.

Quality assessment

The methodological quality and risk of bias of the included in vivo studies were assessed using the SYRCLE's Risk of Bias (RoB) tool [42]. This checklist, adapted from the Cochrane RoB tool, is specifically designed for animal intervention studies and contains ten domains addressing selection bias (sequence generation, baseline characteristics, allocation concealment), performance bias (random housing, blinding), detection bias (random outcome assessment, blinding), attrition bias (incomplete outcome data), and reporting bias (selective outcome reporting). In addition to the standard SYRCLE domains, three items critical for the accurate interpretation of bioaccumulation studies were evaluated:

- Reported Ti Background: Whether the study reported titanium levels in the control (non-exposed) group.
- Reported Detection Limit: Whether the detection limit was specified.
- Ethical Statement: Whether a statement regarding ethical approval was provided.

The results for each domain were judged as "Low risk," "High risk," or "Unclear risk" of bias according to the tool's guidelines and are summarized in the results section and presented graphically.

Results

Search results

The literature search yielded a total of 3012 bibliographic records across three databases (1068 from Scopus, 1180 from PubMed, and 764 from Web of Science). The study selection process is summarised in Fig. 1 using the PRISMA flow diagram. After excluding 731 duplicates, 2281 records remained. Following the title and abstract screening, 1981 studies were removed based on predetermined exclusion criteria. In the second selection phase, after full-text screening of the remaining 300 articles, 54 studies were included in the systematic review. The included studies were published between 1994 and 2025.

Study characteristics

The characteristics of the reviewed studies, as shown in Table 1, varied widely. Despite the importance of

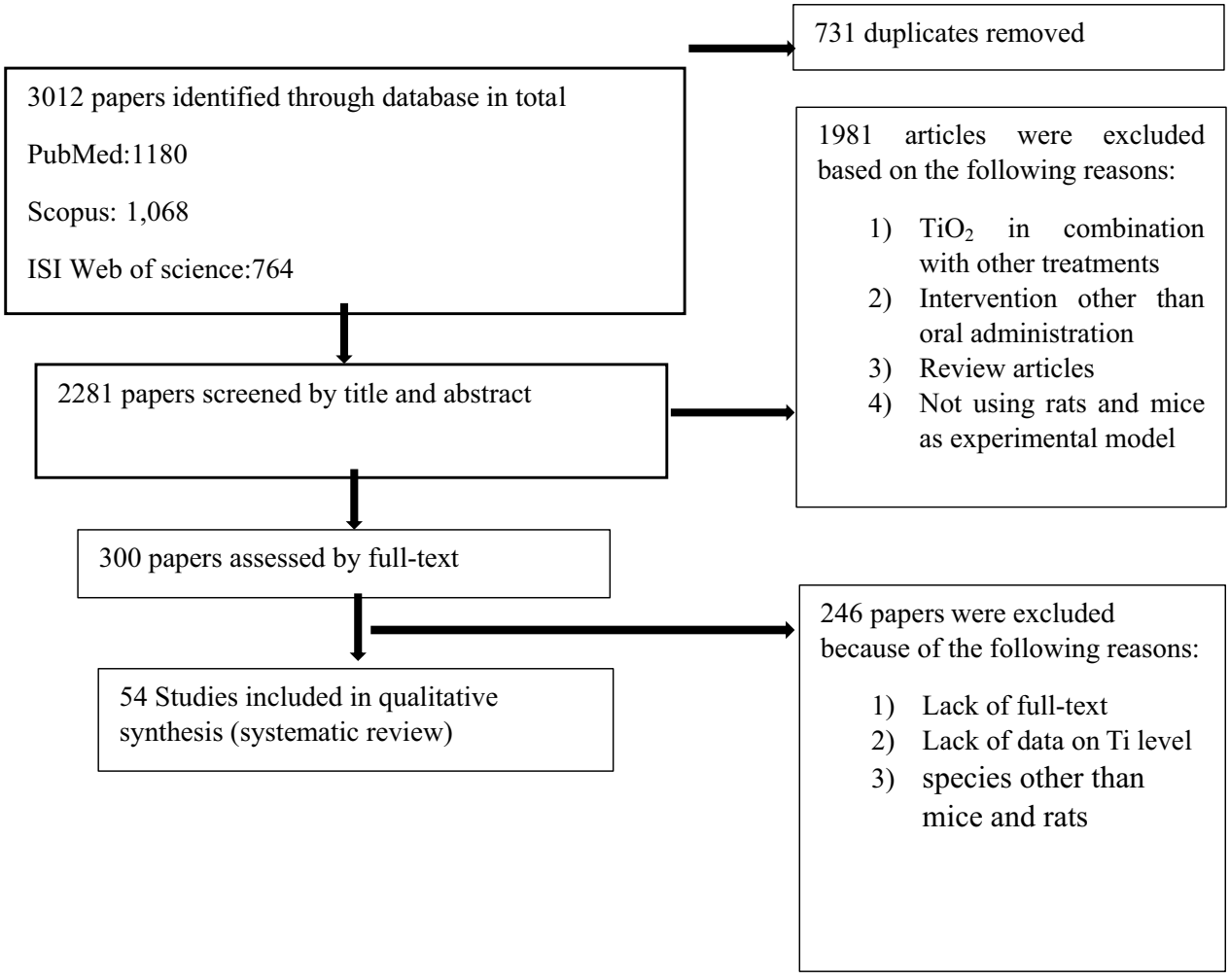

Fig. 1 PRISMA flow diagram of systematic literature search. Overview of the study identification, screening, eligibility assessment, and inclusion process based on PRISMA guidelines

Table 1 Overview of physicochemical characteristics of TiO_2 NPs from 50 selected studies

TiO_2-NP characteristics	Categories*	No. of Studies
Crystalline phase	Anatase	26
	Mixture	9
	Rutile	8
	NS	11
Size (nm)	< 100	42
	> 100	12
Hydrodynamic size (DLS size, nm)	100 >	10
	> 100	17
	NS	27
Specific surface area (SSA, m^2/g)	100 >	15
	> 100	8
	NS	31
Surface charge (mV)	Negative	17
	Positive	8
	NS	29

*NS Not Specified

reporting the crystalline phase in nanoparticle studies, 20% of the research (11 out of 54) did not include this information. Of the studies that included this detail, the anatase phase was the most commonly investigated and featured in 48% (26 studies). In addition, 16 (9 studies) analysed a combination of anatase and rutile, whereas 14 (8 studies) focused exclusively on the rutile phase. Most of the research, or 77 (42 out of 54 studies), focused on TiO_2 NPs smaller than 100 nm. In contrast, 22 (12 studies) examined TiO_2 particles exceeding 100 nm in size, highlighting the widespread interest in the nanoscale properties of TiO_2 for various applications and research. Details about the hydrodynamic size, specific surface area, and surface charge of TiO_2 NPs were provided in 50% (27 out of 54), 42% (23 out of 54), and 46% (25 out of 54) of the reviewed papers, respectively (Table 1). The use of dispersion methods prior to biological assays varied, with 61% (33 out of 54) employing sonication

or ultrasonication. However, 39% (21 out of 54) of the papers did not specify the dispersion techniques used.

Out of the 54 papers reviewed, 16 studies used various rat strains: Sprague–Dawley (n = 11), Wistar (n = 4), and Fisher (n = 1). The remaining 38 studies used different mouse strains: ICR (n = 15), C57Bl/6 (n = 9), Kunming (n = 10), Swiss (n = 2), BALB/c (n = 1), and one study employed genetically modified strains, including WT, db/db, Nrf2^{-}/$^{-}$, and Ces2h^{-}/$^{-}$ mice (n = 1). Twenty-eight studies used only males, 11 studies used only females, 9 studies included both sexes, and six studies did not specify the sex of the subjects. The studies reviewed employed a wide range of doses, from 0.5 mg/kg to 5000 mg/kg body weight per day, highlighting the variability in experimental approaches. Exposure durations varied widely, ranging from a single oral exposure to daily exposure lasting up to 28 weeks (Table 2).

Quality assessment and risk of bias

The results of the methodological quality and risk of bias assessment for the included studies are presented in Fig. 2. Evaluation of the standard SYRCLE domains revealed that, overall, reporting was generally poor, resulting in unclear methodological quality for many studies. Key domains, such as blinding of caregivers and researchers (performance bias) and blinding during outcome assessment (detection bias), were consistently rated as high risk or unclear in the majority of studies. This reflects common practices in animal toxicological research, where blinding is often not reported.

In contrast, most studies showed a low risk of bias in other critical areas, including baseline characteristics (94%), ethical statements (93%), and incomplete outcome data (93%). Sequence generation (randomization) was reported in 59% of the studies, while allocation concealment was rarely reported (4%). The detailed risk of bias assessment for all included studies is provided in Supplementary Table S1.

Furthermore, for the specific aims of this systematic review on titanium accumulation, the assessment of supplementary items revealed that most studies (89%) reported titanium background levels in control animals, but only a minority (37%) explicitly reported the detection limit of the analytical method used to quantify titanium.

Analysis of Ti accumulation in organs and tissues

Ti accumulation was evaluated across various organs in the reviewed studies. The distribution of these studies by organ is as follows: 37 studies focused on the liver, 23 on the kidneys, 22 on the spleen, 14 on the brain, 10 on the lungs, 9 on the cardiovascular system, and 19 on the gastrointestinal tract. Additionally, 5 studies examined the pancreas, 9 investigated the reproductive system (including the uterus, ovaries, and testes), and 9 explored other organs, such as the mesenteric lymph nodes, bones, bladder, thyroid, gastrocnemius muscle, and Peyer's patches. Of the 54 studies reviewed, 29 (54%) reported the tissue amount used for Ti analysis, ranging from 0.01 to 2.0 g. The remaining 25 did not specify tissue quantity. Only 20 out of 54 studies (37%) reported the detection limit for Ti measurements, with variation across studies. The remaining studies did not include this detail. An analysis of the reporting of Ti background levels in control animals, which is essential for confirming accumulation above baseline, revealed that 48 out of 54 studies (89%) provided this fundamental information (Table 2). Study data for each organ were categorized into four groups based on the duration and frequency of TiO_2 particle exposure: Acute Oral Exposure (a single exposure or dose, typically lasting less than 24 h), Subacute Oral Exposure (repeated exposures over up to 28 days), Subchronic Oral Exposure (repeated exposures over a moderate duration, usually 1–3 months or 90 days), and Chronic Oral Exposure (repeated exposures over a prolonged period, often lasting a significant portion of the animal's lifespan—typically from 3 months up to 2 years) [43]. The subsequent paragraphs provide an overview of the findings from these studies.

Gastrointestinal tract

Out of the nineteen studies on gastrointestinal biodistribution of Ti, three investigated acute oral exposure, eleven addressed subacute exposure, three focused on subchronic exposure, and two examined chronic oral exposure. These studies assessed the distribution of titanium across various gastrointestinal regions, revealing significant differences based on particle size, dose, and duration of exposure.

Acute oral exposure In a study investigating Ti particle translocation, a single low dose of 5 mg/kg bw TiO_2 NPs was administered, and titanium concentrations in the gastrointestinal tract were measured over 96 h using ICP-MS. Titanium levels in treated rats were comparable to those in the control group, ranging from 0.06 mg Ti/kg tissue in controls to 0.15 mg Ti/kg tissue in the treatment group [32]. Another study, involving a single oral dose of 100 mg/kg bw via gavage in SD rats (24-h follow-up), found no significant titanium accumulation in the small intestine compared to the control group (~ 2.0 mg Ti/kg tissue in the treatment group vs. 1.8 mg Ti/kg tissue in the control group) [44]. Additionally, a single dose of 40 mg/kg bw TiO_2 NPs was administered, and titanium absorption was analysed at 2, 4, 8, and 24 h in the jejunum, ileum, and colon. Significant accumulation was observed in the jejunum and ileum at 4 h, with a peak titanium absorption of approximately 0.007% in the entire intestine at that

Table 2 Study characteristics

Study	Species	Strain	Dose(s)	Route of exposure	Exposure frequency	Exposure duration	Sex	Age	Time points of analysis	Organs examined for Ti accumulation	Tissue amount	Methods used for Ti quantification	Detection limit	Ti background level (µg/mL)
Jani et al. [28]	Rat	SD	12.5 mg/kg	Oral gavage	Single dose	10 days	Female	12–14 weeks	24 h post-last treatment	Colon, small intestine, stomach, liver, lungs, spleen, heart, kidney; Peyer's patches, mesenteric lymph nodes, peritoneal tissue	NS	ICP-AES	NS	No control group
Wang et al. [29]	Mouse	CD-1	0, 5 g/kg bw	Oral gavage	Once	Single dose	Male and female	NS	2 weeks post-treatment	Liver, spleen, kidney, lung, brain	0.1–0.3 g	ICP-MS	0.074 ng/mL	Liver: 0.08–0.1
Zhang et al. [62]	Mouse	Kunming	5 g/kg	Oral gavage	Daily	7 days	Male and female	6–8 weeks	At the end of 7 days of exposure (post-sacrifice)	Liver, kidney, brain	NS	ICP-MS	NS	Liver: 0.04
Onishchenko et al. [66]	Rat	Wistar	0, 1, and 100 mg/kg	Oral gavage	Daily	28 days	Male	NS	At the end of 28 days of exposure (post-sacrifice)	Liver	NS	ICP-MS	NS	0
Gui et al. [80]	Mouse	CD-1	0, 2.5, and 5 mg/kg bw	Oral gavage	Daily	90 days	Male	5 weeks	At the end of 90 days of exposure (post-sacrifice)	Kidney	~0.1 g	ICP-MS	NS	Control group: 0.38–0.5
Wang et al. [68]	Rat	SD	0, 10, 50, and 200 mg/kg bw/d	Oral gavage	Daily	30 days	Male	3 weeks and 8 weeks	At the end of 30 days of exposure (post-sacrifice)	liver, kidney, spleen	0.1–0.3 g	ICP-MS/ICP-OES	NS	Liver: 0.38; Kidney: 0.29; Spleen: 0.2

Table 2 (continued)

Study	Species	Strain	Dose(s)	Route of exposure	Exposure frequency	Exposure duration	Sex	Age	Time points of analysis	Organs examined for Ti accumulation	Tissue amount	Methods used for Ti quantification	Detection limit	Ti background level (µg/mL)
Cho et al. [30]	Rat	SD	0, 260.4, 520.8, 1041.5 mg/kg bw/d	Oral gavage	90 days	6 weeks 90 days	Male and female	6 weeks	At the end of 90 days of exposure (post-sacrifice)	Liver, spleen, kidney, brain	NS	ICP-MS	0.1–1 ng/L	Control group: 0.456
Geraets et al. [24]	Rat	Wistar	10.2,11.4, 13.1,15.2 mg/kg bw/d	Oral gavage	Daily	5 days	Female and male	9 weeks	24 h post-last treatment	Liver, Spleen, Mesenteric lymph nodes	0.5–1 g	ICP-MS	0.03 µg/g	Control group: ≤ 0.03
Janer et al. [44]	Rat	SD	100 mg/kg bw	Oral gavage	Once	Single dose	Male	NS	24 h post-last treatment	Spleen, Liver, Small intestine, Mesenteric lymph nodes, Peyer's patches	NS	ICP-MS	NS	Control group: 1–3.5
Donner et al. [59]	Rat	SD	0, 500, 1000, 2000 mg/kg bw	Oral gavage	Once	Single dose	Male and female	NS	48 or 72 h post-last exposure	liver	NS	ICP-MS	NS	Liver: 0.29; Kidney: 44
Kim and Park [82]	Rat	SD	0,10, and 100 mg/kg bw/day	Oral gavage	Daily	5 days	Male	NS	At the end of 5 days of exposure (post-sacrifice)	Liver, kidney, and lung	NS	ICP-MS	NS	NS
Pérez-Campaña et al. [87]	Rat	Wistar	10 mg/kg bw	Oral administration	Daily	7 days	Male	Adult	0–4 min, 4–8 min, 36–44 min, 160–200 min, 408–464 min, 10 h	Liver, lungs, kidneys, bladder, stomach, spleen, brain, bones, small intestine, esophagus	NS	ICP-MS	NS	Control group: 0.036
Tassinari et al. [83]	Rat	SD	0, 1, 2 mg/kg bw/d	Oral gavage	Daily	5 days	Male and female	60 days	24 h after the last treatment	Thyroid, Ovary, Uteri, Spleen	NS	ICP-MS	0.009 µg/g	NS
MacNicoll et al. [32]	Rat	SD	0 and 5 mg/kg bw	Oral gavage	Once	Single dose	Male	8 weeks	1–96 h post-administration to 7 days	Liver, Brain, Heart, Kidney, Spleen, GI tract (large and small intestine)	NS	ICP-MS	8 ng/g	Control group: 0.5; Pancreas: 0.7

Table 2 (continued)

Study	Species	Strain	Dose(s)	Route of exposure	Exposure frequency	Exposure duration	Sex	Age	Time points of analysis	Organs examined for Ti accumulation	Tissue amount	Methods used for Ti quantification	Detection limit	Ti background level (µg/mL)
Gu et al. [35]	Mouse	CD-1	0, 64 mg/kg bw	Oral administration using a syringe	Daily	28 weeks	Male	6 weeks	At the end of 28 weeks of exposure (post-sacrifice)	Liver, Pancreas	0.1 g	ICP-OES	NS	Liver: 1; Spleen: 2; Kidney: 2.9; Pancreas: 1; Small intestine: 1.2; Muscle: 0.5
Hu et al. [56]	Mouse	CD-1	0, 0.52, 2.6, 13, 64, 320 mg/kg bw	Oral administration	Daily	14 weeks	NS	6 weeks	At the end of 14 weeks of exposure (post-sacrifice	Liver, Spleen, Small intestine, Gastrocnemius muscle, Kidney, Pancreas	0.2 g	ICP-MS	NS	Liver: 0.00147; Kidney: -0.00085; Brain: -0.00049; Heart: 0.00233; Spleen: 0.00606; GI tract: 0.06769
Mohamed [33]	Mouse	Swiss Webster	0, 5, 50, 500 mg/kg bw/d	Oral gavage	Daily	5 days	Male	NS	1, 7, or 14 days post-treatment	Gastric cells	0.3 g	ICP-MS	NS	Control group: 0
Hu et al. [75]	Mouse	CD-1	64 mg/kg bw/d	Oral gavage	Daily	18 weeks	Male	6 weeks	At the end of 18 weeks of exposure (post-sacrifice)	Liver, pancreas	0.1 g	ICP-MS	NS	Liver: 0.8; Pancreas: 1
Jo et al. [58]	Rat	SD	500 mg/kg TiO_2 NPs (f-TiO_2 and g-TiO_2)	Oral gavage	Once	Single dose	Male	5 weeks	6 h, 1, 2, 3 days post-administration	Kidneys, liver, lungs, spleen	NS	ICP-AES	NS	Liver: 1.9; Kidney: 5.5; Spleen: 5.5; Lung: 5.5
Chen et al. [47]	Mouse	CD-1	2.5 mg/kg bw/day	Oral gavage	Daily	7 days	NS	7 weeks	At the end of 7 days of exposure (post-sacrifice)	Heart, liver, spleen, lung, kidney, stomach, duodenum, ileum, jejunum, colon	NS	ICP-MS	NS	NS
Farrell and Magnuson [64]	Rat	SD	200 ppm (~30 mg/kg bw/day)	Oral administration	Daily	7 days	Male, Female	7–10 weeks	1-, 24-, and 72-h post-exposure	liver, kidney, muscle	NS	ICP-AES	0.1–0.2 mg/kg	< 0.1
Ammendolia et al. [45]	Rat	SD	0,1, and 2 mg/kg bw	Oral gavage	Daily	5 days	Male and female	NS	After 5 days	Small intestine (jejunum histopathology)	NS	ICP-	NS	Control group: 0.08

Table 2 (continued)

Study	Species	Strain	Dose(s)	Route of exposure	Exposure frequency	Exposure duration	Sex	Age	Time points of analysis	Organs examined for Ti accumulation	Tissue amount	Methods used for Ti quantification	Detection limit	Ti background level (µg/mL)
Yang et al. [90]	Mouse	C57/BL6	250 and 500 mg/kg bw	Oral administration	Daily	14 days	Male	5–7 weeks	At the end of 14 days of exposure (post-sacrifice)	liver	NS	ICP-MS	NS	Placenta: 0.08; Foetus: 0.01
Hong et al. [84]	Mouse	CD-1	25, 50, and 100 mg/kg BW	Oral gavage	Daily	Gestational day 0 to 17	Female	NS	End of gestational day 17	Placenta and fetus	0.1 g	ICP-MS	0.074 ng/mL	Liver: 0.00021; Other tissues: < 0.074
Kreyling et al. [109]	Rat	Wistar Kyoto	30–80 µg/kg bw	Oral gavage	Once	Single oral exposure	Female	Adult	1 h, 4 h, 24 h, 7 d post-gavage	Liver, lungs, kidneys, brain, spleen, uterus, skeleton	NS	ICP-MS	NS	0
Morgan et al. [81]	Rat	Albino	100 mg/kg bw	Oral gavage	Daily	Two months	Male	55 days	At the end of 2 months of exposure (post-sacrifice)	Kidney	0.1 g	ICP-MS	0.074 ng/mL	NS
Martins et al. [69]	Rat	Wistar	0, 0.5 mg/kg bw	Oral gavage	Daily	45 days	Male	NS	At the end of 45 days of exposure (post-sacrifice)	Kidney, liver	50–75mg	ICP-MS	NS	Control group: 0
Chen et al. [73]	Rat	SD	0, 2, 10, 50 mg/kg bw	Oral gavage	Daily	90 days	Female	3 weeks	At the end of 90 days of exposure (post-sacrifice)	Liver	1 mL homogenate	ICP-MS	NS	Control group: 0.01
Hu et al. [57]	Mouse	ICR	0, 10, 20, 50, 100, 200 mg/kg bw	Oral gavage	Daily	26 weeks	NS	NS	At the end of 26 weeks of exposure (post-sacrifice)	Liver, pancreas, spleen, kidney, small intestine, muscle	0.1 g	ICP-OES	NS	Liver: 1.8; Pancreas: 1; Spleen: 2; Kidney: 2; Small intestine: 2; Muscle: 1

Table 2 (continued)

Study	Species	Strain	Dose(s)	Route of exposure	Exposure frequency	Exposure duration	Sex	Age	Time points of analysis	Organs examined for Ti accumulation	Tissue amount	Methods used for Ti quantification	Detection limit	Ti background level (µg/mL)
Li et al. [67]	Mouse	C57BL/6	100 mg/kg bw	Oral gavage	Daily	28 days	Male	8 weeks	At the end of 28 days of exposure (post-sacrifice)	Liver, spleen, kidney, lung, heart, brain, jejunum, colon	NS	ICP-MS	NS	Liver: 0.025; Spleen: 0.07; Kidney: 0.012; Lung: 0.05; Brain: 0.012
Vasantharaja and Ramalingam [78]	Rat	Wistar	50, 100 mg/kg bw	Oral gavage	Daily	14 days	Male	NS	24 h after the last treatment	Brain (cerebrum, cerebellum, medulla oblongata)	0.2–0.3 g	ICP-MS	0.074 ng/mL	Brain: Not detected
Chen et al. [74]	Rat	SD	0, 2, 10, 50 mg/kg bw	Oral administration	Daily	90 days	Male and female	3 weeks	At the end of 90 days of exposure (post-sacrifice)	Liver	1 mL	ICP-OES	NS	Liver: 0.0008
Lee et al. 9 [65]	Rat	SD	0, 100, 300, 1000 mg/kg bw	Oral administration	Daily	GDs 6 to 19	Female	9 weeks	On GD 20	Maternal tissues (liver, brain, blood) and fetal tissues (liver, brain, placenta)	200 mg	ICP-MS	0.0002 mg/kg	Liver: 0.2; Brain: 0.4
Talamini et al. [34]	Mouse	NFR	0, 5 mg/kg b	Oral administration – dripped in mouth	3 times/week	3 weeks	Male	NS	3 days after last dose	Stomach, small intestine, large intestine, liver, lung, kidney, brain, testes, spleen	NS	ICP-MS	0.002–0.009 mg/kg	Stomach: 0.4; Small intestine: 0.4; Large intestine: 0.5; Liver: 0.2; Lung: 0.02; Spleen: 0.04
Chen et al. [54]	Mouse	C57BL/6	25 mg/kg bw	Oral gavage	once	Single oral exposure	Male	3 weeks	24 h after exposure	Bone marrow, liver	0.5 g	ICP-MS	0.032 µg/g	Liver: 0.1; Intestine: 0.5; Colon: 1; Spleen: 0; Heart: 0; Lung: 0.1; Kidney: 0.4; Testicle: 0.4
Coméra et al. [17]	Mouse	C57BL/6	40 mg/kg bw	Oral gavage	once	Single dose exposure	NS	12–18 weeks	2, 4, 8 h after single oral administration	Jejunum, ileum, colon	NS	ICP-MS	0.02 mg/kg	0
Grissa et al. [99]	Rat	Wistar	50, 100, 200 mg/kg bw	Oral administration	Five times per week	8 weeks	Male	NS	One day post last treatment	Brain (frontal lobes)	0.25 g	ICP-OES	0.02 mg/L	Brain: 0

Table 2 (continued)

Study	Species	Strain	Dose(s)	Route of exposure	Exposure frequency	Exposure duration	Sex	Age	Time points of analysis	Organs examined for Ti accumulation	Tissue amount	Methods used for Ti quantification	Detection limit	Ti background level (µg/mL)
Hu et al. 0 [76]	Mouse	ICR-1	0, 50 mg/kg bw	Oral gavage	Daily	26 weeks and 8 weeks	Male	3 weeks and 10 weeks	At the end of 26 weeks and 8 weeks of exposure (post-sacrifice)	Liver, pancreas	0.3 g	ICP-OES	0.1 ng/mL	Liver: 0.5–1.2; Pancreas: 0.6–1.0
Yan et al. [51]	Mouse	ICR-1	160 mg/kg bw	Oral gavage	Daily	28 days	Male	NS	12 h after the last treatment	Heart, liver, cerebral cortex, stomach, duodenum, colon	NS	ICP-MS, ICP-OES	NS	Heart: 2; Liver: 1; Cortex: 1.5; Stomach: 1; Duodenum: 0.5; Colon: 1.5
Yao et al. [50]	Mouse	Kunming	300 mg/kg bw, 1200 mg/kg bw	Oral gavage	Daily	28 days	Female	8 weeks	At the end of 28 days of exposure (post-sacrifice)	Ileum, jejunum, duodenum	0.1–0.5 g	ICP-AES	NS	Ileum: 2.5
Chen et al. [85]	Mouse	Kunming	0, 10, 50, 250 mg/kg bw	Oral gavage	Daily	GD3.5 to GD17.5	Female	6–8 weeks	GD18	Placenta, fetus	0.1–0.5 g	ICP-MS	NS	Placenta: 1.5; Foetus: 1.2
Zhao et al. [48]	Rat	SD	100 mg/kg bw	Oral gavage	Daily	14 days	Male	3 weeks	24 h after 14th exposure	Colon, liver	0.2–0.4 g	ICP-MS	NS	Liver: 0.2; Intestine: 1
Han et al. [55]	Rat	SD	0, 10, 100, 1,000 mg/kg	Oral gavage	Daily	90 days	Male and female	5 weeks	At end of 90 days	Colon, spleen, kidneys, stomach	NS	ICP-MS	NS	Colon: 13.94–23.77; Kidney: 8.04–12.61; Kidney: 8.04; Spleen: 7.96–14.90
Mortensen et al. [60]	Rat	SD	10 mg/kg	Oral administration	Daily	Between postnatal days (PND) 7–10	Male and female	PND 2–3	Four hours after administration	Liver, brain, duodenum, jejunum, ileum, and colon	2.58 ng/g	ICP-MS	NS	0
Nie et al. [63]	Rat	SD	150 mg/kg	Oral gavage	Daily	7 days	Female	4 weeks	24 h after the last exposure	liver	0.05–0.1g	ICP-MS	NS	Liver: 2.2
Zhao et al. [71]	Mouse	Kunming	2 mg/kg bw and 20 mg/kg bw	Oral gavage	Daily	8 weeks	Male	5 weeks		Liver	0.4–0.5 g	ICP-AES	NS	Liver: 7

Table 2 (continued)

Study	Species	Strain	Dose(s)	Route of exposure	Exposure frequency	Exposure duration	Sex	Age	Time points of analysis	Organs examined for Ti accumulation	Tissue amount	Methods used for Ti quantification	Detection limit	Ti background level (µg/mL)
Karthika et al. [53]	Rat	SD	5 mg/kg bw	Oral administration	5 days per week	70 days	Male	NS	At the end of 70 days of exposure (post-sacrifice)	Colon	0.1–0.5 g	ICP-MS	NS	Control group: 0
Akagi et al. [52]	Rat	F344/DuCrlCrlj	28-day study: 10, 100, 1000 mg/kg bw/day; 90-day study: 100, 300, 1000 mg/kg bw/day	Oral gavage	Daily	28 days, 90 days	Male and female	6 weeks	At the end of 28 days and 90 days of exposure (post-sacrifice)	Liver, kidneys, spleen, and intestine	NS	ICP-MS	NS	Liver: 0.01; Kidney: 0.03; Spleen: 0.03
Lin et al. [72]	Rat	SD	0, 10, 100, 1000 mg/kg bw/day	Oral gavage	Daily	90 day	Male and female	4 weeks	At the end of 90 days of exposure (post-sacrifice)	Brain, liver, spleen, kidneys, ovary/testis, mesenteric lymph nodes	0.01–2.0g	ICP-MS	NS	Brain: 0.7; Liver: 0.9–1.8; Spleen: 1–1.5; Kidney: 1–1.5; Testis: 0.5; Ovary: 2
Cai et al. [49]	Mouse	WT, db/db, Nrf2$^{-/-}$, Ces2h$^{-/-}$	0.72, 1.8, 18 mg/kg/day	Oral gavage	Daily	21 days	Male	8 weeks	0, 1, 3, 7, 14, 21 days	Heart, liver, spleen, lung, kidney, intestine, brain	NS	ICP-MS	NS	Control group: 0.5
Herrera-Rodríguez et al. [79]	Rat	Wistar	10 mg/kg	Oral	Every other day	3 months	Male	NS	End of study	Heart	0.04–0.3g	ICP-OES	NS	0
Wang et al. [46]	Mouse	ICR	5.74 mg/kg	Oral	Daily	5 days	Male	6 weeks	End of study	Intestine	NS	ICP-MS	NS	Control group: 0.2802
Abd-Elhakim et al. [70]	Rat	SD	50 mg/kg	Oral gavage	Daily	60 days	Male	12 weeks	End of study	Liver	NS	ICP-OES	NS	Control group: 0.16
Zhang et al. [86]	Mouse	C57BL/6	10 mg/kg	Oral gavage	Daily	28 days	Female	7 weeks	End of study	Ovary, Colon	0.01–0.03 g	ICP-AES	NS	NS

NS Not specified

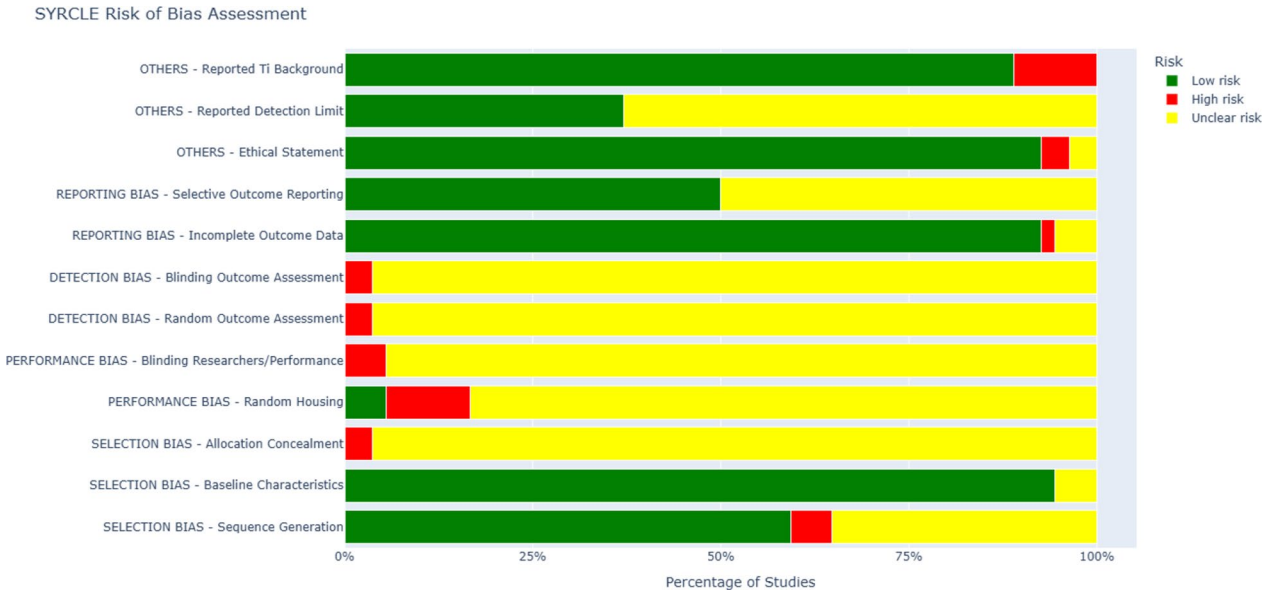

Fig. 2 Risk of bias and quality assessment by means of the SYRCLE's tool. The score (%) represents risk of bias for each component of the tool

time. Titanium concentrations in the jejunum (18 mg Ti/kg tissue) and ileum (38 mg Ti/kg tissue) were significant, but no significant titanium was found in the colon at any time point compared to the control group [17].

Subacute oral exposure In a study, mice were orally administered TiO_2 NPs at doses of 5, 50, or 500 mg/kg bw for five consecutive days. Titanium accumulation in gastric tissue was measured 24 h, 1 week, and 2 weeks after the final treatment. Significant accumulation was observed in the treatment groups compared to controls, with approximately 100 mg Ti/kg tissue detected at the 5 mg/kg dose, 175 mg Ti/kg tissue at the 50 mg/kg dose, and 375 mg Ti/kg tissue at the 500 mg/kg dose. The levels of titanium accumulation were unaffected by the sampling time [33]. Another short-term study exposed animals to nano-sized TiO_2 for 5 days at doses of 1 and 2 mg/kg bw, with a control group receiving ultrapure water. While titanium concentrations in the small intestine were similar in the control (0.08 mg Ti/kg tissue) and 1 mg/kg group (0.09 mg Ti/kg tissue), a significant increase was observed in the 2 mg/kg group (0.13 mg Ti/kg tissue), indicating dose-dependent titanium accumulation [45]. In line with this, another 5-day study reported no significant titanium level in the intestines of mice orally exposed to 5.74 mg/kg TiO_2 NPs, with titanium levels of 2.2 mg/kg tissue in the TiO_2 group versus 2.8 mg/kg tissue in controls [46]. In a 7-day study, CD-1 mice were administered 2.5 mg/kg bw/day of TiO_2 via oral gavage, and titanium content was assessed in the stomach, small intestine, and colon. Trace amounts were detected in the stomach and small intestine of the treated group (0.01–0.02 mg Ti/kg tissue); however, a significant titanium level was observed only in the colon (0.17 mg Ti/kg tissue) compared to the control group [47]. A 10-day study with a 12.5 mg/kg dose found minimal TiO_2 uptake in the small intestine (6.81 mg Ti/kg tissue), representing 0.11% of the administered dose, and in the stomach (6.89 mg Ti/kg tissue), representing 0.06%. High Ti content was detected in the colon, accounting for approximately 4% of the administered dose [28]. In a separate study, a 14-day exposure to 100 mg/kg TiO_2 led to significant titanium accumulation in the colon, measured at 3.5 mg Ti/kg tissue [48]. Oral administration of TiO_2 E171 resulted in a 1.8-fold increase in titanium levels in the colon of mice (1.07 mg Ti/kg tissue vs. 0.6 mg Ti/kg tissue in controls) after 3 weeks of 5 mg/kg bw, given three times a week. However, titanium levels in the small intestine and stomach were not significantly higher than control group [34]. Similarly, in a 21-day oral exposure study using low (0.72 mg/kg/day), medium (1.8 mg/kg/day), and high (18 mg/kg/day) doses of TiO_2 NPs, only slight increases in intestinal titanium levels were observed compared to controls (1.0–1.2 mg Ti/kg tissue vs. 0.5 mg/kg), indicating minimal accumulation [49]. In another study, mice orally exposed to 300 and 1200 mg/kg bw TiO_2 for 28 days showed titanium accumulation in the small intestine, with levels reaching 0.4 and 0.8 mg/kg tissue, respectively, indicating a dose-dependent increase following subacute exposure [50]. Mice exposed daily to 160 mg/kg micro- or nano-TiO_2 for 28 days showed increased Ti accumulation in the gastrointestinal tract compared to controls. Ti levels in the intestine ranged from 0.5 to 1.0 mg/kg (control: 0.6 mg/kg), in the stomach from 1.6 to 1.8 mg/kg (control: 1.1 mg/kg), and in the colon from 1.2 to 1.8 mg/kg (control: 2.1 mg/kg) across the treatment groups, with micro-TiO_2 generally resulting in higher accumulation in the

intestine and stomach, and nano-TiO_2 showing elevated levels in the stomach and colon [51]. In a 28-day study, mice were administered TiO_2 by oral gavage at doses of 10, 100, and 1000 mg/kg bw/day. Titanium accumulation in the intestine was minimal and not statistically significant, with levels ranging from 0.01 to 0.05 mg/kg tissue across all treatment groups [52].

Subchronic oral exposure A study involving the oral administration of TiO_2-NPs (5 mg/kg bw/d) to rats for 70 days showed that titanium predominantly accumulated in the colon, with a concentration of 5.1 mg Ti/kg tissue [53]. In a 90-day study involving repeated low-dose TiO_2 NP exposure (0, 2, 10, and 50 mg/kg), a significant increase in titanium levels was observed only in the colon of rats treated with 50 mg/kg, where titanium levels reached 2 mg Ti/kg tissue compared to 0.1 mg Ti/kg tissue in the control group. No clear dose–response relationship was noted, and titanium accumulation in the small intestine was minimal [54]. In a 90-day oral exposure study, rats were administered E171 at doses of 0, 10, 100, or 1,000 mg/kg bw/day, and titanium accumulation was measured in the colon of both sexes. Titanium levels in the colon were around 15 mg/kg tissue in controls, with minimal changes at 10 and 100 mg/kg/day (15–23 mg/kg tissue). However, levels rose sharply at 1,000 mg/kg/day, reaching up to 88 mg/kg. In females, titanium increased from 13 mg/kg tissue (control) to 70 mg/kg at the highest dose [55].

Chronic oral exposure In a 14-week study, mice in both the low-dose (64 mg/kg) and high-dose (320 mg/kg) groups showed dose-dependent increases in TiO_2 accumulation in the small intestine, with levels of 5.2 and 5.6 mg Ti/kg tissue, respectively [56]. Consistently, a 26-week study with ICR mice administered TiO_2 NPs via oral gavage at doses of 10 and 50 mg/kg bw/day showed significant titanium accumulation in the small intestine. Titanium levels in the small intestine were approximately ~ 3.5 mg Ti/kg tissue at the 10 mg/kg dose and ~ 5 mg Ti/kg tissue at the 50 mg/kg dose [57].

To summarize, Ti accumulation in the gastrointestinal tract is influenced by dose, with the colon being the site where accumulation is most consistently observed during subacute and subchronic exposures.

Liver

Thirty-seven studies have explored the biodistribution of Ti in the liver, with seven examining acute exposure, fifteen investigating subacute exposure, ten focusing on subchronic exposure, and five analysing chronic exposure. This section will summarise the findings of these studies, highlighting the differences observed in Ti accumulation across varying exposure durations and doses.

Acute oral exposure Significant liver accumulation of Ti was observed in mice following a single oral gavage dose of 5 g/kg bw of 80 nm TiO_2 NPs, with liver titanium concentrations reaching 4 mg Ti/kg tissue. In contrast, lower and statistically insignificant titanium concentrations were detected in groups exposed to 25 nm TiO_2 NPs (1.1 mg Ti/kg tissue) and fine TiO_2 particles (1.1 mg Ti/kg tissue), compared to 0.1 mg Ti/kg tissue in the control group [29]. Other studies reported no significant Ti retention in the liver following acute exposure to moderately low doses. For instance, no evidence of Ti translocation or liver accumulation was found after a single oral gavage dose of 5 or 100 mg/kg TiO_2 NPs. Similarly, a single dose of 500 mg/kg of TiO_2 NPs administered to rats did not lead to elevated liver titanium concentrations at 6 h, 1 day, 2 days, or 3 days post-administration [32, 44, 54, 58]. Another study found that rats exposed to 2.3 mg/kg bw of TiO_2 NPs for 1 or 5 days had liver titanium levels below the detection limit of 0.03 mg Ti/kg tissue [24]. In an experiment with six types of TiO_2 (nanoparticle and pigment-grade forms), no dose-dependent increase in liver titanium content was detected in rats given single doses of 0, 500, 1000, or 2000 mg/kg bw, with mean concentrations ≤ 0.364 mg Ti/kg tissue at 48 or 72 h post-exposure [59].

Subacute oral exposure Rats exposed to 10 mg/kg bw of TiO_2 E171 for three consecutive days, or to TiO_2 NPs at 10 and 100 mg/kg bw/day for 5 days, showed only a minimal increase in liver titanium levels (up to 0.3 mg/kg) compared to vehicle controls [60, 61]. However, significant Ti liver accumulation was observed in rats exposed to 50 nm or 120 nm TiO_2 NPs at 5 g/kg bw/day for 7 days, with levels reaching 0.9 and 0.6 mg Ti/kg tissue, respectively for each group [62]. Elevated liver titanium levels (3.8 mg Ti/kg tissue) were also reported in rats exposed to 150 mg/kg bw/day TiO_2 NPs for 7 days, compared to 2.2 mg Ti/kg tissue in controls [63]. Other studies found no detectable liver titanium in rats exposed to 200 mg/kg TiO_2 NPs or no significant changes in mice exposed to 2.5 mg/kg bw/day TiO_2 NPs during the same period [47, 64]. Rats exposed to 12.5 mg/kg bw per day of TiO_2 NPs for 10 days showed moderate uptake, with liver concentrations reaching 2.4% of the administered dose [28]. Significant liver accumulation was observed in rats exposed to 50 and 100 mg/kg bw/day of TiO_2 NPs for 7 and 14 days, respectively, with titanium levels reaching 1.2 and 3.7 mg Ti/kg tissue, compared to 0.3 mg/kg in the control group [48]. Likewise, Pregnant rats exposed to 1000 mg/kg bw/day TiO_2 NPs during gestation exhibited elevated liver accumulation (0.8 mg Ti/kg tissue) compared to controls (0.2 mg Ti/kg tissue), while no significant increase was observed at lower doses (100 and 300 mg/kg bw/day) [65]. Studies with longer exposure durations (21–28 days) also produced mixed findings. In rats exposed to 100 mg/kg bw

per day of three types of TiO_2 (Rutile NPs, Anatase NPs, and E171) for 28 days, liver titanium accumulation significantly increased to 0.94 mg Ti/kg tissue only in the Rutile NPs group. The control group showed a concentration of 0.46 mg Ti/kg tissue, while the Anatase NPs and E171 groups did not differ significantly from the control [66]. Also, elevated liver titanium levels (0.94 mg Ti/kg tissue) were observed in mice treated with repeated oral doses of E171 at 5 mg/kg bw, three times per week for 3 weeks, representing a 1.8–3.6-fold increase over the control group [34]. Consistent with these findings, another 21-day study showed a significant, dose-dependent increase in liver titanium content following oral administration of TiO_2 at 0.72, 1.8, and 18 mg/kg/day, with liver Ti levels rising from 0.5 mg/kg tissue in controls to 1.0 mg/kg tissue in the low and middle dose groups, and ~ 1.5 mg/kg tissue in the high-dose group [49]. In contrast, no significant titanium accumulation in the liver was observed following prolonged TiO_2 exposure in other studies. For example, titanium levels remained unchanged in mice exposed to 160 mg/kg bw TiO_2 NPs for 28 days, and similar results were reported in rats exposed to 100 mg/kg bw/day TiO_2 NPs for the same duration [24, 51, 67].

Subchronic oral exposure In a repeated oral exposure study, male and female rats were given TiO_2 NPs at doses of 10, 100, and 1,000 mg/kg bw/day for 28 days, and 100, 300, and 1,000 mg/kg bw/day for 90 days. In the 28-day study, only female rats in the 1,000 mg/kg group showed a slight increase in liver titanium levels (0.03 mg/kg tissue) compared to controls (0.01 mg/kg tissue), with other groups showing no meaningful differences. Similarly, in the 90-day study, an increase was observed in one female rat at 1,000 mg/kg (0.09 mg/kg vs. 0.02 mg/kg tissue in controls), while other doses remained comparable to control levels [52]. In a 30-day study on rats exposed to TiO_2 NPs, no significant changes in liver titanium levels (0.1–0.4 mg Ti/kg tissue) were observed across control and treatment groups at doses of 10, 50, or 200 mg/kg bw/day [68].

In a separate 45-day study, a significant increase of 2.1 mg Ti/kg tissue of titanium in liver tissue was observed in rats exposed to 0.5 mg/kg bw/day of TiO_2 NPs via oral gavage [69]. In a 60-day study, rats orally exposed to 50 mg/kg bw/day of TiO_2 NPs showed a significant increase in hepatic titanium levels, reaching 0.98 mg/kg compared to 0.16 mg/kg in controls [70]. In an 8-week study, mice exposed to 20 mg/kg bw of TiO_2 NPs showed substantial titanium accumulation in the liver, reaching 7 mg Ti/kg tissue [71].

Differing from this, a 90-day study found no increase in liver titanium content in rats exposed to TiO2 NPs at doses of 10, 100, and 1,000 mg/kg bw/day; titanium levels remained consistent across all groups [72]. Additionally, very low and insignificant liver accumulation was observed in rats exposed to 2, 10, or 50 mg/kg bw TiO_2 NPs for 30 or 90 days [54, 73, 74]. Also, rats exposed to TiO_2 NPs at doses of 260.4, 520.8, or 1041.5 mg/kg bw/day for 91 days showed slight, non-significant liver accumulation, with titanium concentrations of approximately 0.10 mg Ti/kg tissue in controls and 0.12 mg Ti/kg tissue in all exposed groups [30].

Chronic oral exposure In a 14-week study, significant liver accumulation was observed in mice administered TiO_2 NPs, with titanium concentrations reaching 2.5 mg Ti/kg tissue at a dose of 64 mg/kg bw/day and 4.5 mg Ti/kg tissue at a dose of 320 mg/kg bw/day [56]. Also, an 18-week oral gavage study, mice were administered 64 mg/kg bw/day of TiO_2 NPs, resulting in considerable titanium accumulation in the liver. The liver titanium concentration in treated mice was 5.34 mg Ti/kg tissue, compared to undetectable levels in the control group, indicating a substantial increase in titanium following exposure [75]. In a 26-week study, mice were exposed to 10 and 50 mg/kg bw/day of TiO_2 NPs, leading to significant liver titanium concentrations of 2 mg Ti/kg tissue in the 10 mg/kg group and 3 mg Ti/kg tissue in the 50 mg/kg group [57]. A study involving 26-week and 8-week oral gavage exposures to TiO_2 at doses of 0 and 50 mg/kg bw/day revealed elevated titanium retention in the liver at both time points. In the 26-week exposure, liver titanium concentrations were approximately 1.2 mg Ti/kg tissue in the control group (0 mg/kg) and 2.2 mg Ti/kg tissue in the 50 mg/kg group. Similarly, in the 8-week exposure, liver titanium levels were around 0.6 mg Ti/kg tissue in the control group and 1.1 mg Ti/kg tissue in the 50 mg/kg group, indicating a dose-dependent increase in titanium accumulation with prolonged exposure [76]. In a different study, male CD1 mice were exposed to 0 and 64 mg/kg bw/day of TiO_2 in both its FP (130 nm) and NP (21 nm) forms for 28 weeks. The results revealed liver titanium concentrations of around 0.6 mg Ti/kg tissue in the control group and the 64 mg/kg bw/day TiO_2 FP (130 nm) group. In comparison, the group exposed to TiO_2 NPs (21 nm) at the same dose showed a marked increase in liver titanium levels, reaching 1.9 mg Ti/kg tissue. This highlights the differential accumulation based on the form of TiO_2 [35].

Generally, the findings from these studies indicate a complex pattern of Ti accumulation in the liver, with significant accumulation observed at higher doses and with prolonged exposure. However, several studies report no substantial changes in liver titanium levels.

Brain

The accumulation of Ti in the brain has been investigated under different exposure durations, including acute, subacute, and subchronic oral exposure, with three, eight,

and three studies, respectively. The subsequent paragraph highlights the detailed outcomes of these studies.

Acute oral exposure The distribution of three TiO_2 NP variants, anatase (40 nm), rutile (40–50 nm), and anatase (120 nm), in male Sprague–Dawley rats was examined after a single oral dose of 5 mg/kg bw, and tissue accumulation was assessed over a 2 to 96-h period. No significant differences in nanoparticle distribution were found among the TiO_2 variants, and brain accumulation remained comparable to that of the water-treated control group [32]. Trace amounts of titanium in the brain were also observed in rodents following acute exposure to 30–80 μg of TiO_2 NPs [77]. On the other hand, a single dose of 5 g/kg bw of TiO_2 suspensions (80 nm) led to a significant accumulation of ~ 0.62 mg Ti/kg tissue in the brains of mice, whereas no accumulation was observed in the groups treated with TiO_2 particles of 25 nm or 155 nm [29].

Subacute oral exposure In a study assessing repeated exposure to TiO_2 NPs (50 nm), mice were administered a dose of 5 g/kg bw/day for 7 days. The results showed a significant increase in titanium accumulation, with levels reaching 0.1 mg Ti/kg tissue in the cortex and hippocampus of the TiO_2-50 (50 nm) group. However, no significant changes were observed in the TiO_2-120 group (120 nm), and titanium levels were generally lower in the TiO_2-120 nm group compared to the TiO_2-50 nm group [62]. In a separate study, significant dose-dependent titanium accumulation was observed in various brain regions of rats exposed to 50 mg/kg and 100 mg/kg bw/day for 14 days. Specifically, in the cerebrum, titanium levels were 0.17 mg Ti/kg tissue at 50 mg/kg and 0.22 mg Ti/kg tissue at 100 mg/kg. In the cerebellum, levels were 0.16 mg Ti/kg tissue at 50 mg/kg and 0.22 mg Ti/kg tissue at 100 mg/kg. For the medulla oblongata, levels were 0.14 mg Ti/kg tissue at 50 mg/kg group and 0.16 mg Ti/kg tissue at 100 mg/kg group. These increases in titanium levels were correlated with histological changes, including neurocyte calcification and ependyma proliferation. No detectable titanium was found in the brain regions of the control group [78]. Pregnant SD rats orally exposed to 1000 mg/kg bw TiO_2 NPs from gestational days 6 to 19 also exhibited significant accumulation of 1.1 mg Ti/kg tissue in the maternal brain [65]. However, no meaningful increase in titanium accumulation was reported in the brain following 28 days of oral gavage with 100 mg/kg bw/day of TiO_2 NPs [67]. Consistent with this, in a 21-day study, mice orally treated with TiO_2 NPs at doses of 0.72, 1.8, and 18 mg/kg/day exhibited only minor increases in brain titanium levels, ranging from approximately 0.5 mg Ti/kg tissue in controls to 0.8 mg Ti/kg tissue in the low- and middle-dose groups, and 1.0 mg Ti/kg tissue in the high-dose group [49]. Also, in mice treated with repeated oral doses of E171 at 5 mg/kg bw, three times per week for 3 weeks, brain titanium content was below the limit of quantification (LOQ) of 0.03 mg Ti/kg tissue [34]. Furthermore, a dose of 160 mg/kg bw/day of TiO_2 for 28 days led to an insignificant increase in titanium levels in the cerebral cortex of mice, from 0.7 mg Ti/kg tissue in the control group to 2.6 mg/kg in the micro-TiO_2 group and 2.4 mg/kg in the nano-TiO_2 group [51]. Scant titanium accumulation (0.01–0.05 mg/kg tissue) was observed in the brains of rats after 28 days of oral exposure to TiO_2 NPs at doses of 10, 100, and 1000 mg/kg bw/day [52].

Subchronic oral exposure In an investigation of long-term exposure, rats were administered TiO_2 NPs for 8 weeks, during which significant accumulation was observed in the frontal lobe. Titanium levels increased in a dose-dependent manner, suggesting that higher doses of TiO_2 NPs contribute to greater titanium deposition in the brain. At a dose of 50 mg/kg bw, the titanium concentration was 0.7 mg Ti/kg tissue, rising to 0.8 mg Ti/kg tissue at 100 mg/kg bw and peaking at 5.3 mg Ti/kg tissue at 200 mg/kg bw, indicating substantial TiO_2 accumulation at higher doses. No titanium was detected in the control group [24]. Similarly, rats exposed to 260.4, 520.8, or 1041.5 mg/kg bw/day of TiO_2 NPs for 90 days showed very low and insignificant accumulation in the brain [30]. No significant increase in brain TiO_2 content was observed in rats following 90 days of oral gavage at doses of 10, 100, and 1000 mg/kg bw [72].

In summary, Ti accumulation in the brain is influenced by exposure duration and dose, with acute exposures generally resulting in minimal accumulation, while subacute and subchronic exposures demonstrate significant, dose-dependent deposition. Particularly, histological changes, including neurocyte calcification and ependyma proliferation, were associated with higher doses, though some studies reported undetectable titanium levels even after prolonged exposure.

Heart

A limited number of studies have focused on Ti accumulation in the heart following oral exposure, with two examining acute exposure, five subacute, and two subchronic.

Acute oral exposure The biodistribution of three types of TiO_2 NPs (anatase 40 nm, rutile 40–50 nm, and anatase 120 nm) was studied in male SD rats following a single oral gavage dose of 5 mg/kg bw over 96 h. The study found no significant increase in titanium levels in the heart compared to the control group treated with water [32]. Also, Rodents acutely exposed to 30–80 μg of TiO_2 NPs exhib-

ited detectable, though minimal, titanium levels in heart tissue [77].

Subacute oral exposure In a 7-day study, daily oral gavage of 2.5 mg/kg/day of TiO_2 NPs to mice showed no significant findings in heart tissue [47]. Furthermore, trace amounts of titanium were detected in the hearts of rats following the administration of 12.5 mg/kg TiO_2 NPs for 10 days (0.06% of the administered dose), but these levels were not statistically significant [28]. Also, a 21-day oral exposure study using 0.72, 1.8, and 18 mg/kg/day of TiO_2 NPs showed no meaningful dose-dependent increase in heart titanium content, with all exposed groups having approximately 1.0 mg/kg tissue compared to 0.5 mg/kg tissue in controls [49]. Titanium accumulation in the heart ranged from below detection limits to 0.0006 mg Ti/kg tissue, depending on the dose administered (30–80 µg/kg bw) for 7 days [77]. Furthermore, mice administered 10 or 50 mg/kg bw of TiO_2 NPs for 30 consecutive days showed only slight, non-significant changes in titanium levels in the heart, with values ranging from 2.1 mg/kg in controls to 1.2 mg/kg in the micro-TiO_2 group and 1.8 mg/kg in the nano-TiO_2 group [51].

Subchronic oral exposure In a 90-day study, daily administration of 50 mg/kg TiO_2 resulted in very low titanium content in the heart, indicating negligible accumulation [54]. In another 3-month study, rats administered E171 at 10 mg/kg bw every other day showed no detectable titanium accumulation in the heart [79].

In conclusion, despite the varied doses and exposure durations, Ti accumulation in the heart is consistently minimal across acute, subacute, and subchronic oral exposures.

Kidney

Regarding kidney Ti biodistribution, three studies focused on acute exposure, nine on subacute exposure, nine on subchronic oral exposure, and two on chronic exposures. These findings are further detailed in the following paragraphs.

Acute oral exposure Several studies have examined TiO_2 NPs accumulation in the kidneys following acute oral exposure. A study examining the biodistribution of TiO_2 NPs of different sizes (25 nm, 80 nm, and 155 nm) in mice following a single oral gavage dose of 5 g/kg bw and a 2-week follow-up period found significant accumulation of TiO_2 in the kidneys. The titanium content in the kidneys was ~ 0.2 mg Ti/kg tissue in both the control group and the 155 nm NP group. In comparison, significantly higher levels of titanium were found in the kidneys of the 25 nm NP group (~ 0.4 mg Ti/kg tissue) and the 80 nm NP group (~ 0.45 mg Ti/kg tissue) [29]. Another study reported an increase in titanium levels in the kidneys (2.3 mg Ti/kg tissue) six hours after a single oral dose of 500 mg/kg bw TiO_2. However, these elevated levels returned to baseline (0.6 mg Ti/kg tissue) within 3 days post-administration [58]. Trace titanium levels were also found in the kidneys 96 h after a single oral gavage dose of 5 mg/kg bw TiO_2 [32].

Subacute oral exposure In a 5-day study of rats, no significant changes in kidney titanium levels were observed, increasing only from 0.4 mg/kg in the control group to 0.9 mg/kg in animals exposed to TiO_2 NPs at doses of 10 and 100 mg/kg/day [61]. Another study with mice exposed to 50 nm TiO_2 at 5 g/kg bw for 7 days showed substantial accumulation of titanium in the kidneys (0.86 mg Ti/kg tissue). In contrast, the 120 nm TiO_2 group showed lower accumulation (0.63 mg Ti/kg tissue) [62]. Titanium levels in the kidneys were below detection limits (< 0.2 and 0.0002 mg Ti/kg tissue) in rats exposed to 200 mg/kg TiO_2 for 7 days [64], and in rats exposed to 30–80 µg/kg bw for 7 days [77]**.** Similarly, no measurable increase was observed in mice exposed to 2.5 mg/kg/day TiO_2 for 7 days [47]. No TiO_2 particles were detected in the kidneys of rats exposed to 12.5 mg/kg bw/day TiO_2 NPs for 10 days [28]. In mice subjected to a 21-day oral gavage of TiO_2 nanoparticles at doses of 0.72, 1.8, and 18 mg/kg/day, kidney titanium levels showed only slight increases, with values ranging from approximately 0.5 mg Ti/kg tissue in controls up to 1.2 mg Ti/kg tissue in the highest dose group [49]. Additionally, mice receiving repeated administrations of E171 (5 mg/kg bw, three times per week for 3 weeks) showed no evident increase [34]. However, a significant accumulation of 0.15 mg Ti/kg tissue was reported in mice treated with 100 mg/kg bw/day rutile TiO_2 NPs for 28 days, while no accumulation was found in the anatase group under the same conditions [67].

Subchronic oral exposure Subchronic exposure to 0.5 mg/kg bw TiO_2 NPs for 45 days resulted in 2.8 mg Ti/kg tissue accumulation in the kidneys [69]. In another study where rats were exposed to 200 mg/kg bw TiO_2 NPs daily for 30 consecutive days, no significant titanium accumulation (0.1–0.4 mg Ti/kg tissue) was observed in the kidneys of either control or treated groups [68]. A substantial increase in kidney Ti levels was observed in mice following 90 days of exposure, with concentrations recorded at 0.1 mg Ti/kg tissue for a dose of 2.5 mg/kg bw, 0.2 mg Ti/kg tissue for 5 mg/kg bw, and 0.36 mg Ti/kg tissue for 10 mg/kg bw [80]. In a study with rats exposed to 100 mg/kg bw TiO_2 for 2 months, significant accumulation of titanium was observed in the kidneys (0.3 mg Ti/kg tissue) [81]. However, in a study with rats exposed to TiO_2 NPs at 260.4, 520.8, and 1,041.5 mg/kg bw/day for

13 weeks, no increase in accumulation in the kidneys was observed [30].

Furthermore, administration of 2, 10, and 50 mg/kg bw TiO_2 NPs [54], and 10, 100, and 1000 mg/kg bw food-grade TiO_2 [55, 72] for 90 days did not result in significant accumulation of these particles in the kidneys of rats relative to the control group. Similarly, administration of 10, 100, and 1000 mg/kg bw/day for either 28 days or 90 days did not elevate Ti concentrations in the kidneys of rats [52].

Chronic oral exposure A study investigating the effects of 64 and 320 mg/kg TiO_2 NPs in mice over a 14-week period showed a dose-dependent increase in titanium accumulation in the kidneys, with levels of 4.5 mg Ti/kg tissue in the 64 mg/kg group and 4.9 mg Ti/kg tissue in the 320 mg/kg group [56]. Likewise, a study examining chronic exposure reported significant titanium accumulation in the kidneys of mice administered 10 and 50 mg/kg bw/day for 26 weeks, with concentrations of approximately 4 mg Ti/kg tissue at both doses [57].

In summary, kidney titanium levels generally remain low, typically falling within the range of background levels (often < 0.5 mg Ti/kg tissue) or showing only minor increases (e.g., < 1.0 mg Ti/kg tissue) in many acute, subacute, and subchronic exposure studies. Noticeable accumulation (often > 2 mg Ti/kg tissue) was observed primarily after high-dose acute or long-term chronic exposures, while intermediate-term studies frequently showed limited uptake.

Lung

The titanium content in the lungs following oral TiO_2 exposure in rodents was evaluated in two acute, seven subacute, and one subchronic study.

Acute oral exposure The biodistribution of three TiO_2 NPs sizes (25 nm, 80 nm, and 155 nm) was studied in a SD rat model following a single oral gavage at a dose of 5 g/kg. Significant particle accumulation was detected in the lungs, with the extent of accumulation varying by particle size. Titanium concentrations were measured at ~ 0.15 mg Ti/kg tissue for 25 nm particles, ~ 0.17 mg Ti/kg tissue for 80 nm particles, and ~ 0.22 mg Ti/kg tissue for 155 nm particles [29]**.** Moreover, elevated titanium concentrations were observed in the lungs of rats six hours after a single oral dose of 500 mg/kg of food-grade TiO_2 particles. Although these levels decreased after 24 h, they remained significantly higher than those in the control group [58].

Subacute oral exposure In a 5-day toxicity study, rats administered TiO_2 NPs by oral gavage at doses of 10 and 100 mg/kg bw showed no significant change in lung titanium levels, which increased slightly from 0.2 mg/kg in controls to 0.4 mg/kg in the highest dose group [82]. Similarly, a 7-day exposure to 2.5 mg/kg/day of TiO_2 NPs resulted in no measurable changes in titanium levels in the lungs of mice [47]. Furthermore, minimal Ti levels (ranging from below detection to 0.0023 mg/kg of tissue) were found in the lungs of rats following a 30–80 µg/kg bw dose for 7 days [77]. A moderate degree of uptake (1.2% of the administered dose) was observed in the lungs of rats after a 12.5 mg/kg bw dose for 10 days [28]. No appreciable increase in titanium levels (0.4 vs. 0.3 mg/kg in controls) was reported in the lungs of mice following administration of 5 mg/kg TiO_2 E171 doses three times per week for 3 weeks [34]. In a 21-day study, oral administration of TiO_2 NPs to mice at doses of 0.72, 1.8, and 18 mg/kg/day resulted in increased lung titanium levels, rising from about 0.5 mg Ti/kg tissue in control animals to 1.0 mg Ti/kg tissue in the low- and middle-dose groups, and reaching 1.5 mg Ti/kg tissue in the high-dose group [49]. In contrast, oral administration of 100 mg/kg/day rutile TiO_2 NPs for 28 days led to significantly higher titanium deposition in the lungs of the rutile-treated group, whereas no significant accumulation was observed in the anatase-treated group [67].

Subchronic oral exposure Subchronic exposure studies show minimal titanium accumulation in lung tissue, even after prolonged oral exposure. For instance, negligible Ti content was reported in the lungs of mice following daily oral administration of 50 mg/kg bw/d of TiO_2 NPs for 90 days [54].

The degree of Ti accumulation in the lungs varies, with significant uptake observed under some acute and subacute exposure conditions, nevertheless most results show insignificant accumulation.

Pancreas

Very few studies have investigated titanium levels in the pancreas after oral exposure, comprising one subchronic and four chronic studies. The results from these studies are summarized below.

Chronic oral exposure Chronic exposure studies also reported significant Ti accumulation in the pancreas. In one study, after 14 weeks of exposure to 64 and 320 mg/kg bw/day of TiO_2 NPs, titanium concentrations in the pancreas were measured at 2.3 and 2.6 mg Ti/kg tissue, respectively [56]. Mice treated with 64 mg/kg bw/day of TiO_2 NPs for 18 weeks exhibited significant titanium accumulation, with concentrations of 2.3 mg Ti/kg tissue in the pancreas [75]. In addition, exposure to 10 or 50 mg/kg bw/day of TiO_2 NPs for 26 weeks resulted in titanium concentrations of 1.9 and 2 mg Ti/kg tissue in the corresponding groups [57]. Further investigations with 50 mg/kg bw TiO_2 NPs over both 8-week and 26-week periods

showed dose-dependent accumulation in the pancreas. In the 26-week study, Ti concentrations in the pancreas were approximately 1.0 mg Ti/kg tissue in the control group and 2.8 mg Ti/kg tissue in the TiO_2 group. Similarly, for the 8-week exposure, titanium levels were about 0.7 mg Ti/kg tissue in the control group and 1.3 mg Ti/kg tissue in the TiO_2 group [76].

Lastly, a 28-week study with mice administered 64 mg/kg bw/day of TiO_2 NPs (with particle sizes < 100 nm) also revealed significant Ti accumulation in the pancreas, with concentrations of 2.3 mg Ti/kg tissue. In comparison, titanium levels in the pancreas of mice exposed to fine particles (FP) were similar to the control group and lower than those in the NP group [35].

These findings suggest a consistent dose-dependent accumulation of Ti in the pancreas following chronic exposure.

Reproductive system

Seven studies have measured titanium distribution and accumulation in the reproductive system of both male and female rodents, including seven studies focusing on subacute exposure and two on subchronic exposure. The following section delves into these results in greater detail.

Subacute oral exposure A significant increase in Ti tissue levels (0.28 mg Ti/kg tissue) was found in the ovaries of rats treated with 2 mg/kg bw TiO_2 NPs per day following short-term exposure (5 days) compared to the control group (0.12 mg Ti/kg tissue) [83]. Titanium accumulation in the uterus ranged from below the detection limit to 0.0005 mg Ti/kg tissue in rats after 7 days of exposure to a range of 30–80 µg/kg bw TiO_2 NPs [77]. An insignificant, dose-dependent increase in placental titanium levels was observed in pregnant rats orally exposed to TiO_2 NPs for 13 days (gestational days 6–19), with levels measured at 0.2, 0.2, 0.4, and 0.7 mg Ti/kg tissue at doses of 0, 100, 300, and 1000 mg/kg bw/day, respectively [65]. Titanium levels in the testes were reported as below the limit of quantification (LOQ) of 0.03 mg Ti/kg tissue) in mice treated with 5 mg/kg bw (3 days/week) for 3 weeks [34]. In pregnant mice administered 25, 50, or 100 mg/kg bw TiO_2 NPs daily from gestational day 0 to 17, significant titanium accumulation was observed in both placentas and foetuses. Placental Ti levels increased from 0.015 mg Ti/kg tissue (25 mg/kg bw/d) to 0.035 mg Ti/kg tissue (100 mg/kg bw/d), while fetal Ti levels ranged from 0.01 mg Ti/kg tissue (25 mg/kg bw/d) to 0.025 mg Ti/kg tissue (100 mg/kg bw/d) [84].

In mice with gestational diabetes mellitus (GDM) administered 250 mg/kg TiO_2 NPs orally for 14 days, significant increases in Ti content were observed in the placenta (3 mg Ti/kg tissue) and foetus (2 mg/kg) on gestational day 18, while no significant changes were observed in lower dose groups (10 and 50 mg/kg BW) [85]. In another study, 4 weeks of oral exposure to 10 mg/kg TiO_2 NPs in mice did not lead to a notable increase in titanium levels in the ovary [86].

Subchronic oral exposure In rats treated with 50 mg/kg bw/day TiO_2 NPs for 90 days, titanium concentration in the testicles was reported as very low, indicating minimal accumulation [54]. No significant differences in Ti content were observed in the ovaries and testes between the control and 1000 mg/kg bw/day groups after 90 days, indicating that TiO_2 NPs did not distribute significantly in the ovary/testis [72].

Ti accumulation in the reproductive organs is observed at varying levels, with significant accumulation in the ovaries following subacute exposure. However, long-term exposure studies show minimal Ti distribution in the ovaries and testes.

Spleen

Titanium biodistribution studies in the spleen span a range of exposure durations, including seven acute, eight subacute, five subchronic, and two chronic oral exposure studies. The following sections provide detailed findings for each exposure type.

Acute oral exposure A single oral gavage of TiO_2 at doses of 5, 25,100, and 500 mg/kg bw led to only limited titanium presence in the rat spleen [32, 44, 54, 58]. Acute exposure to 30–80 µg/kg bw of TiO_2 NPs also resulted in very low titanium concentrations being detected in the spleens of rodents [77]. Similarly, titanium concentrations in the spleens of rats exposed to 2.3 mg/kg bw TiO_2 for one or five consecutive days remained below the detection limit of 0.03 mg Ti/kg tissue [24]. In contrast, a single oral gavage dose of 5 g/kg bw TiO_2 NPs (25 nm, 80 nm, 155 nm) caused significant spleen titanium accumulation in mice. The measured levels were ~ 0.55 mg Ti/kg tissue for 25 nm particles, ~ 0.5 mg/kg for 80 nm, and ~ 0.6 mg Ti/kg tissue for 155 nm particles, compared to ~ 0.2 mg Ti/kg tissue in the control group [29].

Subacute oral exposure Subacute studies showed varying degrees of Ti accumulation in the spleen depending on the dose and particle type. A five-day exposure to anatase TiO_2 NPs at doses of 0, 1, and 2 mg/kg bw/day resulted in spleen titanium levels of 0.036, 0.040, and 0.046 mg Ti/kg tissue, respectively, with significant accumulation observed in the 2 mg/kg bw/day group [83]. However, no significant accumulation was reported in mice exposed to 2.5 mg/kg bw/day for seven days [47] or in rats exposed to 30–80 µg/kg bw for 7 days [77].

Minimal uptake was observed in rats exposed to 12.5 mg/kg bw for 10 days, with titanium concentrations being only 0.02% of the administered dose [28]. Similarly, repeated administration of 5 mg/kg bw of the food additive E171, three times per week for 3 weeks, did not result in significant increases in spleen titanium levels in mice [34]. In line with these findings, a 21-day oral exposure study in mice using TiO_2 at 0.72, 1.8, and 18 mg/kg/day showed only a slight increase in spleen titanium levels, with concentrations rising from 0.5 mg/kg tissue in controls to 1.0–1.2 mg/kg tissue in treated groups [49]. On the contrary, significant Ti spleen accumulation was observed in mice exposed to 100 mg/kg of anatase and rutile TiO_2 NPs for 28 days, with titanium concentrations of 0.15 and 0.14 mg Ti/kg tissue in each group, compared to the untreated group [67]. However, negligible absorption was reported in rats exposed to 200 mg/kg bw/day for 30 consecutive days [68].

Subchronic oral exposure Minimal titanium absorption was reported in the spleen of rats administered 260.4, 520.8, and 1041.5 mg/kg bw/day of TiO_2 NPs for 13 weeks [30]. In addition, no increase accumulation was observed in the spleen of rats treated with 2, 10, and 50 mg/kg bw/day TiO_2 NPs for 90 days [54] or in those exposed to 10, 100, and 1000 mg/kg bw/day TiO_2 NPs over the same period [55, 72]. Similarly, no increase in pancreatic titanium concentrations was detected in rats exposed to 10, 100, or 1000 mg/kg bw/day of TiO_2 NPs for either 28 or 90 days [52].

Chronic oral exposure Mice administered 64 and 320 mg/kg bw/day of TiO_2 NPs for 14 weeks showed significant spleen titanium levels of 3.6 mg Ti/kg tissue and 4.5 mg Ti/kg tissue, respectively [56]. Another study reported notable spleen accumulation after 26 weeks of exposure, with titanium concentrations of approximately 3 mg Ti/kg tissue in the 10 mg/kg bw/day group and 4 mg Ti/kg tissue in the 50 mg/kg bw/day group [57].

Significant spleen accumulation of titanium was primarily observed in acute high-dose and some chronic exposure studies, while most subacute and subchronic studies reported minimal or inconsistent accumulation. This suggests that spleen biodistribution of titanium is dose- and duration-dependent, with notable accumulation occurring mainly under high or prolonged exposure conditions.

Other organs and tissue

In less studied organs and tissues, titanium concentration was analysed in two acute oral studies, six subacute oral studies, and one chronic exposure.

Acute oral exposure Rats given a single oral dose of 100 mg/kg bw TiO_2 NPs showed elevated titanium levels in the mesenteric lymph nodes (1.6 mg Ti/kg tissue) and Peyer's patches (~2.2 mg Ti/kg tissue) [44]. Significant Ti levels were detected in the bladder of rats administered 50 mg/kg bw for 24 h, with approximately 3.5 ± 0.4% of the administered dose [87].

Subacute oral exposure In the thyroid, a slight but non-significant increase in Ti concentration (0.24 mg Ti/kg tissue) compared to controls (0.22 mg Ti/kg tissue) was observed in female rats treated with 2 mg/kg bw/day TiO_2 NPs for five days [83].

In mesenteric lymph nodes, administration of 10.2–11.4 mg/kg bw/day TiO_2 NPs in males and 13.1–15.2 mg/kg bw/day in females, either as a single oral dose or over 5 days, resulted in total titanium levels ranging from 0.07 to 0.36 mg Ti/kg tissue. Recovery rates from dosing ranged from 60 to 92% in males and 66–95% in females [24].

Titanium levels in the skeleton (0.0002 mg Ti/kg tissue) and soft tissues (0.00002 mg Ti/kg tissue) were minimal following 7 days of exposure to 30–80 µg/kg bw TiO_2 NPs [77].

In peritoneal tissue, rats exposed to 12 mg/kg bw TiO_2 NPs for 10 days showed an accumulation of 1.68% of the administered dose. Significant Ti accumulation was also observed in Peyer's patches, the mesenteric network, and lymph nodes, with concentrations reaching 2.86% of the dose [28].

Slight Ti accumulation in muscle tissue was noted in rats administered 10–100 TiO_2 NPs mg/kg bw/day for 14 days, but concentrations were generally below the limit of detection (< 0.2 mg/kg wet weight) [64].

No statistically significant increase in Ti levels was found in the mesenteric lymph nodes of rats treated with 10 mg/kg bw/day TiO_2 NPs for 28 days [44].

Subchronic oral exposure A low amount of TiO_2 NPs was identified in bones of rats exposed to 50 mg/kg bw/day TiO_2 NPs for 90 days [87].

Chronic oral exposure A 26-week study found no significant increase in Ti concentration in muscle tissue of rats exposed to 10 or 50 mg/kg bw/day TiO_2 compared to controls [57].

Overall, in less-studied organs, such as the mesenteric lymph nodes, there was high Ti retention, but the thyroid and adrenals showed minimal or undetectable levels across most exposure scenarios.

Quantitative summary of accumulation findings

A comprehensive summary of studies reporting either significant or non-significant titanium accumulation is

Table 3 Summary of significant and non-significant titanium accumulation findings across vital organs and tissues

Organ	# of studies reporting	# Reporting significant accumulation	# Reporting no significant change
Liver	37	28 (75%)	9 (25%)
Kidneys	23	13 (57%)	10 (43%)
Spleen	22	16 (73%)	6 (27%)
Gastrointestinal Tract	19	16 (84%)	3 (16%)
Brain	14	6 (43%)	8 (57%)
Lungs	10	6 (60%)	4 (40%)
hear	9	3 (33%)	6 (67%)
Reproductive System	9	6 (67%)	3 (33%)
Pancreas	5	4 (80%)	1 (20%)
Other Organs and tissues	9	7 (78%)	2 (22%)

presented in Table 3. Each study–organ outcome was classified as showing a statistically significant increase ("Positive") or no significant difference ("No Change") compared with controls. The results indicate a clear organ-dependent pattern of Ti accumulation. Organs such as the liver, spleen, and gastrointestinal tract most consistently showed dose-dependent accumulation, while the brain and heart showed greater variability, with many studies reporting no measurable accumulation above background levels.

Summary of titanium content across organs by exposure group

To provide a visual summary of reported Ti content across all organs and exposure durations, heatmaps were generated for each exposure group: acute (Fig. 3), subacute (Fig. 4), subchronic (Fig. 5), and chronic (Fig. 6). These visualisations display the reported Ti content (mg/kg tissue) from all individual studies, regardless of significance, organised by dose range (x-axis) and Ti content range (y-axis) for each organ. Each cell represents the Ti content of the number of study results that fall within the corresponding dose range. Cells shaded in grey represent organ–dose combinations for which no study was identified. These visualisations complement the organ-specific findings described above and highlight broader trends in organ accumulation across increasing exposure durations.

Discussion

In recent years, an increasing number of researchers have reported the distribution of Ti in vital organs and tissues following oral administration. This has raised concerns about the safety and toxicity of TiO_2 NPs, particularly due to their widespread use and the subsequently increasing likelihood of human exposure, especially through oral ingestion. Despite these concerns, no systematic review had been conducted prior to this study to specifically investigate TiO_2 exposure and its distribution in vital organs. This review aims to address this gap by comprehensively examining the uptake and tissue distribution of titanium in these organs.

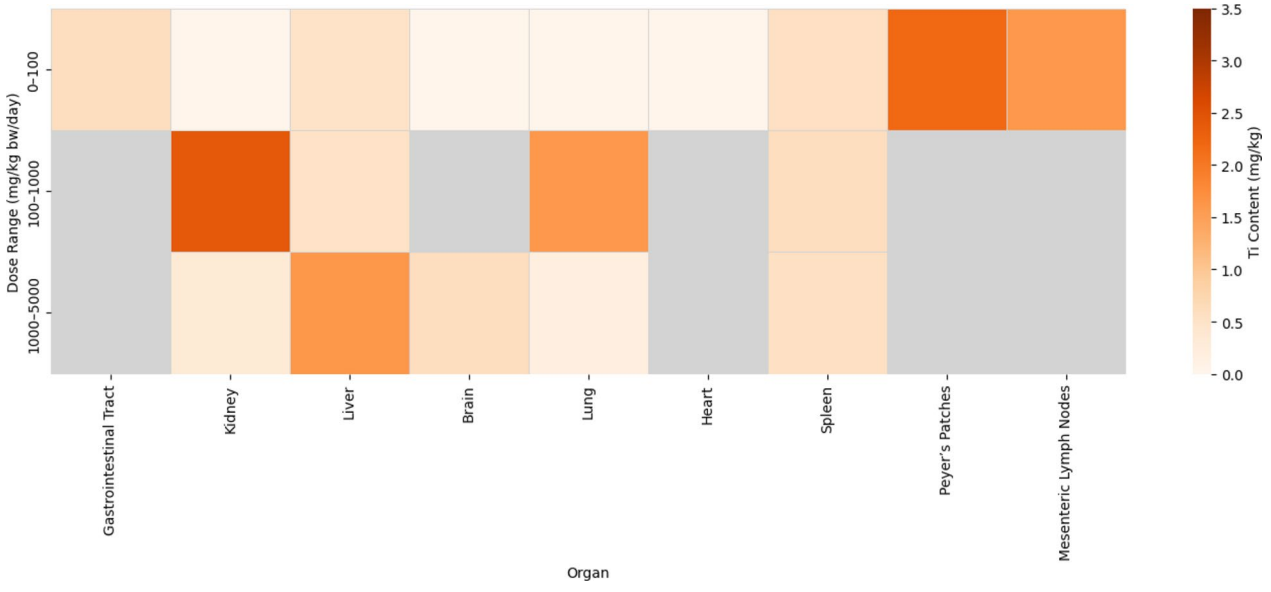

Fig. 3 Titanium content in organs after acute oral TiO_2 exposure. Heatmap illustrating Ti content in vital organs following acute oral exposure to TiO_2. Ti content values (mg Ti/kg tissue) reported in individual studies are organised by dose range on the y-axis and organ type on the x-axis. Colour intensity reflects the extent of Ti accumulation. Cells shaded in grey represent organ–dose combinations for which no study was identified

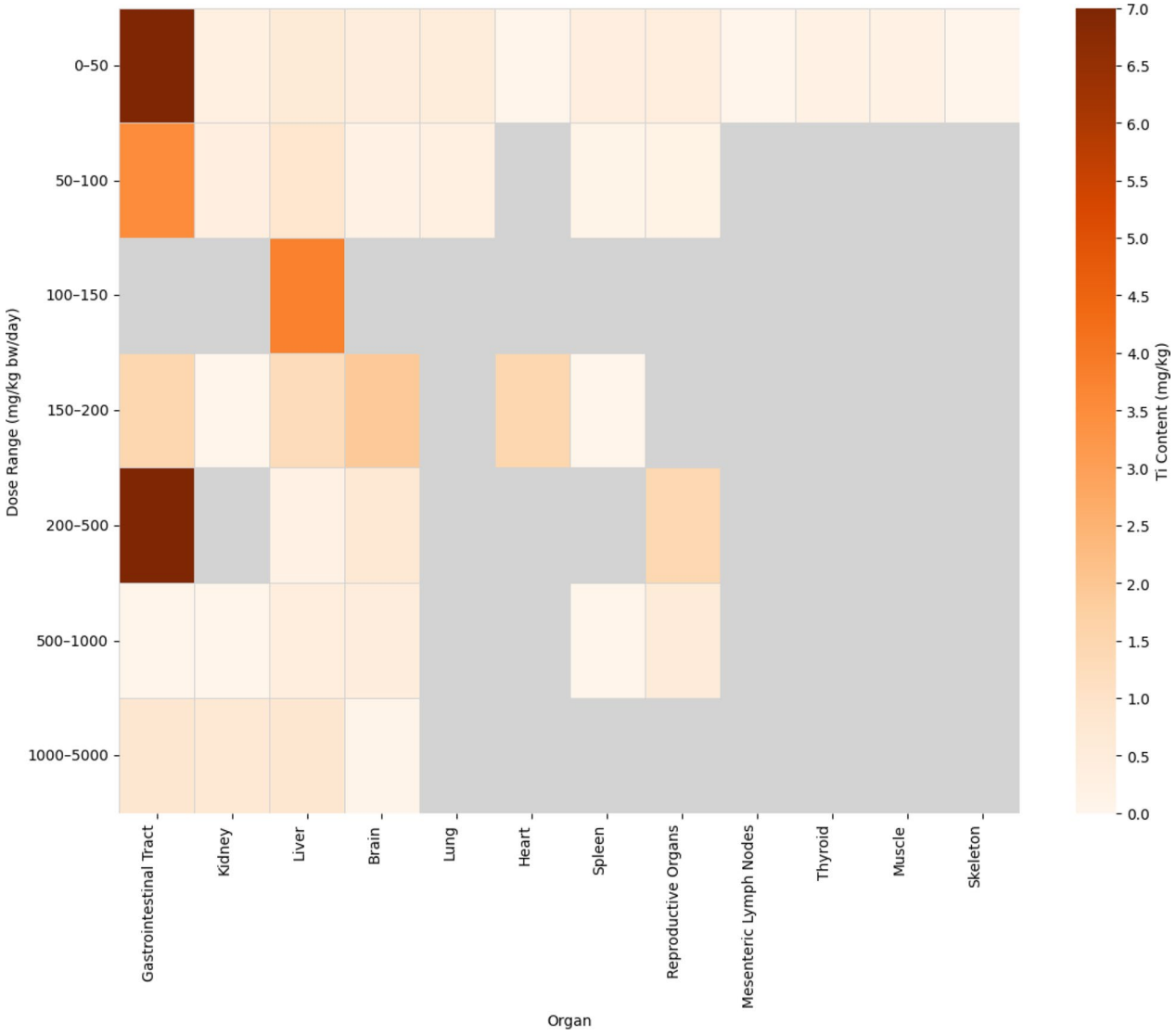

Fig. 4 Titanium content in organs after subacute oral TiO_2 exposure. Heatmap showing reported titanium content (mg Ti/kg tissue) in vital organs after subacute oral exposure to TiO_2. Dose groups are categorised along the y-axis, and organs are displayed across the x-axis. Colour shading represents reported Ti levels from individual studies. Cells shaded in grey represent organ–dose combinations for which no study was identified

Effects of acute oral exposure

Acute oral exposure to TiO_2 demonstrates minimal organ accumulation across various studies. Research consistently indicates that a single oral administration, regardless of dosage (e.g., 2.3, 5 mg/kg, or 100, 500 mg/kg bw), does not lead to significant translocation or buildup of titanium in critical organs such as the liver, kidneys, spleen, lungs, heart, or brain [24, 32, 44, 58, 59]. A biokinetics study using [48 V]-radiolabeled TiO_2 NPs in rats further supports these findings. Following a single oral dose (30–80 mg/kg bw), particle retention was monitored at intervals of 1 h, 4 h, 24 h, and 7 days post-gavage. These findings indicate that the minimal amount of titanium which crosses the gastrointestinal barrier after a single acute dose does not lead to significant accumulation in systemic organs. Instead, it is efficiently cleared from the body, primarily through faecal excretion, with no evidence of long-term persistence [77]. The transition from minimal accumulation after acute exposure to detectable accumulation with prolonged exposure is likely not due to detection limits but rather to the kinetics of uptake and elimination. Although the fraction of TiO_2 absorbed across the intestinal barrier during each exposure is small, the continuous daily intake of TiO_2 from the diet results in a steady, low-level entry into systemic circulation [88]. Once in the bloodstream, particles can distribute to various tissues. Importantly, elimination from these organs appears to be exceptionally slow, as evidenced by the persistence of Ti in vital tissues following longer exposure durations [75, 76].

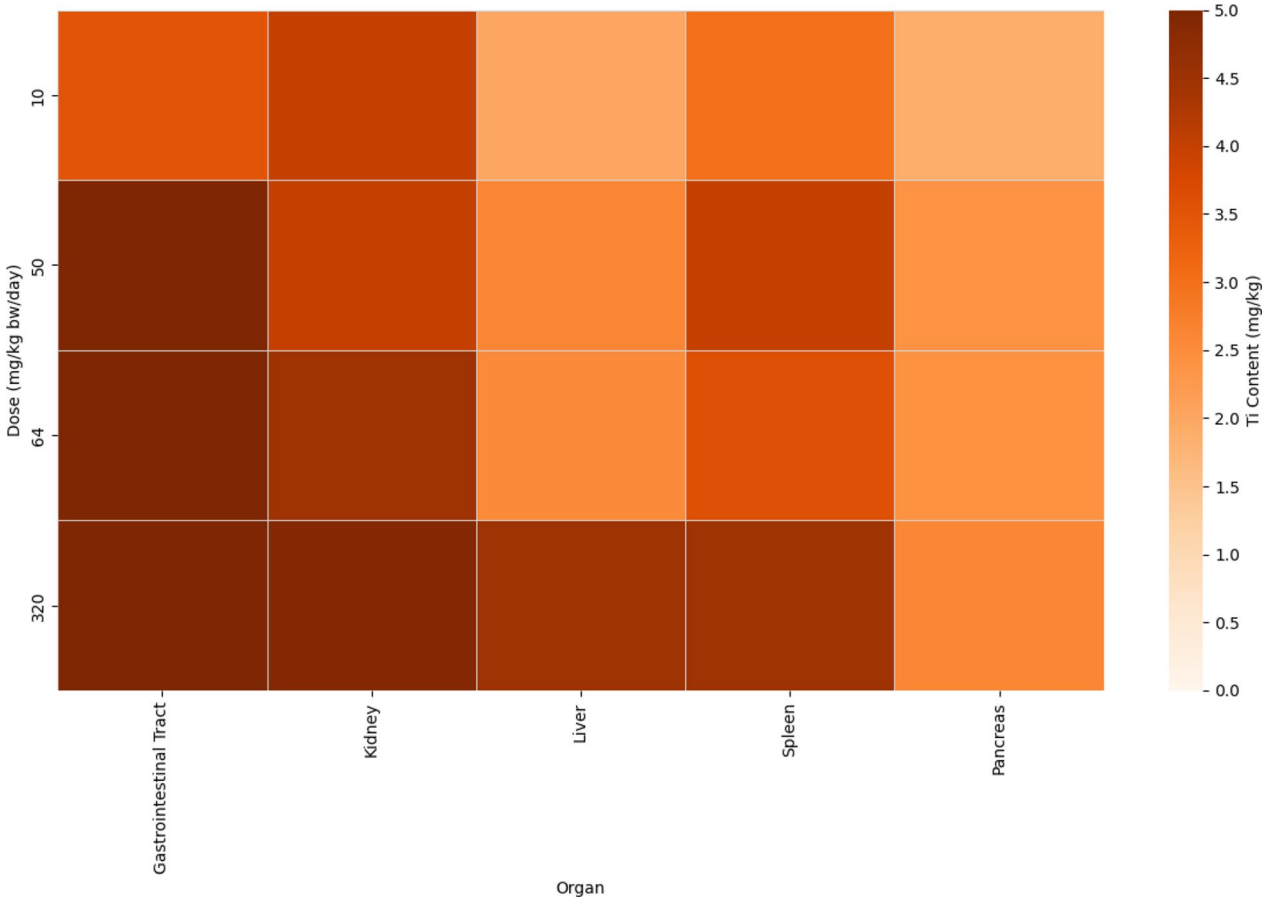

Fig. 5 Titanium content in organs after subchronic oral TiO_2 exposure. Reported Ti content in vital organs after subchronic oral exposure to TiO_2, visualised as a heatmap. Each cell reflects the Ti concentration (mg/kg tissue) extracted directly from individual studies. Cells shaded in grey represent organ–dose combinations for which no study was identified

Effects of prolonged oral exposure

With increased exposure time, subacute, subchronic, and chronic toxicity effects of TiO_2 were significantly enhanced, causing significant Ti accumulation and damage to various organs or systems of the body, including liver, gastrointestinal tract, kidney, spleen, brain as well as pancreas and reproductive system.

In the liver, subacute oral exposure led to an increase in titanium levels, ranging from 0.5 mg Ti/kg tissue [62] to 3.8 mg Ti/kg tissue [63]. In subchronic exposure studies, titanium levels were reported to range from 0.03 mg Ti/kg tissue [52] to 7 mg Ti/kg tissue [71]. For chronic exposure, titanium levels varied between 1.1 mg Ti/kg tissue [76] and 5.3 mg Ti/kg tissue [75]. The liver's substantial blood volume and slow sinusoidal blood flow make it a primary site for the distribution of Ti [89]. Additionally, liver macrophages (Kupffer cells) are directly exposed to circulating NPs, enabling their uptake through various phagocytosis and endocytosis pathways, which contributes to their accumulation in the liver [89]. Pathological changes associated with TiO_2 NP exposure in the liver include sinusoidal dilatation, hepatocyte derangement, and necrosis [62]. Tissue damage such as fibre fractures [35] and cellular alterations, including mitochondrial proliferation and endoplasmic reticulum oedema, have been documented [90]. Chronic inflammation, marked by macrophage infiltration and necroinflammation, may progress to fibrosis or hepatocellular carcinoma [34].

In the gastrointestinal (GI) tract, titanium levels ranged from 0.01 mg Ti/kg tissue in the small intestine [47] to 375 mg Ti/kg tissue in gastric cells following subacute oral exposure [33]. In subchronic exposure, titanium accumulation ranged from 2 mg Ti/kg tissue [54] to 88 mg Ti/kg tissue in colon [54]. In chronic oral exposure studies, accumulation ranged from approximately 3.4 mg to 5.3 mg Ti/kg tissue in the small intestine [56, 57]. In prolonged exposure, accumulation was particularly pronounced in the colon, where several studies reported increases in titanium levels, while no such changes were observed in other parts of the intestinal tract [28, 34, 47]. This is likely due to the slower transit time in the large intestine, which facilitates accumulation, especially with repeated oral administration [6]. Titanium accumulation in the gastrointestinal tract is facilitated by various

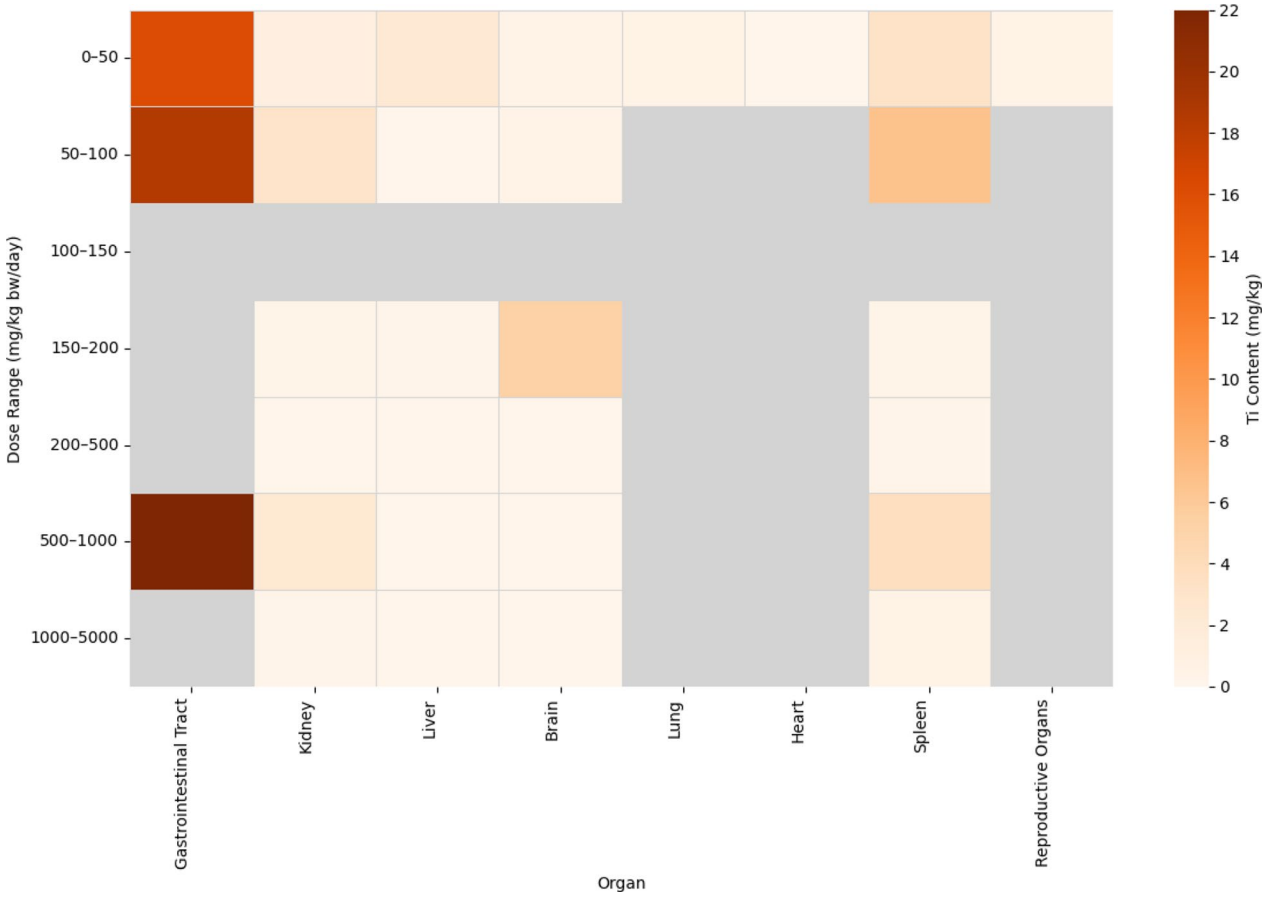

Fig. 6 Titanium content in organs after chronic oral TiO_2 exposure. Heatmap depicting reported Ti accumulation (mg/kg tissue) in vital organs following chronic oral exposure to TiO_2. The data are organised by dose range (y-axis) and target organs (x-axis), with colour intensity corresponding to reported Ti content in each organ. Cells shaded in grey represent organ–dose combinations for which no study was identified

mechanisms. The gut epithelium, composed of enterocytes and mucus-secreting Goblet cells, plays a key role in particle uptake. In the small intestine, M cells within Peyer's patches of the ileum and jejunum drive nanoparticle absorption [19, 91–93]. In the colon, however, accumulation predominantly occurs within the epithelium, particularly in goblet cells, where TiO_2 NPs are trapped by the thick mucus layer and may be internalized. Evidence also suggests limited translocation to the submucosa and mesenteric lymph nodes, especially under chronic exposure or inflammation, indicating deeper tissue involvement [19, 94, 95].

Titanium accumulation in the kidneys has been observed across varying exposure durations and doses. Subacute exposure studies reported titanium levels up to 1.2 mg Ti/kg tissue [49]. In subchronic exposure studies, accumulation levels were lower, spanning from 0.1 [68] to 2.8 mg Ti/kg tissue [69]. Chronic exposure studies demonstrated higher levels of accumulation, with titanium concentrations recorded between 4 and 5 mg Ti/kg tissue [57]. The kidney's primary functional unit, the nephron, consists of the glomerulus and the tubule system. The glomerulus, consisting of blood capillaries supported by mesangial tissue, facilitates nanoparticle filtration from the blood, which are subsequently excreted in the urine [96].

Titanium accumulation in the spleen also varied by exposure duration. Subacute studies recorded titanium concentrations ranging from 0.046 [83] to 1.2 mg Ti/kg tissue [49]. Subchronic exposure resulted in minimal titanium accumulation in the spleen. Chronic exposure studies confirmed titanium presence, with concentrations ranging from 3 to 4 mg Ti/kg tissue [56, 57]. Histological alterations in the spleen due to TiO_2 exposure included an increased white-to-red pulp ratio, indicative of potential immune or inflammatory responses [83]. Such changes suggest that TiO_2 NPs may impact spleen structure and function.

Subacute oral exposure to TiO_2 NPs resulted in significant titanium accumulation in the brains of mice and rats, with levels ranging from 0.01 [52] to 2.6 mg Ti/kg tissue [51]. These levels were highest in the cortex, hippocampus, cerebrum, cerebellum, and medulla oblongata, accompanied by histological changes such as neurocyte

calcification and ependymal proliferation [62, 65, 78]. Subchronic exposure studies reported titanium accumulation levels of 0.7 to 5.3 mg Ti/kg tissue [24]. While nanoparticles generally do not cross the blood–brain barrier (BBB) and thus do not usually induce significant brain toxicity, smaller NPs have been shown to accumulate in the brain through several mechanisms. These include uptake by glial cells, such as astrocytes or pericytes, which support the BBB; passage through the semipermeable basal membrane located between endothelial and glial cells; and transcytosis within endothelial cells where the basal membrane does not provide coverage, allowing direct release into the cerebrospinal fluid [97]. Additionally, NPs may reach the brain through transsynaptic transport or by disrupting the BBB, resulting in central nervous system toxicity [98]. Histological damage in the brain due to TiO_2 exposure has been extensively documented. Observations include vacuoles, necrosis, pyknosis, deranged cells, and acidophilic lesions, particularly in the cortex and hippocampus [62]. Additionally, glial cell proliferation, filamentous neurons, and necrosis were reported [99], suggesting significant neurotoxicity and potential cognitive impairments.

Titanium accumulation in the pancreas ranged from 1.3 to 2.8 mg Ti/kg tissue following chronic exposure [76]. Histopathological changes in the pancreas due to TiO_2 exposure include destruction of islet architecture and hyalinization [35]. TiO_2 exposure at higher doses also induced reactive oxygen species (ROS), contributing to insulin resistance and elevated plasma glucose levels [56]. These findings suggest a critical role of TiO_2 nanoparticles in disrupting pancreatic function and increasing the risk of metabolic disorders.

In long-term exposure studies, titanium accumulation in the lungs, heart, thyroid, skeletal tissue, muscle tissues, and mesenteric lymph nodes was negligible [24, 64, 77, 83].

Subacute oral exposure to TiO_2 NPs led to significant titanium accumulation in the reproductive tissues of mice and rats, with levels varying by tissue type and sex. In placental tissue, Ti concentrations ranged from 0.016 to 3 mg Ti/kg tissue, indicating notable deposition [84]. Female reproductive tissues showed differential accumulation: the ovaries exhibited Ti levels of 0.28 mg/kg tissue after exposure to 2 mg/kg/day TiO_2 NPs for 5 days [83], whereas the uterus had minimal accumulation (0.0005 mg/kg) following a lower dose of 30–80 µg/kg/day for 7 days [77]. In males, no detectable titanium was found in the testes (< 0.03 mg/g) after exposure to 5 mg/kg TiO_2 NPs three times weekly for 3 weeks, indicating limited retention in male reproductive tissues [34]. These findings highlight tissue- and sex-specific differences in Ti biodistribution, with female reproductive tissues appearing more susceptible to accumulation. Exposure to TiO_2 NPs has been linked to various adverse effects on reproductive health, including increased apoptosis in ovarian granulosa cells, alterations in the thyroid follicular epithelium, and necrosis of the adrenal cortex [83]. Gender-specific hormonal changes were also observed, pointing to potential endocrine disruptions. Additionally, maternal weight gain was reduced in TiO_2-exposed groups, with significant developmental delays reported in offspring. These developmental effects included a reduced number of live foetuses and delayed foetal skeletal development, particularly at a dose of 100 mg/kg TiO_2 NPs [84]. Collectively, these findings suggest that TiO_2 NPs may negatively impact reproductive health and foetal development.

Discrepancies and influencing factors

Conversely, minimal or non-significant titanium accumulation has been reported in vital organs following prolonged oral exposure to various doses of TiO_2 (e.g., 50–160 mg/kg/day for 28–90 days) [24, 34, 51, 60, 64]. The discrepancies observed could be attributed to the following factors. Firstly, the differences in titanium accumulation across studies can be attributed to the physicochemical properties of TiO_2 NPs, including particle size, crystal structure, and surface coating, which influence their absorption, distribution, and elimination. Smaller particles generally show enhanced tissue penetration [100]. For instance, significant brain accumulation (100 ng/g) was found with 50 nm TiO_2 NPs, while larger particles (120 nm) showed minimal accumulation [62]. Similarly, higher liver (1.9 mg Ti/kg tissue) and pancreas (2.3 mg Ti/kg tissue) titanium levels were found in mice exposed to < 100 nm TiO_2 NPs compared to fine particles [35]. Crystal structure also plays a crucial role. Higher liver accumulation (0.94 mg Ti/kg tissue) was found with rutile TiO_2 NPs compared to anatase NPs or E171 [66]. Significant lung deposition was also measured with rutile TiO_2 NPs, but not with anatase NPs [67]. Moreover, different TiO_2 types may be absorbed to varying extents, distributed differently across tissues, and exhibit different levels of toxicity. For example, some studies explicitly used food-grade TiO_2, while others used different types of TiO_2 NPs, which may have distinct properties compared to other TiO_2 formulations [17, 34]. Secondly, the way TiO_2 is formulated plays a crucial role in its bioavailability. Different studies have used various administration methods, with some suspending TiO_2 in water and others incorporating it directly into the animals' food. These delivery methods can modify the properties of the particles, including the potential formation of a protein corona, which can influence intestinal uptake and subsequent outcomes [101]. Moreover, administration of TiO_2 via oral gavage may affect its absorption, bioavailability, and metabolism, as gavage bypasses oral mucosa

interactions [102]. For instance, administering donepezil to rodents via gavage resulted in lower concentrations of the drug in the blood and brain compared to when it is taken via an oral syringe [103]. Variations in dispersion protocols for preparing TiO_2 suspensions are another factor contributing to study inconsistencies. A review by McCormack et al. [104] shows that methods such as sonication or vortexing can alter TiO_2 properties, including agglomeration state and particle size, thereby affecting bioavailability and toxicity. Inadequate dispersion can lead to the formation of larger agglomerates, reducing absorption and underestimating organ accumulation. In our dataset, 61% of studies used sonication, whereas 39% did not specify the dispersion method, which may partly explain the variability in reported results. Lastly, other aspects that may lead to discrepancies between studies include differences in rodent species and strains, as well as exposure concentrations and duration, as these variations can influence how nanoparticles undergo dynamic chemical and physical changes in the biological system, altering the dose–response relationship [105].

In a number of experiments, even with high doses and prolonged exposure e.g., 1000–2260 mg/kg bw/day for 28–90 days), no significant increase in titanium levels compared to controls was observed [30, 52, 55, 72]. This can be attributed to the complex relationship between dose and gastrointestinal uptake, which is not fully understood. It is known that high concentrations of TiO_2 can lead to the formation of large agglomerates, which complicates uptake and reduces bioavailability. Studies on silica particles have shown that agglomeration increases at high concentrations, resulting in lower absorption [105], a finding that aligns with rat studies where the lowest concentration resulted in the highest absorption [106]. Therefore, a high degree of agglomeration in the gastrointestinal tract may limit the absorption of TiO_2, preventing linear increases in internal concentrations with higher doses. This could explain the lack of effects observed in high-dose studies, in contrast to the effects seen in low-dose studies.

Strengths and limitations

This systematic review on the oral exposure to TiO_2 NPs and their accumulation in vital organs offers several strengths. First, it provides a comprehensive synthesis of the available literature, covering a broad range of studies that explore various exposure durations, doses, and organ systems, offering valuable insights into the biodistribution of TiO_2 NPs. By examining multiple organs, including the liver, kidney, spleen, brain, and others, the review highlights the organ-specific variations in TiO_2 accumulation, providing a nuanced understanding of its impact on biological systems. Another strength lies in its focus on acute, subacute, subchronic, and chronic exposure studies, offering a balanced perspective on the temporal effects of TiO_2 exposure. Additionally, the review addresses the biological and histopathological changes associated with TiO_2 exposure, offering a thorough examination of the potential health risks posed by these nanoparticles.

However, certain limitations should be taken into account when interpreting the results. A key issue is the heterogeneity of exposure conditions across studies, including variations in the administered doses, exposure durations, and nanoparticle physicochemical properties (e.g., size, shape, surface charge). These inconsistencies make it challenging to establish dose–response relationships or identify clear patterns of accumulation. Furthermore, many studies lacked detailed characterisation of the TiO_2 NPs used, such as agglomeration state or solubility, which are critical for understanding their biodistribution and toxicity. Also, the quality assessment using SYRCLE's tool highlights methodological limitations, such as under-reporting of detection limits (37% of studies), which may contribute to variability in reported accumulation. These biases were considered in interpreting discrepancies, emphasizing the need for improved reporting in future studies.

Another limitation is the scarcity of chronic exposure data, leaving gaps in understanding long-term accumulation and associated health risks. Additionally, the reliance on animal models, which may not fully replicate human physiology, restricts the translation of findings to human health contexts. Finally, the review is constrained by the limited availability of studies examining specific organs, such as the pancreas, heart and reproductive tissues, resulting in potential underrepresentation of these areas. Addressing these limitations in future research would enhance our understanding of TiO_2 NP behaviour and risks following oral exposure.

A critical limitation in the current body of research on TiO_2 NPs accumulation following oral exposure is the predominant reliance on mg/kg as a measure of tissue concentration. While this unit quantifies total mass, it overlooks critical factors such as the number of particles per cell and their size distribution, which are likely more significant drivers of toxicological effects. The biological impact of TiO_2 NPs, particularly their capacity to induce inflammation, appears closely tied to these particle characteristics [107]. To address this gap, we recommend that future studies report Ti accumulation not only in mg/kg but also as the number of particles per kilogram for specific size ranges, including < 100 nm, < 25 nm, and < 2.5 nm. This multi-unit approach would offer a more detailed insight into how particle size and abundance influence biodistribution and toxicity, leveraging the assumption that tissue weight (in kg) correlates with cell number. In contrast to trace elements, where mg/kg

adequately reflects biological activity due to their atomic-scale dispersion, TiO_2 particles exert effects through their physical presence and quantity [108], making particle number per weight a more toxicologically relevant metric. By identifying this methodological weakness and proposing a standardized reporting framework, this systematic review underscores a critical need for consistency in unit usage. Such a shift could enhance the comparability of findings across studies, resolve apparent contradictions in the literature, and ultimately advance our understanding of TiO_2 NPs toxicity to better inform safety assessments and regulatory frameworks.

Future research directions

In line with this, future research on the oral exposure to TiO_2 NP and their accumulation in vital organs should address several key areas to deepen our understanding of their potential health risks. First, studies that explore the long-term effects of TiO_2 NP exposure, particularly in relation to chronic health outcomes, are needed. While the current review includes chronic exposure studies, further investigation into the cumulative effects over extended periods, particularly across the lifespan of organisms, is essential for assessing potential chronic toxicity. Second, the underlying mechanisms of TiO_2 NPs' biodistribution and tissue accumulation require further elucidation. More research is needed to understand how these nanoparticles interact with specific cellular structures, the blood–brain barrier, and other organ-specific barriers, which may help clarify the differential accumulation observed in different tissues. Additionally, the role of TiO_2 NP size, surface coating, and agglomeration in modulating their accumulation and toxicity should be better defined, as these factors can significantly influence their biological behaviour. Finally, gender- and age-related differences in the uptake and distribution of TiO_2 NPs, particularly in reproductive and developmental contexts, warrant additional study to understand the sex- and age-specific risks associated with exposure. Addressing these areas will help establish more comprehensive safety guidelines for the use of TiO_2 NP in consumer products and industrial applications.

Conclusion

This systematic review provides a thorough evaluation of the accumulation of Ti in vital organs and tissues following oral exposure in rats and mice. The findings reveal that while the acute oral toxicity of TiO_2 NPs is relatively low, their subacute and subchronic toxic effects intensify with prolonged exposure, highlighting the potential for more serious long-term risks. However, most existing studies have focused on high-dose, short-term exposure (acute and subacute), leaving significant gaps in our understanding of the effects of low-dose, long-term exposure (chronic), which are more representative of real-world human exposure.

The results demonstrate a clear dose-dependent and time-dependent pattern of Ti NP accumulation, particularly in the liver, kidneys, spleen, gastrointestinal tract, and brain during extended exposure periods. Conversely, minimal accumulation was observed in the lungs and heart, even after prolonged exposure. However, these findings must be interpreted cautiously due to the limited number of studies available for certain organs and the variability in experimental designs, nanoparticle sizes, surface properties, exposure regimens, and analytical techniques across the studies.

These inconsistencies highlight the urgent need for standardized and validated experimental protocols and thorough nanoparticle characterization in future research. Improving methodological consistency will enhance the comparability and reliability of findings, ultimately contributing to a more accurate understanding of the long-term health effects of TiO_2 NP exposure.

While this review synthesizes data from rodent studies, it highlights important implications for human exposure to TiO_2, primarily through food additives, pharmaceuticals, and other consumer products. Current evidence indicates that acute exposure in animal results in minimal organ accumulation. However, repeated or chronic exposure leads to measurable titanium buildup and associated tissue effects.

In humans, exposure typically occurs at much lower doses but over longer durations, raising concerns about subtle, cumulative effects that may not be captured in high-dose, short-term animal studies. This gap underscores the urgent need for well-designed human biomonitoring and toxicokinetic studies to determine real-world exposure levels, bioavailability, and potential long-term health outcomes. Particular attention should be given to vulnerable populations, such as children, who may experience higher exposure relative to body weight. Addressing these gaps is essential for developing accurate risk assessments and evidence-based regulatory guidelines regarding TiO_2 use.

Abbreviations

bw	Body weight
DLS	Dynamic light scattering
E171	Food additive code for titanium dioxide
EFSA	European Food Safety Authority
FDA	Food and Drug Administration
FP	Food-grade pigment
GIT	Gastrointestinal tract
ICP-MS	Inductively coupled plasma mass spectrometry
LOQ	Limit of quantification
MeSH	Medical subject headings
mg/kg bw/day	Milligrams per kilogram of body weight per day
mg Ti/kg tissue	Milligrams of titanium per kilogram of tissue
ng/g	Nanograms per gram
NPs	Nanoparticles
NS	Not Specified

PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RES	Reticuloendothelial system
Ti	Titanium
TiO_2	Titanium dioxide
TiO_2 NPs	Titanium dioxide nanoparticles

Supplementary Information

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Supplementary material 1.

Author contributions

VM: Conceptualization, methodology, literature search, screening, data extraction, data analysis, writing—original draft, reviewing and editing, visualization. JK: Literature search, screening assistance. PT: Critical feedback, reviewing and editing, final approval. NK: Methodological guidance, critical feedback, reviewing and editing, final approval. MK: Critical feedback, reviewing and editing, final approval. CB: Feedback during project development, data analysis/visualisation feedback BM: Supervision, project administration, critical feedback, reviewing and editing, final approval. All authors reviewed the manuscript.

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Data availability

All data generated or analysed during this study are included in this published article and its supplementary files. Additional information is available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

The authors declare no competing interests.

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