



Toxicological Consequences of Titanium Dioxide Nanoparticles (TiO₂NPs) and Their Jeopardy to Human Population

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Abstract

Titanium dioxide nanoparticles (TiO₂ NPs) are the most produced nanomaterial for food additives, pigments, photocatalysis, and personal care products. These nanomaterials are at the forefront of rapidly developing indispensable nanotechnology. In all these nanomaterials, titanium dioxide (TiO₂) is the most common nanomaterial which is being synthesized for many years. These nanoparticles of TiO₂ are widely used at the commercial level, especially in cosmetic industries. High usage in such a way has increased the toxicological consequences of the human population. Several studies have shown that TiO₂ NPs accumulated after oral exposure or inhalation in the alimentary canal, lungs, heart, liver, spleen, cardiac muscle, and kidneys. Additionally, in mice and rats, they disturb glucose and lipid homeostasis. Moreover, TiO₂ nanoparticles primarily cause adverse reactions by inducing oxidative stress that leads to cell damage, inflammation, genotoxicity, and adverse immune responses. The form and level of destruction are strongly based on the physical and chemical properties of TiO₂ nanoparticles, which administer their reactivity and bioavailability. Studies give indications that TiO₂ NPs cause both DNA strand breaks and chromosomal damages. The effects of genotoxicity do not depend only on particle surface changes, size, and exposure route, but also relies on the duration of exposure. Most of these effects may be because of a very high dose of TiO₂ NPs. Despite increased production and use, epidemiological data for TiO₂ NPs is still missing. This review discusses previous research regarding the impact of TiO₂ NP toxicity on human health and highlights areas that require further understanding in concern of jeopardy to the human population. This review is important to point out areas where extensive research is needed; thus, their possible impact on individual health should be investigated in more details.

Keywords Nanomaterials · Titanium dioxide nanoparticles · Jeopardy · Nanotechnology · Nanoparticles · TiO₂

Abbreviations

TiO₂ Titanium dioxide

NPs Nanoparticles
PSLT Poorly soluble low-toxicity
ALT Alanine aminotransferase
LDH Lactic acid dehydrogenase
BUN Blood urea nitrogen

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1 Introduction

Nanoparticles are small materials ranging in size from 1 to 100 nm and can be classified into various categories depending on their shape and size [1]. These particles have unique physicochemical properties due to their large surface area and small size that makes this material excellent in many areas of human research activities, cosmetic products, and agriculture [2].

TiO₂ is one of the most used nanoparticles nowadays. It is a white pigmented additive with high opacity and coating properties. It is generally used in dye, clothing, rubber, paper,

ceramic, metallurgy, drug, cosmetic, pharmaceuticals, food industries, car materials, and other biomaterials for sterilization and industrial photolytic processes regarding the decomposing of organic matters [3]. These particles have several adverse effects at the cellular level, such as oxidative stress and DNA damage [4]. Meanwhile, oxidative stress is a significant determinant of nanoparticle (NPs) induced injuries. So, it is essential to illustrate the reactive oxygen species (ROS) response resulting from NPs [5]. Hence, increasing human exposure to nanoparticles has an increasing concern for their safety and health. In experimental animals, TiO₂ cause severe pulmonary response [6]. The situation is more worse after turning into anatase form under UV irradiation [7]. It has been proved that TiO₂ anatase form has more toxic properties than TiO₂ rutile form, because smaller particles with larger surface enhance the side effects [8]. So, the overall safety of TiO₂ NPs is still at the initial stage. It is generally known that TiO₂ NPs have a higher biological activity than ordinary bulk materials due to their large surface area to volume ratio [8]. So, these unique NPs features raise concerns about human safety regarding health [9]. Therefore, additional efforts are still needed to understand the interaction between these NPs and the human body. In this regard, nanotoxicology and nanolithography attract the attention of toxicologists and regulatory scientists [6].

So, it is necessary to evaluate the adverse health effects and environmental biosafety of TiO₂ NPs and highlights those areas where further understanding in concern of jeopardy to the human population is needed.

2 Physical Properties of Nanostructured TiO₂

TiO₂ is naturally found in various rocks and mineral sands. Naturally occurring TiO₂ comes in a red or brownish red or black color. The color is usually due to the presence of impurities such as iron, vanadium, zirconium, and chromium, which can make up 10% of all titanium [10]. Titanium is the ninth common element in the Earth's crust. Titanium dioxide belongs to the family of transition metal oxides. TiO₂ is a white, odorless, nonflammable powder with a 79.9-g/mol molecular weight, a boiling point of 2972 °C, a melting point of 1843 °C, and a relative density of 4.26 g/cm³ at 25 °C. TiO₂ is entirely insoluble in water, common organic solvent, and dilute acids. It is soluble in concentrated sulfuric acid or hydrofluoric acid at high temperatures [11]. TiO₂ has excellent electrical properties due to its high dielectric constant. It does not react with oxygen, sulfur dioxide, hydrogen sulfide, ammonia, and carbon dioxide. TiO₂ has chemical stability, biocompatibility, and a robust photocatalytic activity. It exists in three common polymorphs in nature, i.e., brookite, anatase, rutile, and a few common structures of TiO₂ [12]. It is a wide-band semiconductor with anatase, rutile, and brookite

bandgap of 3.2, 3.02, and 2.96 eV, respectively [13]. The small size of TiO₂ renders it more genetically toxic regardless of its crystalline levels. According to many studies, the small sizes of nanoparticles allow easy entry and accumulation inside the cell's of cytoplasm and nucleus [14]. Several pieces of literature show that the anatase form of nanoparticles cause more toxic effects than rutile nanoparticles because the anatase form has more photocatalytic properties. TiO₂ NPs produce large size agglomerates that cause DNA damage in different cell lines [15]. These small size nanoparticles express higher toxicity than large size particles. Moreover, the nanorods of TiO₂ exhibit more toxicity than spherical particles having the same surface area and size, indicating the role toward cytotoxicity [16].

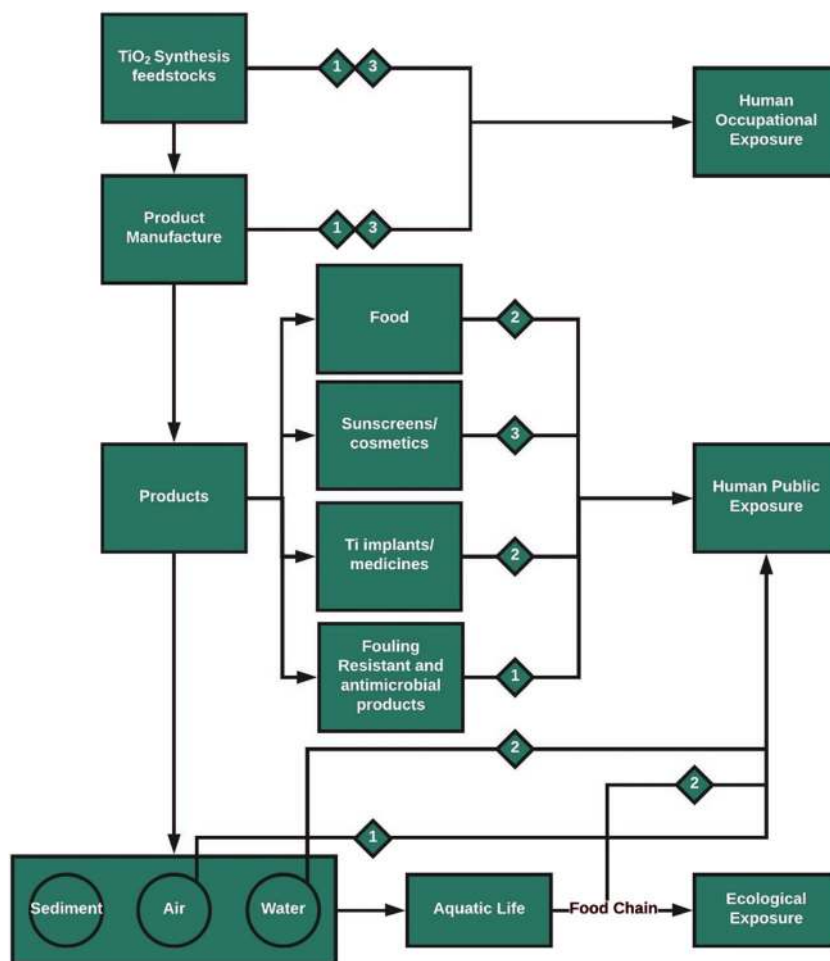
3 Mass Production and Filthiness of TiO₂ NPs

In 2006, the USA manufactures 40,000 tons of TNPs [17]. Due to increased market demand, the annual production of TiO₂ NPs is expected to reach 2.5 million tons by 2025 [18]. Therefore, a huge quantity of TiO₂ NPs will be released into the environment (Fig. 1). The widespread distribution of TiO₂ nanoparticles is released into the air, soil, and/or water throughout and can affect all components of the environment, including humans, animals, and plants on direct exposure to these pollutants [20]. Transfer in food chain usually happens from plants to animals, because plants are a primary food chain source that consumes nutrients and waste from the environment with toxins. Accumulation and translocation were studied by analytical tools to detect NPs in numerous plant tissues that trigger leaf necrosis, inhibit seedlings' root elongation, and influence root growth [21]. However, ecological life examinations, potential bioaccumulation, and especially the transfer of NPs within the food chain and air remain restricted. Because TiO₂ NPs are inevitable in the production, use, and disposal of waste through the air, soil, and water, the natural environment's ecological environment has attracted considerable attention both domestically and internationally [22]. Employees and academic researchers of production industries may experience the highest threat to exposure of these NPs through inhalation and skin penetration [11]. Wastewater treatment plants contain 100–3000g TiO₂ NPs with a ratio of 5–15g Ti/L [23]. Most countries standardize solid waste disposal, but non exclusively addresses nanoparticle removal. Thus, the nanoparticle pollution hazard is important and cannot be unnoticed.

4 Potential Toxicity of TiO₂ NPs and Its Accumulation

Recently, growing interest in nanotechnology applications has been observed in various fields like agriculture, medicine,

Fig. 1 Possibility of release, absorption, and impact of TiO₂ NPs in an ecosystem (1) inhalation; (2) ingestion; and (3) deposition [19]



pharmacy, and materials science. Due to its crystal structure, size, and coating, use of TiO₂ NP is inevitable. Particle size, crystalline structure, and coating affect the surface charge, agglomeration, and sedimentation, thereby making the TiO₂ NP very toxic to human cells. Previous researches show that TiO₂ NPs disturb glucose and lipid homeostasis in mice and rats [24]. Available data on TiO₂ NP toxicity to humans is limited, so the potential risk is still in doubt. For that reason, investigators are using numerous toxicological models, such as human cells, animals, and aquatic organisms to generate desirable facts to avoid toxicity (Fig. 2).

Previous *in vitro* and *in vivo* tests have confirmed the toxic effects of TiO₂ NPs on the human body like altered cell cycle, nuclear stenosis, and apoptosis [26–29]. Studies have also shown that TiO₂ NPs causes DNA damage and causes rupturing of the small intestine epithelium, which is involved in the absorption of nutrients [30]. This damage is due to various ways, mainly by inhalation, injection, and skin contact, as well as digestion and absorption [11]. In print plant manufacturing units, workers were found to have symptoms such as shortness of breath because they were exposed to polyacrylate nanoparticles coupled with TiO₂ NPs without any protective procedures

[31]. Other clinical signs of TiO₂ toxicity may include rashes on the face, hands, and forearms [32], and pleural effusion [33]. Some even suffered from pericardial effusions [34], hypoxemia [31], and cancer [35–37]. *In vivo* testing of such exposure revealed that inhalation or oral exposure of TiO₂ NPs may accumulate in different places like the liver, heart, spleen, lungs, kidneys, alimentary tract, and cardiac muscle (Table 1) [47–49].

5 Bio Distribution and Systemic Toxicity in the Different Organ System

All nanomaterials can differ considerably in composition, charge, morphology, specific surface area, and state of matter, which influences different organs (Fig. 3) [51] and may be found in the lung, kidney, lymph node, liver, and spleen [52]. TiO₂ NPs can be transported through the digestive tract to other organs or tissues, which can lead to liver damage and myocardial damage [53].

Among the routes by which lung toxicity can occur, some investigations favor the hypothesis that the surface area may be the most appropriate dose indicator for TiO₂ NPs [11]. The

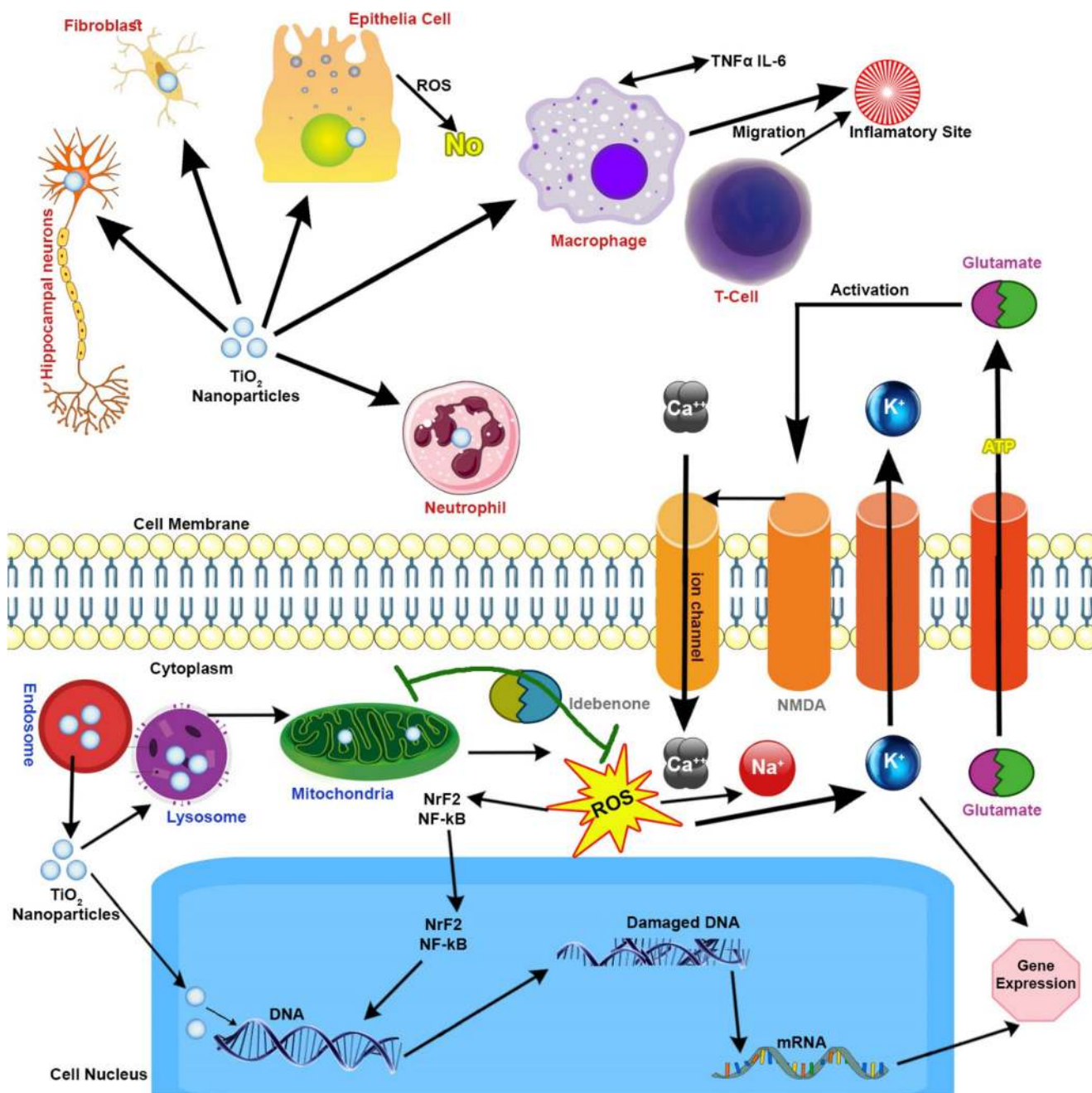


Fig. 2 Schematic diagram of the toxicity of TiO_2 NPs [25]

ultrafine TiO_2 NPs have high quality or low volatility that can damage the lungs at low dose [54] as compared to fine PSLT particles that increase inflammatory reaction and lung retention, e.g., nano-PSLT particles [55, 56]. After treating the rats with the TiO_2 NPs, a high level of inflammatory reactions were observed due to increased NP surface size as compared to particles having a large surface area. According to some researches, TiO_2 NPs cause a more significant pulmonary inflammation than large particles of TiO_2 , when a similar mass dose is introduced [57, 58]. However, when the dose is normalized in the surface area, the lungs' response is the same

because of nanosized and fine TiO_2 particles. Therefore, in the study of lung toxicity, particles of different sizes of the same chemistry proved to be better.

Moreover, other investigations suggest that inflammatory reactions are likely to be more severe with the large surface area of nanoparticles [7, 59]. However, many studies proved that TiO_2 NPs has more side effects [42]. These nanoparticles may cause immunological and pathological changes after accumulation [60] and can induce hepatic injury by altering biochemical parameters of serum (ALT, LDH, and BUN) depending on the amount and size of particles [53].

Table 1 In vivo studies conducted to evaluate the toxic properties of TiO₂ NPs

Crystal phase composition (particle size in nm)	Type of exposure	Body system under evaluation	Type and number of animals	Results	Reference
Anatase TiO ₂ (15)	Oropharyngeal aspiration: ~0.8 mg/kg TiO ₂	Respiratory system	5–6 male BALB/c mice	Increased airway reactivity by TiO ₂ in toluene diisocyanate sensitized mice. TiO ₂ increased neutrophils and alveolar macrophages in bronchoalveolar lavage of toluene diisocyanate sensitized mice.	[38]
Anatase + Brookite TiO ₂ (20)	Inhalation: 8–30 mg/m ³ for 0.5 h (acute exposure); 30 mg/m ³ for 1 h a day, 4 days a week for 4 weeks (sub-chronic exposure)	Respiratory system	4–6 male Crl:OF1 mice per group	Reduced expiratory flow in all the exposure situations	[39]
TiO ₂ (35)	IV injection of 0.8 mg TiO ₂ for 2 consecutive gestational days.	Nervous system	Pregnant mice	TiO ₂ found in fetal brain	[40]
Rutile TiO ₂ (15, 50, 100)	ID injections of 20 µg TiO ₂ with/without mite allergen	Dermal and mucosal system	11 male mice per group	Atopic dermatitis, increased ear thickness, increased IL-4, IL-13, M-CS, and EOSS. Decrease IFN-γ	[41]
Rutile Fe-doped TiO ₂ (diameter, 7; length, 80)	1 and 5 mg/kg TiO ₂ intratracheal instillation	Cardiovascular system	4 male Wistar rats per group	Heart rate and systolic blood pressure increased	[42]
Anatase TiO ₂ (5, 10, 60, 90)	IP injection 5, 10, 50, 100, 150, and 200 mg/kg once a day for 14 days	Liver	10 ICR mice per group	Accumulation of titanium in the spleen, lung, brain, and heart; apoptosis of hepatocytes, damage to mitochondria, generation of ROS, and expression disorders of protective genes in the liver of mice	[43]
Anatase TiO ₂ (5 ± 1)	Intratracheal instillation: 0.8–20 mg/kg TiO ₂ .	Renal system	8 male Sprague-Dawley rats per group	Blood urea nitrogen increased; Ketone bodies, choline, low-density lipoprotein, alanine, and glutamic acid increased; lactate, pyruvate, and creatine decreased. TEM analysis: tubule epithelial cell damage, vascular deformity	[44]
Anatase TiO ₂ (thickness: 10–15; diameter: 45.87 ± 7.75)	Intraarticular injection of TiO ₂ of 0.2, 2, 20 mg/kg in knee joints every other day for 4 times	Musculoskeletal system	10 male Sprague-Dawley rats per group	Glutathione peroxidase, oxidized glutathione, malondialdehyde, and superoxide dismutase increased.	[45]
Anatase TiO ₂ (25–70)	SQ injection of 100 µL of TiO ₂ at 1mg/mL at 3, 7, 10 and 14 days post-coitum.	Reproductive system	6 pregnant Slc:ICR mice per group	Decreased sperm production, number of Sertoli cells, and epididymal sperm motility; disorganized and disrupted seminiferous tubules; a little mature sperm.	[46]

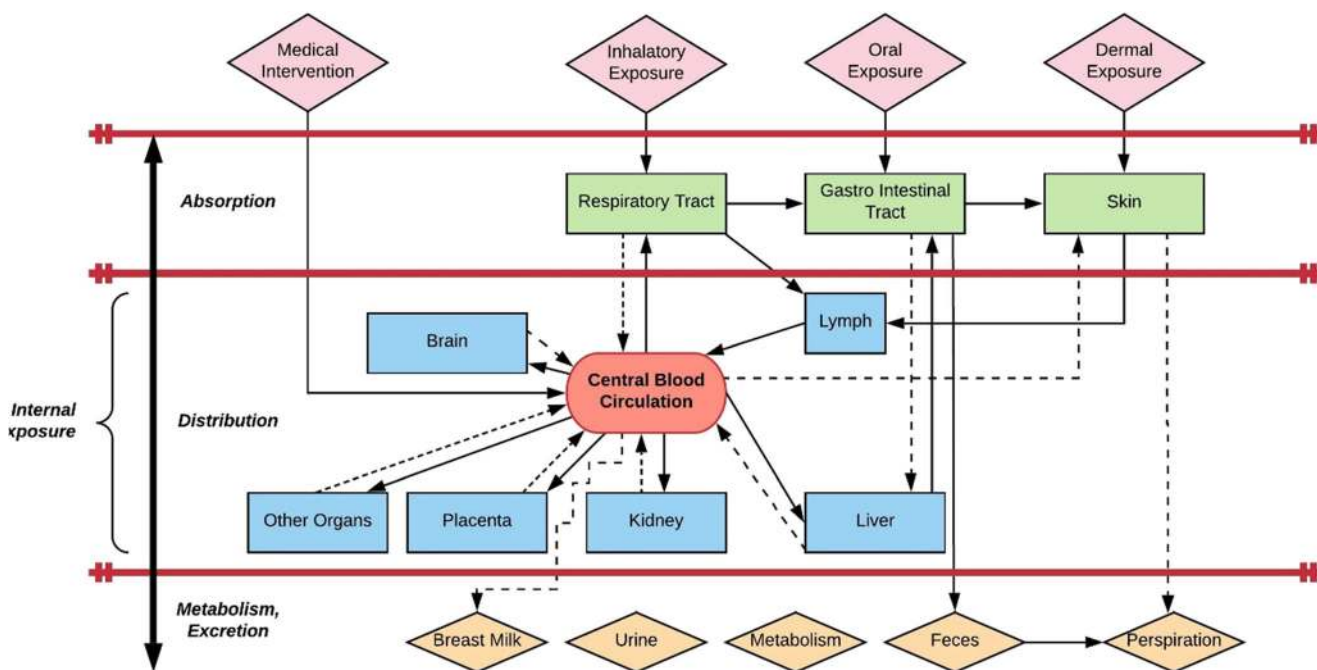


Fig. 3 The dynamic nature of NPs in vivo. Radiation penetrates the systemic circulation. The black line represents the nanoparticle confirmation path, and the dotted line represents the virtual path (other organs = spleen, heart, genitals) [50]

TiO₂ NPs also induce brain injury because of their high vulnerability to oxidative stress [61–63]. Olfactory nerve and hippocampal neurons are considered the pathways for NPs when administered through nasal route under oxidative stress that decreases mice's spatial recognition memory ability [64]. Moreover, TiO₂ NPs can also decrease special recognition memory by disturbing the homeostasis of neurotransmitters, trace elements, and enzymes [19]. Many studies revealed a toxic effect depending on the duration of exposure and the dose of NPs [65–68]. These NPs elicit apoptosis and may accumulate in the brain, causing the increment in malondialdehyde (MDA), superoxide, water, 8-hydroxy-2'-deoxyguanosine, and carbonyl protein [69]. In addition, changes in the expression of associated genes also occur [70] that stimulate brain microglia to disturb mitochondrial energy with the production of ROS [71]. These particles also have a toxic effect on the glial cells by inducing morphological changes, with an increase in mitochondrial membrane potential (MMP) [72].

6 Oxidative Stress Induced by TiO₂ NPs

Oxidative stress is considered a key mechanism for harmful biological effects by NPs [73]. This mechanism is confirmed by the increase in ROS production, oxidative products, and depletion of cellular antioxidants [74]. Oxidative stress is generally considered to be one of the major mechanisms of TiO₂ NPs [73] which is associated with hydroxyl (OH) formation,

DNA damage [75], and a high level of glutathione and liver's malondialdehyde [76].

TiO₂ mediates oxidative stress to produce different amounts of hydroxyl radicals with or without UV light exposure [77]. These hydroxyl radicals are the major destructive species that enhance DNA damage [75]. After initial exposure to ultraviolet light, anatase TiO₂ particle sizes decrease cell viability in rats, resulting in DNA strand breaks and oxidative damage to DNA [78]. This is an important discovery, showing for the first time that photo-activated TiO₂ particles retain higher cytotoxic and genotoxic potential regardless of particle size when UV irradiation is stopped because ROS is also a vital signal regulator [78]. Exposure of NPs to cells can also affect the cellular signaling cascade that controls processes such as cell proliferation, inflammation, and cell death by increasing ROS formation [79]. ROS production depends on the activation of inflammatory cascades such as phosphorylation of the Extracellular Signaling-Regulated Kinase ERK1/2 (ERK_{1/2}), Tumor Necrosis Factor alpha (TNF α), and macrophage production together. High level of TiO₂ NP stress leads to cell damage associated with the moderation of oxidative stress and inflammatory signaling pathways [71, 79–81].

7 Cellular Uptake of TiO₂ NPs

From a toxicological point of view, the main characteristics of TiO₂ NPs are its surface area, size, chemical properties, solubility, crystallinity, and the accumulation of particles [82]. Cell

Table 2 Overview of past studies on genotoxicity of TiO₂ NPs

Sr. no.	Crystalline structure	Dosage	Cause	Result	Exposure type	Reference
1	Anatase	50, 250, and 500 mg/kg body weight of TiO ₂ NPs	Chromosomal aberrations in mice spinal cord bone marrow genetic disturbance	+	Intraperitoneal Injection	[90]
2	Anatase	1, 4, and 16 g/kg BW	Liver effect hepatocytes located around the centrilobular veins' oxidative stress	+	Intraperitoneal injection	[91]
3	Anatase	2.5 or 5 mg kg ⁻¹ body weight	by 4-HNE and Kupffer cells Increased numbers of terminal deoxyribonucleotidyl transferase-mediated dUTP nick-end labeling-positive (apoptotic) germ and interstitial space	+	Intraperitoneal injection	[92]
4	Anatase	0.1 to 100 µg mL ⁻¹ . The TiO ₂ NPs	cells, flagellar abnormalities, excess residual cytoplasm, and ROS Decreases cell viability in both A549 and 16HBE cells; Intracellular ROS levels	+	Inhalation administration	[93]
5	Anatase	1.0, 5.0 mg/kg of TiO ₂ NPs	increased and decreased global DNA methylation Alveolus of the lung	+	Intratracheal instillation	[6]
6	Anatase	5, 50, or 500 mg/kg of TiO ₂ NPs	Mutations in <i>p53</i> exons apoptotic DNA fragmentation	+	Orally administered	[94]
7	Anatase	0.8, 7.2, and 28.5 mg/m ³	Genotoxic effects in C57BL/6 DNA damage	+	Intratracheal instillation	[95]
8	Rutile and Anatase	500mg/kg b.w of TiO ₂ NPs	DNA fragmentation point mutation of Presenilin 1 gene at exon 5, Alzheimer's disease	+	Orally administered	[94]
9	Rutile and Anatase	25, 75, and 125 µM of TiO ₂ NPs	DNA intensity in tail, Olive tail moment, and chromosomal aberrations (CA) at 75 and 125 µM but not at 25 µM	+	Short-term human peripheral blood cultures	[96]
10	Anatase	12.5, 25, 50, and 100 µg/mL of TiO ₂ NPs	DNA damage	+	Continuous supply to roots	[97]
11	Anatase	50 mg/kg 10 nm TiO ₂ NPs	DNA damage accumulated in liver and lung tissues, metabolic homeostasis in the liver and by inducing oxidative stress, inflammatory responses, and apoptosis in lung	+	Intraperitoneal injection	[98]
12	Anatase	2, 10, or 50 mg/kg TiO ₂ NPs	Gene mutation assay micronucleus assay	-	Intravenous injections	[99]
13	Anatase	0.08 to 1.60 mg/mL TiO ₂ NPs	Wing spot test DNA damage cytotoxic effects on midgut and imaginal disc tissues of larvae	+	Ingestion	[100]
14	Anatase		Mutagenic effect at 1.5625 and 3.125 mM, while 78.0 nm NCs increased mutant spots no clastogenic/aneugenic effects.	-		[101]
15	Anatase	(0, 140, 300, 645, or 1387 mg/kg of TiO ₂ NPs	No significant acute hematological or genetic toxicity	-	Intravenous Injection	[102]
16	Anatase, rutile, anatase/rutile	18, 54, 162, or 486 µg of TiO ₂ NPs	Increased collagen staining and fibrosis, inflammation neutrophil influx in BALF, pathological effects	+	Intratracheal installation	[103]
17	Anatase TiO ₂ NPs	0, 10, 50 and 200µg/mL	DNA double strand breaks in bone marrow cells	+	Oral administration	[104]
18	Anatase	5mg/kg bw TiO ₂ NPs	DNA strand breaks	+		[105]

Table 2 (continued)

Sr. no.	Crystalline structure	Dosage	Cause	Result	Exposure type	Reference
19	Anatase	20 µg/mL for 24, 48, 72, and 96 h	Formamidopyrimidine DNA glycosylase-oxidized purines SCSA Perm DNA fragmentation kidney proximal tubular cells (NRK-52E ROS mitosis decreased apoptotic cells increased BrdU immunoreactivity reduced	+	Intravenously administration	[106]
20	Anatase	50 mg/kg	DNA damage mainly resulted from oxidized nucleotides, oxidative stress, apoptosis in the lung	+	Intraperitoneal injection	[98]

uptake, subcellular localization, and toxicity depend on the nature of these nanoparticles [83]. There are two main methods for the absorption of NPs in cells: active absorption in endocytosis and passive absorption in free diffusion [84]. Inhalation of TiO₂ NPs may stimulate alveolar macrophages to remove micrometer sized particles (3–6 µm), but not TiO₂ nanoparticles as they have very less size (20 nm) [84]. Phagocytosis normally removes particles larger than 500 nm because they cannot absorb the small particles [85]. So, particles remain in the tissue and cause constant stress on other tissues to endocytosis [86]. Results indicate that uptake of 50 nm nano-TiO₂ by endocytosis with alveolar A549 epithelial cells are limited to aggregate particles [86]. Rothen-Rutishauser et al. [87] used an in vitro model of the airway walls in which membrane-bound aggregates (> 200 nm) and unbound aggregates were observed in the cytoplasm. They found highly aggregated NPs in both the late and early endosomes. TiO₂ NP aggregates of less than 200 nm were able to penetrate red blood cells, but large particles were attached to the cell surface only [87].

8 Genotoxicity

Numerous in vitro and in vivo studies have been conducted to explore the TiO₂ NP genotoxic effects including DNA damage, inflammatory cytokines, gene mutations, DNA deletions, and micronuclei formation that is indicative of chromosomal aberrations in different cell lines [88, 89]. The genotoxic effects depended upon TiO₂ NP size and form [11] (Table 2).

9 Future Prospective

Nanotechnology develops the latest products and materials with improved properties. Existing data on nanoparticles show that these NPs spread throughout the body and accumulate in many organs by avoiding numerous protective barriers.

Different forms of TiO₂ NPs work differently due to the flexibility in shape, particle size, bioavailability, crystal structure, and UV-induced photocatalytic activity. So, it is suggested that TiO₂ nanoparticles should be used with great care, especially in foods and cosmetics. Nanoscale TiO₂ concentration must be declared in these products, so consumers are aware of the side effects of these products because these particles have a detrimental effect at the cellular, intracellular, and protein levels. Therefore, specific measures need to be taken to avoid the risk of disease for researchers, students, and workers during the manufacturing of these nanoparticles.

Declarations

Conflict of Interest The authors declare no competing interests.

Research Involving Human Participants and/or Animals None.

Informed Consent None.

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