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Toxicity of Nano-Titanium Dioxide (TiO_2-NP) Through Various Routes of Exposure: a Review

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Abstract Nano-titanium dioxide (TiO_2) is one of the most commonly used materials being synthesized for use as one of the top five nanoparticles. Due to the extensive application of TiO_2 nanoparticles and their inclusion in many commercial products, the increased exposure of human beings to nanoparticles is possible. This exposure could be routed via dermal penetration, inhalation and oral ingestion or intravenous injection. Therefore, regular evaluation of their potential toxicity and distribution in the bodies of exposed individuals is essential. Keeping in view the potential health hazards of TiO_2 nanoparticles for humans, we reviewed the research articles about studies performed on rats or other mammals as animal models. Most of these studies utilized the dermal or skin and the pulmonary exposures as the primary routes of toxicity. It was interesting that only very few studies revealed that the TiO_2 nanoparticles could penetrate through the skin and translocate to other tissues, while many other studies demonstrated that no penetration or translocation could happen through the skin. Conversely, the TiO_2 nanoparticles that entered through the pulmonary route were translocated to the brain or the systemic circulation from where these reached other organs like the kidney, liver, etc. In most studies, TiO_2 nanoparticles appeared to have caused oxidative stress, histopathological alterations, carcinogenesis, genotoxicity and immune disruption. Therefore, the use of such materials in humans must be either avoided or strictly managed to minimise risks for human health in various situations.

Keywords TiO_2 nanoparticles · Exposure · Toxicity · Routes

Introduction

Titanium dioxide (TiO_2) particles are being synthesized and used in various different sizes including fine particles with the size of approximately 0.1–2.5 μm and nanosize particles with the primary size of <0.1 μm [1]. Humans may be exposed to TiO_2 nanoparticles during manufacturing as well as by their use. The exposure to TiO_2 nanoparticles can be in the form of aerosols, suspensions or emulsions. At the workplace, the major routes through which TiO_2 nanoparticles can be encountered are inhalation and dermal exposure in relevance to toxicology. Robertson et al. [2] reported more than 150 items of manufacturer-identified nanotechnology-based consumer products that would have long-term dermal contact. TiO_2 nanoparticles are the most common of the nanomaterials found in dermally applicable consumer products [2].

Titanium dioxide nanoparticles are being used in toothpaste, food colorants and nutritional supplements on a large scale, and therefore, oral exposure to TiO_2 nanoparticles may happen through consumption of such products. According to a recent study, candies, sweets and chewing gums have a higher amount of TiO_2 nanoparticles (<100 nm) [3]. TiO_2 nanoparticulate carriers are delivered into the human body through intravenous or subcutaneous injection in nanomedicine [4]. Saber et al. [5] investigated that titanium dioxide nanoparticles found in products like paint are less dangerous, unless they become free by sanding. Gao et al. [6] studied testicular damage and alterations in gene expression profiles in male mice induced by intragastric

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administration of TiO_2 nanoparticles (NPs). They observed that TiO_2 NPs crossed the blood-testis barrier to reach the testis and resulted in testicular lesions, sperm malformations and alterations in serum sex hormone levels. Therefore, the production and application of TiO_2 NPs should be carried out cautiously, especially by humans of reproductive age.

Different routes of exposure that can lead to the systemic disposal of these nanoparticles, and the most prevalent ones are oral, subcutaneous, dermal, intravenous and, lastly, respiratory. Respiratory exposure threats are mostly increased in the form of occupational risk. Earlier works showed that over 150 different cosmetic products can lead to long-term dermal exposure of titanium dioxide nanoparticles. The whitening properties of TiO_2 nanoparticles render them useful as a food colorant. It is well known that several common food products have these nanoparticles in them, along with likely daily exposure to humans of various age groups [7]. Continual use of TiO_2 containing nanoparticles can lead to chronic level of exposure and accumulation in numerous organs.

Whatever the route of exposure to TiO_2 nanoparticles is, once they entered into the circulatory system, the nanoparticles are transported into various parts of the body as illustrated in Fig. 1 [8].

It may be concluded that the occupational exposure to TiO_2 nanoparticles may primarily occur by the route of inhalation. The use of antimicrobial spray which contains TiO_2 nanoparticles may possibly be responsible for consumer inhalation. The use of food products containing TiO_2 nanoparticle additives may cause oral exposure. The applications of sunscreens and other cosmetics are the source of dermal contact to TiO_2 nanoparticles. The medical application of TiO_2 nanoparticles may be in the form of their intravenous injections.

In this paper, the main focus will be on current information regarding the toxicity of TiO_2 nanoparticles. In order to gather the knowledge about the toxic effects of TiO_2 nanoparticles on humans, the studies done on rats or other mammalian organisms as experimental models will be reviewed. The main focus will be on the in vivo studies; however, very few of the in vitro studies will also be included in order to comprehend the results. Studies performed on TiO_2 nanoparticles and their mixtures with other substances and the studies that focused on the effects of TiO_2 nanoparticles on aquatic ecosystems or the environment will be avoided to be discussed in this review.

Dermal/Skin and Intradermal Exposure

A number of consumer products like sunscreens and cosmetics may contain TiO_2 nanoparticles, and hence, the study of dermal absorption of these nanoparticles is of interest. The outer layer of the human skin is a tough layer called the stratum corneum. Penetration of a number of inorganic particles through it is difficult. Normally, cosmetics and sunscreens containing TiO_2 nanoparticles are applied on an undamaged skin. However, certain conditions such as sunburn or physical force can cause slight injuries to the skin. Therefore, a number of in vivo and in vitro studies have been performed to investigate the penetration of TiO_2 nanoparticles on both intact skin as well as on the stripped skin to evaluate the effect on the injured skin [9]. Some of these studies, such as those of Newman et al. [10] and Sadrieh et al. [11], have shown no significant penetration of TiO_2 nanoparticles through the intact skin. Sadrieh et al. [11] found that TiO_2 nanoparticles (uncoated submicron sized, uncoated nanosized and

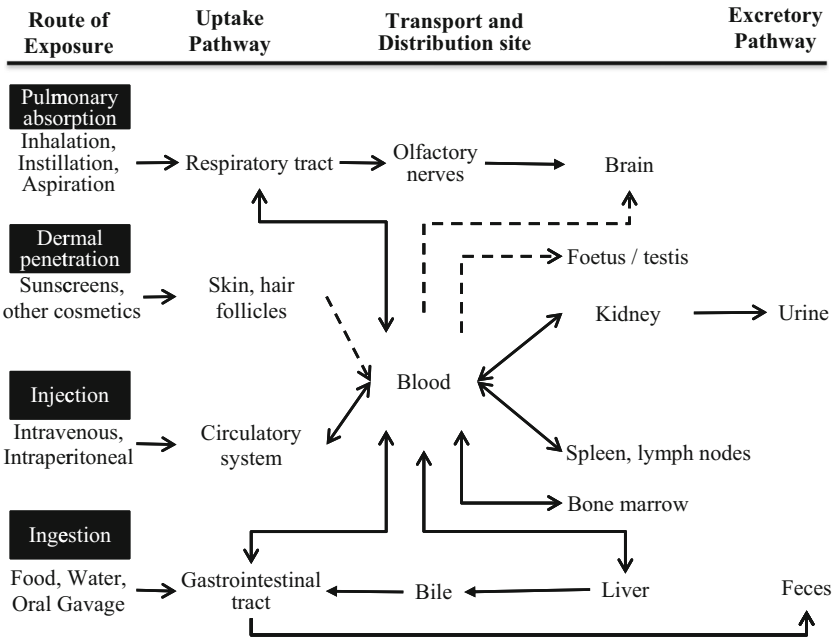

Fig. 1 Toxicokinetics and accumulation sites of TiO_2 nanoparticles. The *dotted line arrows* indicate uncertainties

dimethicone/methicone copolymer-coated nanosized) do not significantly penetrate the intact normal minipigs' epidermis when applied 5 % by weight in a sunscreen topically at 2 mg/cm^2 of the skin at the rate of 4 applications per day and 5 days per week for 4 weeks. No increase in the titanium levels in the lymph nodes and liver was observed; however, an elevated titanium level was observed in the treated epidermis of minipigs with sunscreens containing TiO_2 nanoparticles. The dermis of the abdominal and neck of minipigs treated with coated and uncoated TiO_2 nanoparticles showed increased titanium. Electron microscopy and energy-dispersive X-ray analysis revealed that all types of TiO_2 nanoparticles were observed in the upper follicular lumens and stratum corneum, and most of the particles visible were coated TiO_2 nanoparticles. The presence of isolated titanium particles was also detected at various positions in the dermis of the treated animals with sunscreens containing TiO_2 nanoparticles. However, distribution pattern or pathology suggested that the particles were the result of contamination, and the few isolated particles were a very small fraction of the total TiO_2 nanoparticles applied. Senzui et al. [9] applied 35 nm non-coated TiO_2 nanoparticles, 35 nm coated TiO_2 nanoparticles, 100 nm TiO_2 nanoparticles coated with alumina and silicon and 250 nm TiO_2 nanoparticles non-coated to the intact and stripped skins of Yucatan micropigs at the rate of 2 μL suspension per cm^2 of skin. Results showed no penetration of TiO_2 particles through viable skin, even though the stratum corneum was damaged. Scanning electron microscopy (SEM) showed the presence of some TiO_2 nanoparticles in vacant hair follicles; however, there was no penetration through the dermis or viable epidermis. The method of tape stripping using adhesive tape is widely used for studying the localization and distribution of drugs in the stratum corneum [12].

Filipe et al. [13] found that coated 20 nm TiO_2 nanoparticles dispersed in three sunscreen formulations were not likely to penetrate the stratum corneum towards the underlying keratinocytes in normal human skin even after 48 h under realistic in vivo conditions in normal and altered skin. However, deposition of the nanoparticles was observed in the openings of the pilosebaceous follicles. Similarly, Monteiro-Riviere et al. [14] demonstrated both in vivo and in vitro in pigs that penetration of TiO_2 nanoparticles of sunscreen formulations was slightly enhanced in the skin damaged by ultraviolet B. However, they detected no transdermal absorption. Sunscreens containing rutile crystallite-coated TiO_2 nanoparticles (90–460 nm) with the primary particle size 10×50 nm and mean agglomerates of 200 nm and 10 % oil/water or water/oil emulsion were dermally applied on skin in flow-through diffusion cells for 24 h. Skin exposed to ultraviolet B had typical sunburn histology.

Bennat and Müller-Goymann [15] showed that TiO_2 nanoparticles can penetrate through hairy skin on application as oil-in-water emulsion. They applied 5 % TiO_2 nanoparticles to human skin with the size of 20 nm as aqueous suspension or oil-in-water emulsion via tape stripping method and observed penetration of the TiO_2 nanoparticles through the hair follicles or pores. Furukawa et al. [16] found that there was no penetration of titanium dioxide nanoparticles through topical application in the epidermis of up to 20 mg silicon-coated TiO_2 nanoparticles and up to 100 mg non-coated TiO_2 nanoparticles in c-Ha-ras. Analysis of rat skin indicated that both doses of TiO_2 nanoparticles were not able to penetrate through either of the healthy or damaged skin. Furthermore, silicon-coated TiO_2 nanoparticles could not penetrate the human epidermis model in vitro [17, 18].

Wu et al. [19] found no penetration of TiO_2 nanoparticles of various sizes through the stratum corneum of isolated porcine skin exposed for 24 h in vitro. However, in vivo studies showed quite different results. Titanium dioxide nanoparticles (4 and 60 nm) penetrated through the horny layer and reached the deep layer of pig ear epidermis applied topically for 30 days. Similarly, TiO_2 nanoparticles penetrate through the skin of hairless mice dermally exposed for 60 days. These nanoparticles reached different tissues and caused various pathological lesions in many major organs. Interestingly, 21-nm-sized TiO_2 nanoparticles exhibited wider tissue distribution and even reached the brain. However, they did not induce any pathological changes. TiO_2 nanoparticles caused the most severe pathological changes in the skin and liver than all other organs studied and significant alterations in malondialdehyde (MDA) and superoxide dismutase (SOD) levels. These findings revealed that the deposition of the TiO_2 nanoparticles caused the oxidative stress that resulted in the pathological lesions. In this way, the collagen content represented as HYP content in mouse skin samples also reduced significantly. This indicates that topically applied TiO_2 nanoparticles in the skin for a long period can induce skin ageing. This study revealed that dermal exposure to TiO_2 nanoparticles over a relatively prolonged time may pose a health risk in humans [19].

Furukawa et al. [16] investigated that titanium dioxide nanoparticles do not possess post-initiation potential for mouse skin carcinogenesis. Topical application of up to 20 mg silicon-coated TiO_2 nanoparticles and up to 100 mg non-coated TiO_2 nanoparticles in c-Ha-ras protooncogene transgenic mice and rats, sensitive to skin carcinogenesis, respectively, and their wild-type siblings that were initially treated with a single dose of 7,12-dimethylbenz[*a*]anthracene showed no carcinogenesis-promoting effects in the skin due to no penetration through the epidermis. Analysis of rat skin indicated that both formulations of TiO_2 nanoparticles were not able to penetrate through either of the healthy or damaged skin. Furthermore, silicon-coated TiO_2 nanoparticles could not penetrate the human epidermis model in vitro [17]. A similar study was performed by Xu et al. [18] with similar results on the c-Ha-ras protooncogene transgenic (Hras128)

rats (sensitive to skin carcinogenesis) and their wild-type siblings treated with ultraviolet B-initiated skin carcinogenesis. TiO_2 nanoparticles were present in the upper stratum corneum. However, they were not detected in the underlying skin tissue layers. TiO_2 particles were also not able to penetrate a human epidermis model in vitro.

Warheit et al. [20] investigated acute dermal irritation in the local lymph node assay in mice and rabbits using 0, 5, 25, 50 or 100 % anatase/rutile (80/20) TiO_2 nanoparticles with the size of 129.4 nm in H_2O for three consecutive days, and TiO_2 nanoparticles did not cause skin irritation or dermal sensitivity. In another study, at 1, 24 or 48 h post-exposure, acute tests on the dermal, eye and vaginal mucous membrane in mice treated with TiO_2 nanoparticles at the dose of 1000, 2150, 4640 and 10,000 mg/kg body weight showed no significant irritation [21]. Topically applied 14, 28, 42 and 56 mg/kg 20-nm-sized TiO_2 nanoparticles on Wistar rat skin caused short-term toxicity in a 14-day toxicity study by Unnithan et al. [22] at the biochemical level expressed as decreased glutathione S-transferase and catalase activity and increased lactate dehydrogenase activity and lipid peroxidation, and the levels of glutamic pyruvic transaminase and glutamic oxaloacetic transaminase in the serum, blood urea nitrogen and creatinine were also increased. However, there were no observable histopathological effects at the tissue level. They concluded that renal as well as hepatic toxicity was caused due to short-term dermal exposure of rats to 42 mg TiO_2 nanoparticles per kilogram body weight of rats. They investigated that TiO_2 nanoparticles may penetrate into the live skin through the hair follicles.

Exposure of the skin to TiO_2 nanoparticles causes barrier dysfunction or defect which can intensify symptoms of atopic dermatitis through T helper-2 immune responses. TiO_2 nanoparticles can initiate and/or promote skin diseases after the barrier dysfunction/defect due to histamine release even when there is no allergen present. On treating male NC/Nga mice with 20 μg of 15, 50 or 100 nm rutile TiO_2 nanoparticles by intradermal injections, allergen+TiO_2 showed atopic dermatitis, enhanced ear thickening and inflammatory action (increased eosinophils, interleukin-4, mast cells and decreased interferon-γ; TiO_2 increased interleukin-13) [23]. They observed that intradermal injection of TiO_2 nanoparticles can decrease the local expression of interferon-γ in the presence of allergen. In serum, expression of interleukin-13 and histamine levels significantly increased the ear thickness in mice.

Pulmonary Absorption

The pulmonary system consists of the nose and nasal cavity, paranasal sinuses and pharynx (the upper respiratory tract), larynx, trachea, bronchi and the lungs (the lower respiratory tract). Here is a brief review of the studies performed on the effects of TiO_2 nanoparticles through inhalation, intranasal (oropharyngeal) exposure and intratracheal instillation (Fig. 2).

Figure 2 explains the distribution of TiO_2 nanoparticles after inhalation based on the study by Simkó and Mattsson [24]. Arrows denote downward movement of the nanoparticles through the respiratory tract. Most of the particles with size ranging 1–5 nm are distributed all through the three regions. Twenty-nanometer particles are mostly distributed in the alveolar regions. Particles of 0.5–10 μm size remain on the epithelial surface of the airways and alveoli.

Inhalation

Inhalation may be included in the major routes of exposure of the human body to TiO_2 nanoparticles especially at workplaces during handling processes. A number of studies have been performed to find out the cellular, genetic or physiological toxicity of TiO_2 nanoparticles using inhalation as the exposure route.

Up till now, there is no data available regarding the absorption of TiO_2 nanoparticles through inhalation in humans. However, a large number of studies have been performed on rodents [25]. Mühlfeld et al. [26] observed the transportation of a small fraction of 20-nm-sized TiO_2 nanoparticles from the airway lumen to the interstitial connective tissue of adult male rats exposed to 0.11 mg/m^3 TiO_2 aerosols for 1 h and released into their systemic circulation (capillary lumen) after 1- and 24-h dose. Similarly, van Ravenzwaay et al. [27] exposed male Wistar rats to anatase-rutile TiO_2 mixture (20–30 nm) and rutile TiO_2 (200 nm) in the form of aerosols of 100 and 250 mg/m^3 of uncoated and pigmentary TiO_2, respectively, for 6 h per day and five consecutive days via inhalation. TiO_2 nanoparticles were distributed in the lungs and mediastinal lymph node.

In response to ultrafine titanium dioxide particles in the form of aerosols, rats and mice developed progressive epithelial and fibroproliferative changes in the lung and lymph

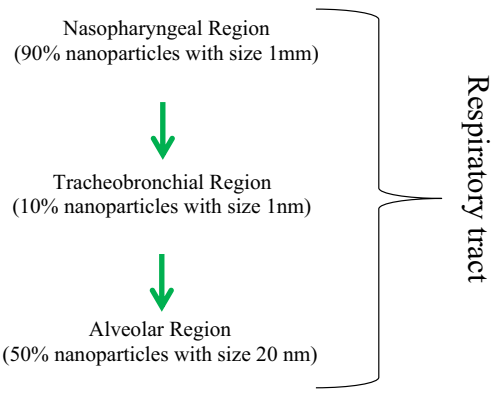

Fig. 2 Distribution of TiO_2 nanoparticles of different sizes in the respiratory tract

nodes. There was a marked impairment in the clearance of TiO_2 nanoparticles from the lung of exposed mice and rats [28]. A dose-dependent deposition of Ti in the lung tissue and an increase in neutrophils in bronchoalveolar lavage (BAL) fluid indicated an inflammatory effect in mice [29]. TiO_2 nanoparticles produced reactive species and endogenous nitric oxide. Nanoparticle exposure considerably enhanced microvascular oxidative stress up to about 60 % and elevated nitrosative stress 4-fold in conjunction with microvascular dysfunction. Spinotrapezious arteriolar endothelium dilation was impaired in male Sprague-Dawley rats exposed to 1.5–16 mg/m^3 P25 anatase-rutile TiO_2 (21 nm) for 240–720 min [30]. Nanoparticle exposure via inhalation significantly impairs endothelium-dependent vasodilation in sub-epicardial arterioles [31]. Nanoparticle exposure as aerosol inhalation reduces bioavailability of the microvascular nitric oxide and alters the vasoreactivity. In addition, the greater adrenergic receptor sensitivity indicates an amplified sympathetic responsiveness [32]. The significant impairment of endothelium-dependent vasoreactivity in coronary arterioles has also been observed by LeBlanc et al. [33]. Inhalation exposure to 6 mg/m^3 P25 anatase-rutile TiO_2 (21 nm) for 240 min impaired coronary arteriolar endothelium dilation and increased oxidative stress (reactive oxygen species (ROS)) in coronary microvascular walls of male Sprague-Dawley rats. Such disturbances in coronary microvascular function may result in the cardiac disturbances associated with the exposure to TiO_2 nanoparticles. Endothelium-dependent arteriolar dilation was significantly decreased in rats exposed to TiO_2 nanoparticles. The production of endogenous microvascular nitric oxide was decreased after inhalation of TiO_2 nanoparticles in a dose-dependent manner. Microvascular oxidative stress was increased significantly [34].

Inhalation of nanoparticulate up-regulates the expression of lung neurotrophins in an age-dependent fashion, and this effect is associated with airway hyperresponsiveness and inflammation vulnerability in earlier stages of lung development, which may lead to a higher risk of developing asthma. Inhalation of P25 Degussa (21 nm) TiO_2 nanoparticles (12 mg/m^3; 5.6 h/day for 3 days) in weanling (2-week-old), newborn (2-day-old) and adult (12-week-old) male and female Fischer 344 rats showed neurotrophin expression (nerve growth factor, brain-derived neurotrophic factor and their receptors), which increased in 2-day-old and 2-week-old rats. The airway resistance was increased in 2-week-old mice [35]. A long-term lung inflammation in time-mated adult female mice was induced by exposure to TiO_2 nanoparticles (42.4± 2.9 mg/m^3 TiO_2; 21 nm, average crystallite size aerosolized powder 97 nm (peak size), rutile elongated modified with Al, Si and Zr and coated with polyalcohols; for 1 h a day on gestation days 8–18). Gestationally exposed offsprings displayed moderate neurobehavioral alterations [36]. The inhalation of the surface-coated TiO_2 nanoparticles causes alterations in the expression of genes in the lungs related with acute phase, inflammation and immune response with concomitant changes in several miRNAs [37]. Noël et al. [38] found that bronchoalveolar lavage fluid (BALF) indicated that large aerosols (>100 nm) caused an acute inflammatory response, as shown by the significantly enhanced number of neutrophils, while small aerosols (5, 10–30 or 50 nm) produced significant oxidative stress damages and cytotoxicity in the lungs of male rats exposed to TiO_2 nanoparticles at 20 mg/m^3 for 6 h. In both aerosols, the 10–30 nm TiO_2 nanoparticles induced the marked pro-inflammatory effects as compared to the controls.

In an inhalation study, rats were exposed to aerosols at the dose of 2, 10 and 50 mg/m^3 rutile-anatase TiO_2 mixture with 25.1-nm-sized TiO_2 nanoparticles by inhalation for 6 h/day for 5 days [39]. Necropsies were performed either immediately after the last exposure or after 3 and 16 days post-exposure. Lung inflammation was associated with a dose-dependent increase in BALF total cell and neutrophil counts, polymorphonuclears, total protein contents, enzyme activities (Γ-glutamyl transpeptidase, lactate dehydrogenase, alkaline phosphatase (ALP), N-acetyl-glucosaminidase) and number of cell mediators. No indications of systemic effects were found by measurement of appropriate clinical pathological parameters. Cell replication increased in bronchi and bronchioles. Similarly, van Ravenzwaay et al. [27] exposed male Wistar rats to anatase-rutile TiO_2 mixture (20–30 nm) and rutile TiO_2 (200 nm) in the form of aerosols of 100 and 250 mg/m^3 uncoated and pigmentary TiO_2, respectively, for 6 h/day on five consecutive days through inhalation. TiO_2 nanoparticles were distributed in the lungs and mediastinal lymph node. Both TiO_2 increased total cell count, polymorphonuclears, total protein content, ALP, lactate dehydrogenase, Γ-glutamyl transpeptidase and N-acetyl-glucosaminidase in bronchoalveolar lavage that are indicators of increased inflammatory action. In an inhalation study on mice by Grassian et al. [40], when exposed to 2–5 nm TiO_2 nanoparticles at the dose of 8.88 mg/m^3 for 4 h per day for 10 days, there were higher counts of total cells and alveolar macrophages in the bronchoalveolar lavage fluid. However, the mice recovered after 3 weeks. These inhalation studies revealed that TiO_2 nanoparticles can cause pulmonary inflammation in both rats and mice at sufficient lung burdens. Nurkiewicz et al. [34] observed that inhalation of nanoparticles (21 nm) or the fine TiO_2 (1 μm) at the dose of 1.5 and 20 mg/m^3 for 24 h post-exposure induced a failure to respond to dilators which indicated microvascular dysfunction of the arterioles in the shoulder muscle. The TiO_2 nanoparticles were 6 to 7 times more potent than the fine particles. In a recent study by the same research group, it was found that the peripheral vascular effects were linked with the exposure to particulate matter of TiO_2 fine particles (710 nm) and nanoparticles (100 nm) inhaled at the dose of 1.5–16 mg/m^3 for 4–12 h inducing the activation of

inflammatory and/or neurogenic mechanisms [34]. In addition, it was observed that the inhalation of 6 mg/m^3 21 nm P25 and a mixture of anatase (80 nm)/rutile (20 nm) TiO_2 nanoparticles for 240 min caused an increased spontaneous arteriolar basal tone, a reduced flow and a reduced responsiveness of the coronary arterioles to dilators 1 day post-exposure in male Sprague-Dawley rats in another study [31]. It is noteworthy that microvascular dysfunction was stated at low lung burdens that did not significantly change the measures of bronchoalveolar lavage fluid in lung inflammation or damage. These findings are interesting as there are known links between particulate matter and cardiovascular diseases. Exposure to particulate matter can result in significant alterations in many cardiovascular indices, like blood pressure, heart rate, heart rate variability and blood coagulability [41].

Surprisingly, Rossi et al. [42] observed that if mice were repeatedly exposed to TiO_2 nanoparticles through the airway, the airway inflammation is modulated. This modulation of the airway inflammation depends upon the immunological condition of the exposed mice. They exposed ovalbumin-sensitized (asthmatic) female mice to silica-coated rutile TiO_2 nanoparticles with 10–40 nm primary particle size in the form of aerosol (10–1000 nm size range) via inhalation at a concentration of 10 mg/m^3 for 2 h per day, 3 days a week and continued for 4 weeks. The results were surprising, that is, a substantial decrease in the levels of antibodies, leucocytes, eosinophils, chemokines and cytokines, alveolar macrophages, periodic acid-Schiff+ goblet cells, interleukin-1β, tumour necrosis factor-α, interleukin-4, interleukin-13 and interleukin-10 was observed that are the characteristics to allergic asthma and inflammatory action. It indicated that the allergic pulmonary inflammation was suppressed dramatically in asthmatic mice exposed to TiO_2 nanoparticles. The airway reactivity was decreased by silica TiO_2, while it was increased by fine TiO_2 (<5 μm). In another study by Rossi et al. [43], the exposure of mice by inhalation for 2 h for four consecutive days for 4 weeks to uncoated rutile and anatase TiO_2 nanoparticles did not induce significant inflammation. Only the rutile TiO_2 nanoparticles coated with SiO_2 caused an obvious pulmonary neutrophilia along with elevated expression level of tumour necrosis factor-α and neutrophil-attracting chemokine in the lung tissue. Almost exclusive accumulation of TiO_2 was observed in the alveolar macrophages. Morimoto et al. [44] also reported that inhaled TiO_2 nanoparticles (rutile 51±9 nm; 2.8×10^5/cm^3 for 4 weeks; 6 h/day) did not induce inflammation or fibrosis in male Wistar rats. Inhalation of titanium dioxide nanoparticles did not induce the gene expression of matrix metalloproteinase-2 (MMP-2) and tissue inhibitor of metalloproteinase-2 (TIMP-2) messenger RNA (mRNA) in rat lungs. There were no changes of the gene expression of MMP-2, TIMP-2 and type I collagen. Another study by Leppänen et al. [45] showed no inflammatory action in male mice exposed to TiO_2 nanoparticles (primary particle size 20 nm; geometric mean diameters of 91, 113 and 130 nm anatase+brookite (3:1); 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)) through inhalation. However, reduction in expiratory flow in all the exposure situations was observed.

A sub-chronic inhalation study comparing pulmonary responses to TiO_2 nanoparticles in several species was performed [28]. Female mice, rats and hamsters that were exposed to different aerosol concentrations of 0.5, 2.0 and 10 mg/m^3 P25 TiO_2 nanoparticles (21 nm) for 13 weeks 6 h per day and 5 days per week showed an increase in retained lung burdens in a dose-dependent fashion in all three groups. However, significant species differences were observed in the pulmonary responses to the inhaled TiO_2 nanoparticles. Rats showed more severe inflammatory responses under similar conditions of lung burdens of TiO_2 nanoparticles than mice and, as a result, developed increased epithelial and fibroproliferative alterations. In the mice and rats that were exposed to 10 mg/m^3 TiO_2 nanoparticles, clearance of particles from the lung was significantly impaired, while in the hamsters, clearance was not affected at any of the administered doses.

Silicon dioxide (SiO_2)-coated 40 nm rutile TiO_2 nanoparticles inhaled at the concentrations of 10 mg/m^3 for 2 h on four consecutive days for 4 weeks induced pulmonary neutrophilia and increased the expression of tumour necrosis factor-α and neutrophil-attracting chemokine CXCL 1 in lung tissues [43]. However, they linked these effects to the surface coating with SiO_2. Minimal inflammatory effects in the lungs, leucopenia and decreased β-glucuronidase by inhalation of TiO_2 nanoparticles have been observed [46].

Chronic lung inhalation studies [47, 48] that exposed pigs or rats, respectively, to TiO_2 nanoparticles have reported findings of pulmonary pathology such as increased incidences of pneumonia, squamous metaplasia [48], sustained pulmonary responses [49], enhanced proliferation of pulmonary cells, defects in macrophage function [50], alveolar epithelial metaplasia, progressive fibroproliferative lesions [51] and accumulation of macrophages in interalveolar septa [8, 47].

Intratracheal Instillation

Intratracheal instillation is a technique in which single or repeated doses of specific volumes of substances are administered directly into the lungs. Although the studies on the exposure through inhalation are thought to be the gold standard, studies on the exposure through intratracheal instillation may prove useful for the assessment of risks [8, 52]. Sager et al. [53] intratracheally instilled 0.26–1.04 mg/rat 21-nm-sized TiO_2 nanoparticles in rats and observed the migration of a significant portion to the interstitial space after 42 days. The extent of migrated TiO_2 nanoparticles to the alveolar

interstitium was significantly greater than their fine counterparts after both inhalation exposure and intratracheal instillation [8, 53]. A small fraction of pulmonary TiO_2 nanoparticles may enter the blood circulation and reach other tissues like the liver and kidneys after 28 days of being intratracheally instilled [54]. When male Kunming mice were treated with 3.3 mg/kg once a week for 4 weeks of anatase TiO_2 (3 nm) nanoparticles by intratracheal instillation, increased Ti brain content, oxidative stress (O^{2-}, OH^-, H_2O_2, MDA) in brain and exudates, inflammatory infiltration and necrosis were observed.

Intratracheal instillation of TiO_2 nanoparticles had shown inflammatory effects in rats and mice [55–59]. The toxic effect of intratracheally instilled TiO_2 nanoparticles in lung tissue exhibited a dose-response relationship. After exposure to TiO_2 nanoparticles, TiO_2 nanoparticles induced standing pulmonary lesions and may suppress the phagocytotic ability of alveolar macrophages. Male and female Sprague-Dawley rats treated with 0.5, 5 or 50 mg/kg TiO_2 nanoparticles with 5, 21, and 50 nm primary particle size by intratracheal instillation showed inflammatory action (lactate dehydrogenase and ALP increased in the lung by 5 and 50 nm TiO_2), inflammatory infiltration, alveolar wall thickening and alveolar macrophage phagocytic ability altered by 5 and 50 nm TiO_2 [57]. Smaller particles induced greater inflammation in the short-term observations. Long-term effects (>1 week post-instillation) include pulmonary inflammation. However, this pulmonary inflammation was remarkably recovered [56]. In two experiments by Kobayashi et al. [56], male rats were treated with 5 mg/kg anatase TiO_2 (4.9 nm) (1st experiment), anatase TiO_2 (23.4 nm) and anatase TiO_2 (154.2 nm) (2nd experiment) by intratracheal instillation. The 4.9- and 23.4-nm TiO_2 nanoparticles increased the total cell count, neutrophils and lactate dehydrogenase, while in the second experiment, agglomerated TiO_2 increased total cell count, neutrophils and lactate dehydrogenase. All treated groups showed epithelium hypertrophy. Inflammatory responses in the lungs have also been reported by Park et al. [55] and Roursgaard et al. [60]. Nemmar et al. [61] also demonstrated that intratracheal exposure to 1.5 mg/kg rutile Fe-doped nanorod TiO_2 (length 80 nm; diameter 7 nm) can promote pulmonary as well as systemic inflammation (neutrophils and interleukin-6 increased) and caused oxidative stress (SOD activity decreased in bronchoalveolar lavage) along with histological changes (inflammatory cell infiltration) in Wistar rats. Blood parameters like WBC, interleukin-6 and SOD were reduced, while glutathione and platelets increased. Lung damage and change in the permeability of the alveolar-capillary barrier have also been observed by Li et al. [54] and Tang et al. [59]. The TiO_2 nanoparticles can enter blood circulation and reach extrapulmonary tissues, e.g. the liver and kidney [54]. Male Kunming mice exposed to 3.3 mg/kg anatase (3 nm) TiO_2 once a week for 4 weeks showed inflammatory action, increased acid phosphatase, ALP in bronchoalveolar lavage and destroyed alveolar walls. Intratracheally instilled TiO_2 nanoparticles may accumulate significantly in the lungs as reported by Sun et al. [62, 63]. TiO_2 nanoparticles may significantly accumulate in the lungs and may increase the lung indices and inflammation and bleeding in the lungs. This may result into severe inflammatory response, pulmonary oedema and pneumonocyte apoptosis for 90 days [62]. With increasing exposure, TiO_2 nanoparticles may significantly accumulate and cause the production of the reactive oxygen species in the lung [63]. A dose-dependent retention of TiO_2 nanoparticles has been observed in the lungs up to 28 days after instillation by Husain et al. [64]. A dose-dependent manner of infiltration of inflammatory cells has also been observed in lung tissues by Nemmar et al. [61]. The intratracheal exposure induced a significant and dose-dependent increase in neutrophils in the bronchoalveolar lavage and an increase in interleukin-6 and caused a dose-dependent decrease of superoxide dismutase activity [61]. A study conducted by Roursgaard et al. [60] showed sub-chronic lung inflammation in a dose-dependent manner due to an increase in BALF macrophages. Histology showed little inflammation overall. Rutile TiO_2 nanoparticles were the most inflammogenic, while amorphous TiO_2 nanoparticles were the most potent in regard to acute tissue damage. There was a dose-dependent acute increase in neutrophils, IL-6 and total protein in BALF in all TiO_2 nanoparticle-treated groups [60].

TiO_2 nanoparticles generate pulmonary inflammation in mice that could be due to the oxidative stress and expression of inflammatory cytokines. Exposure to TiO_2 nanoparticles significantly enhance the reactive oxygen species and lipid peroxidation and decrease the capacity of the antioxidant in the lung [62]. Retention of TiO_2 nanoparticles in the absence of inflammation over time in low-dose groups may possibly upset calcium and iron homeostasis and disturb smooth muscle activities. In high dose, nanoparticles caused lung inflammation. However, in low and medium doses, the inflammation resolved and there was no neutrophil influx in the lung fluid [64].

Low-dose instillation of TiO_2 nanoparticles (5 nm; 0.8, 4, 20 mg/kg) can recoverably impact metabolic function (acetate, valine, dimethylamine, taurine, hippurate and 2-oxoglutarate) because the scattered nanoparticles may be transported from the lung to other organs or tissues like the liver or kidney, but particles in higher doses aggregate and deposit in the lung without migration and cause pulmonary inflammation along with expanded lung gaps and hyperemia [59]. Biochemical parameters showed blood urea nitrogen and creatinine to be increased, while high nuclear magnetic resonance urine analysis showed increases in valine, lactate, acetate, succinate, 2-oxoglutarate, creatinine, taurine, trimethylamine-N-oxide, allantoin and hippurate 1–2 and decreases in citrate and dimethylamine. Oxidative stress may be

induced by the intratracheal exposure to TiO_2 nanoparticles induced in the liver and kidney, but does not affect renal or hepatic functions. Glutathione peroxidase activity of the kidney and superoxide dismutase activity of plasma in the low-dose group significantly decrease, while malondialdehyde levels of the kidney and liver significantly increase. There were no apparent pathological changes in the liver and kidney [65], whereas inflammatory responses have been observed by Park et al. [55]. Liang et al. [65] treated male and female Sprague-Dawley rats with 5 and 21 nm at a dose of 0.5–50 mg/kg by intratracheal instillation. Biochemical parameters such as total protein, albumin, ALT, AST, blood urea nitrogen and creatinine showed no changes. But oxidative stress (decreased SOD and glutathione peroxidase and increased MDA activity) was observed in the liver, kidney and plasma, mostly by 5 nm. Li et al. [54] reported the entrance of intratracheally instilled TiO_2 nanoparticles into the blood and liver and kidney injury due to these particles. Nemmar et al. [61] reported that the liver showed slight infiltration of inflammatory cells, mainly lymphocytes, of few portal tracts. The plasma superoxide dismutase and reduced glutathione activities decreased dose-dependently, while AST and ALT increased [61]. Increased levels of aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase, blood urea nitrogen and creatinine, which indicated a slight injury in the liver and kidney, as well as an increase in alveolar macrophages, expanded lung gaps, hyperemia and alveolar thickness were also reported by Tang et al. [66] in male Sprague-Dawley rats exposed to intratracheal instillation of 0.8, 4 and 20 mg/kg anatase (5± 1 nm) TiO_2 nanoparticles. Biochemical parameters changed (ALT and blood urea nitrogen increased). High nuclear magnetic resonance urine analysis showed increases in valine, lactate, acetate, succinate, 2-oxoglutarate, creatinine, taurine, trimethylamine-N-oxide, allantoin and hippurate 1-2; decreases in citrate, dimethylamine, ketone bodies, choline and low-density lipoprotein; increases in alanine and glutamic acid; and decreases in creatine and pyruvate. TEM analysis of the kidney revealed tubule epithelial cell damage and vascular deformity. Intratracheally instilled TiO_2 nanoparticles may change the permeability of the alveolar-capillary barrier. TiO_2 nanoparticles might pass through the blood-brain barrier and induce brain injury through oxidative stress response in mice [54].

A significant increase in the levels of lactate dehydrogenase and liver enzymes, i.e. aspartate aminotransferase and alanine aminotransferase in plasma, was reported by Nemmar et al. [61]. An increase in the levels of choline, ketone bodies, alanine and low-density lipoprotein; a decrease in the levels of lactate, pyruvate and creatine; an increase in the levels of aspartate aminotransferase, lactate dehydrogenase and alkaline phosphatase; and an increase in the levels of blood urea nitrogen and creatinine in serum indicated a slight injury in the liver and kidney. Transmission electron microscopy revealed particle-related alterations in the structure of the lungs, liver and kidneys. It also revealed apoptosis due to the localization of nanoparticles within cells. Biochemical parameters changed, e.g. albumin and glutamic acid increased. High nuclear magnetic resonance serum analysis showed that ketone bodies, choline, low-density lipoprotein, alanine and glutamic acid increased and lactate, creatine and pyruvate decreased when male Sprague-Dawley rats were treated with anatase (5±1 nm) nanoparticles at 0.8, 4 and 20 mg/kg. TEM analysis showed swollen hepatocytes and congested sinusoids [66].

TiO_2 nanoparticles possibly cause chronic inflammatory diseases in mice. The expressions of genes related with antigen presentation and genes related with the induction of chemotaxis of immune cells markedly increase. ICR mice exposed to 5, 20 and 50 mg/kg of P25 TiO_2 (21 nm) by a single intratracheal instillation showed inflammatory action (interleukin-1, tumour necrosis factor-α, interleukin-6, interleukin-12, interferon-γ, interleukin-4, interleukin-5, interleukin-10 and IgE increased in bronchoalveolar lavage), histology changes (inflammatory proteins, granulomas) and up-regulation of genes involved in antigen presentation and immune cell chemotaxis [55]. Intratracheally instilled TiO_2 nanoparticles caused a transitory response of pro-inflammatory cytokines and T-cell-activating cytokines in the airways, along with an influx of neutrophils and eosinophils. Gustafsson et al. [67] have demonstrated a dynamic response in the lungs of Dark Agouti rats to TiO_2 nanoparticles, starting with an activation of the innate immunity of eosinophils, neutrophils, natural killer cells and dendritic cells, followed by a long-lasting activation of lymphocytes of adaptive immunity. Intratracheal instillation of 1, 5 and 7.5 mg/kg bw P25 Degussa TiO_2 resulted in a transient increase in eosinophils and neutrophils in bronchoalveolar lavage, followed by a recruitment of dendritic cells and natural killers. Elevated levels of interleukin-1, interleukin-2, interleukin-6 and cytokine induced neutrophil chemoattractant-1 and granulocyte-macrophage colony-stimulating factor.

As a result of exposure to intratracheally instilled TiO_2 nanoparticles, both damage to the cell structure and dysfunction of pulmonary alveolar macrophages may happen, leading to a reduction in both specific and non-specific immune responses in individuals exposed to small-sized TiO_2 nanoparticles [68]. Heart rate, systolic blood pressure, plasma interleukin-6 and number of leukocyte and platelet increased [61]. Intratracheal exposure to TiO_2 nanoparticles may trigger the systemic immune response. Immune function response may be considered as an increase in the proliferation of T cells and B cells after mitogen stimulation and increased killing activity of natural killer cell in the spleen, along with an increase in the number of B cells in the blood. There were no significant changes in cytokines [69].

Furthermore, exposure to TiO_2 nanoparticles can induce the expression of heme oxygenase-1, Nrf-2 and glutamate-cysteine ligase catalytic subunit from an exposure of 15 to 75 days, while there were significant decreases in the expression levels of the three factors in the lung at 90-day exposure. The Nrf-2 expression induction is an intracellular adaptive response to oxidative stress induced by TiO_2 nanoparticles in the mouse lung [63]. Furthermore, exposure to TiO_2 nanoparticles activated nuclear factor-κB and increased the levels of cyclooxygenase-2, heme oxygenase-1, tumour necrosis factor-α, interleukin-(IL)-2, IL-4, IL-6, IL-8, IL-10, IL-18, IL-1β and CYP1A1 expression. However, the exposure to TiO_2 nanoparticles reduced nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)-inhibiting factor and expression of heat shock protein 70 in mice [62].

Single intratracheal instillations of 18, 54 and 162 μg per mouse altered approximately 3000 genes as revealed by DNA microarray analysis. Several inflammatory mediators were altered in a dose-dependent and time-dependent manner at the mRNA as well as protein level. Although the low dose did not show influx of neutrophils, alterations in the expression of a number of genes and proteins related with inflammation were observed. Inflammation resolved at the medium dose, while the low dose showed no neutrophil influx in the lung fluid. These effects were associated with down-regulation of genes responsible for ion homeostasis and regulation of muscle [64]. However, Naya et al. [70] found in a study carried out on Sprague-Dawley male rats that intratracheal instillation of anatase TiO_2 nanoparticles is not genotoxic in rats. The comet assay revealed no increase in tail %DNA in the dosage of 1.0 or 5.0 mg/kg body weight and 0.2 or 1.0 mg/kg body weight once a week for 5 weeks. Cho et al. [71] also reported no inflammatory responses in the bronchoalveolar lavage and histology of the lung in rats after 24 h and 4 weeks of treatment of intratracheally instilled 50 and 150 cm^2/rat TiO_2 nanoparticles (30–40 nm). A slight congestion in the spleen and deposition of brown particulate in cervical and axillary lymph node due to exposure to TiO_2 nanoparticles have been demonstrated by Fu et al. [69] in Sprague-Dawley rats.

Zhang et al. [72] evaluated the microdistribution of TiO_2 nanoparticles in the lungs of rats using X-ray fluorescence microscopy. Rats were intratracheally administered with 10 mg/kg TiO_2 nanoparticles with a microsprayer. The intensity of Ti in lung sections was measured using X-ray fluorescence. The beam size was 100 μm. The distribution of TiO_2 nanoparticles was more in the right caudal and accessory lobes, located downstream of the direction of administration and the lower portion of each lobe.

However, Courtois et al. [73] found no altered effect in the intralobar arteries' vasomotor responses to prostaglandin F2α, KCl and acetylcholine in male Wistar or Sprague-Dawley rats exposed to 100 μg TiO_2 in 0.5 mL saline of P25 Degussa TiO_2 nanoparticles (15 nm) by intratracheal instillation.

Liu et al. [57] treated rats by intratracheal instillation with a single dose of 0.5, 5 or 50 mg of 5, 21 and 50 nm TiO_2 nanoparticles per kg body weight. Histopathological examinations of the lung tissue 1 week post-exposure indicated that TiO_2 nanoparticles caused dose-dependent inflammatory lesions. Pulmonary toxicity caused by 5 nm TiO_2 nanoparticles was more severe as compared to those due to 21 or 50 nm TiO_2 nanoparticles. Kobayashi et al. [56] reported the time course of pulmonary responses at 1 and 7 days after intratracheal instillation of 5 mg/ml of 19 and 28 nm TiO_2 nanoparticles in rats. The pulmonary inflammation was greater after 24-h exposure then after 1-week exposure to TiO_2 nanoparticles. The inflammation was dose dependent and locally distributed and recovery was probable. Liu et al. [68] investigated the effects of TiO_2 nanoparticles on the immune function of rat alveolar macrophages exposed to intratracheally instilled 5 and 200 nm TiO_2 nanoparticles at the concentration of 0.5, 5 or 50 mg/kg. They reported damaged cell structure and dysfunction of alveolar macrophages, resulting in reduced non-specific as well as specific immune responses. The phagocytic ability of the macrophages was inversely related to the changes in the dose of TiO_2 nanoparticles. The chemotactic ability of macrophages and expression of some receptors on the cell surface was decreased. Nitric oxide (NO) and expression of TNF-α by the alveolar macrophages were gradually enhanced by the increased dosage of TiO_2 nanoparticles. TiO_2 nanoparticles produced more NO and TNF-α than fine particles [74]. Pulmonary inflammation and airway hyperresponsiveness were increased by low pulmonary doses of 99.9 % anatase 15 nm TiO_2 nanoparticles in toluene diisocyanate-induced asthma mice treated on the dorsum of both ears (20 μL) on days 1 and 8 [75]. The mice were administered oropharyngeally with 40 μL of a TiO_2 nanoparticle suspension (0.8 mg/kg body weight) on day 14. Airway hyperresponsiveness increased 2-fold, and total cell count of bronchoalveolar lavage fluid, mainly consisting of neutrophils and macrophages, increased 3-fold. Inflammation, epithelial damage and edema increased. These studies propose that TiO_2 nanoparticles may be airway irritant.

Sub-acute Study

Zhang et al. [76] intratracheally instilled rats with TiO_2 nanoparticles at the dose of 1 and 10 mg/kg body weight. LDH activity, MDA, total protein and the number of leukocytes as well as pulmonary inflammation were increased significantly at 10 mg/kg body weight dose. Oberdorster et al. [77] observed a significant pulmonary inflammation due to TiO_2 nanoparticles (20 nm) in rats and mice, expressed by the increase of total protein in branchioalveolar lavage fluid, acid-glucosidase and LDH activity. Li et al. [54] studied the effects of intratracheally instilled 3-nm-sized TiO_2 nanoparticles once a week for 28 days in mice after a total dose of 13.2 mg/kg

body weight. Lung damage and change in the permeability of the alveolar-capillary barrier were observed. TiO_2 nanoparticles entered the blood circulation and reached extrapulmonary tissues, e.g. the liver and kidneys, and caused tissue injury. TiO_2 nanoparticles were also found to pass through the blood-brain barrier and caused injury via oxidative stress. In some other areas, TiO_2 nanoparticles at the dose of 1.0, 0.5 and 0.1 mg/ml twice per week for 6 weeks induced dyslipidemia and enhanced the atherosclerosis and plaque rupture in intratracheally instilled mice [78].

Sub-chronic Study

Warheit et al. [79] compared several types of TiO_2 fine particles and nanoparticles with different sizes, surface areas and crystal structures by intratracheal instillation of TiO_2 nanoparticles with the size of 25 or 100 nm and dose of 1 and 5 mg/kg body weight for 24 h, 1 week and 3 months into rats. In the comparison between these particles, even though the surface areas differed as large as 30-fold, the lung inflammation observed was almost similar for the two particle sizes. Therefore, they concluded that toxicity of TiO_2 particles is not dependent on the particle size or surface area through lung instillation. Moreover, the same research group suggested that toxicity depends upon particle surface properties instead of surface areas. When mice were intratracheally instilled with single fixed doses (5, 50 and 500 μg) of TiO_2 fine as well as rutile nanoparticles, Roursgaard et al. [60] found an increase in interleukin-6 and total protein in bronchoalveolar lavage fluid as well as airway inflammation at the highest doses in the acute phase by both fine as well as nanoparticles.

Nasal Exposure (Intranasal Instillation)

Breathing mostly takes place through the nose and is called nasal breathing. The nasal cavity has a respiratory segment and an olfactory segment. The former is lined with pseudostratified ciliated columnar epithelium with much vascularized lamina propria that allows the venous plexuses of the conchal mucosa which helps more blood passing through this segment, controlling airflow and directing air in the nose. The latter is lined with the olfactory epithelium containing receptors for smell. Different types of cells are present here including bipolar neurons and supporting (sustentacular) cells along with basal cells and Bowman's glands. Axons of these bipolar neurons make the olfactory nerve (cranial nerve I) that enters the brain via the cribiform plate.

Titanium dioxide nanoparticles showed a 2-fold increase in airway hyperreactivity and a 3-fold increase in bronchoalveolar lavage total cell counts, mainly comprising neutrophils and macrophages in mice with diisocyanate-induced asthma. Histological analysis revealed increased oedema, epithelial damage and inflammation in the lungs [75]. Yu et al. [80] have also reported that chronic exposure to TiO_2 nanoparticles may result in atherogenesis in combination with pulmonary inflammation. Wang et al. [81, 82] demonstrated on murine brain that intranasally instilled 80 nm rutile and 155 nm anatase TiO_2 nanoparticles at 500 μg/ml for 2, 10, 20 and 30 days can be taken up and translocated by sensory nerves to the brain. Intranasally instilled TiO_2 nanoparticles to female mice directly entered the brain through the olfactory bulb, especially deposited in the hippocampus region and caused the pathological changes in the hippocampus and olfactory bulbs, such as irregular neuronal arrangement and condensated chromatin, and inflammatory action such as increased tumour necrosis factor-α and interleukin-1β levels. The oxidative damage expressed as lipid peroxidation, glutathione peroxidase, glutathione S-transferase, SOD, reduced glutathione and malondialdehyde increased significantly [82]. Nasally instilled TiO_2 nanoparticles can be translocated towards the central nervous system and may cause lesion of the brain. The hippocampus may be the main target within the brain. Female CD-1 (ICR) mice treated with rutile (80 nm) or anatase (155 nm) at the dose of 500 μg every other day for 15 times by intranasal instillation showed Ti brain distribution mainly in the olfactory bulb and hippocampus, causing oxidative stress (decreased catalase activity, malondialdehyde; protein carbonyls increased, SOD), and neurotransmitters like acetylcholinesterase, glutamic acid and NO were increased [81]. Chen et al. [83] have also suggested that the TiO_2 nanoparticles can translocate among the organs and pass through the blood-brain and the blood-heart barrier after long-term exposure.

TiO_2 nanoparticles caused haemorrhage and overproliferation of spongiocytes in the mouse brain. The exposure of mouse to TiO_2 nanoparticles also increased reactive oxygen species production and peroxidation of lipid, protein and DNA [84]. TiO_2 nanoparticles can be translocated and accumulated in the brain, leading to oxidative stress, all glial cell overproliferation, tissue necrosis and hippocampal cell apoptosis. Moreover, TiO_2 nanoparticles showed significant alterations in the expression of certain genes related to oxidative stress, immune response, apoptosis, metabolic process, DNA repair, brain development, signal transduction, memory and learning, response to stimulus and cellular process [85]. TiO_2 nanoparticles induce oxidative damage in the brain of mouse. This damage may take place through the p38-Nrf-2 signalling pathway [84]. Chen et al. [83] studied chronic toxicity of TiO_2 nanoparticles and found obvious adverse effect to zebrafish (*Danio rerio*), including time-dependent and concentration-dependent inhibition of growth and decrease in the liver weight ratio of zebrafish. TiO_2 nanoparticles were also found to be distributed and accumulated in the gill, heart, liver and brain.

Yu et al. [80] observed that the long-term exposure to TiO_2 nanoparticles may cause atherosclerosis and pulmonary inflammation. Chronic exposure to TiO_2 nanoparticles (1.25,

2.5, 5 mg/kg body weight; nasal instillation; 9 months consecutively) caused pulmonary inflammation and atherogenesis, accompanied by alterations of many different serum parameters like carbohydrates, lipid and protein contents and metabolites.

Although there are few studies about the exposure of TiO_2 nanoparticles with respect to inhalation, intratracheal instillation or intranasal exposures, these studies suggest the translocation of the TiO_2 nanoparticles from the lungs into the circulatory system and systemic tissues or from the nasal cavity into the nervous system through the sensory nerves. The available data suggest the low rate of migration of nanoparticles to the circulatory system [8].

Sub-chronic Studies

Wang et al. [86] investigated the influence of intranasally instilled TiO_2 nanoparticles (25, 80 or 155 nm) on monoaminergic neurotransmitters at different post-exposure times at the dose of 50 mg/kg for 2, 10, 20 or 30 days in female mice. The monoaminergic neurotransmitters such as norepinephrine (NE), 3,4-dihydroxyphenylacetic acid (DA), 5-hydroxyindole acetic acid (5-HT), 5-hydroxyindole acetic acid (5-HIAA), dopamine (DOPAC) and homovanillic acid (HVA) were found out by reversed-phase high-performance liquid chromatography with an electrochemical detector. Increased accumulation of TiO_2 nanoparticles was observed in murine brain for the 25-nm group after 10 days (059.3±293.5 ng/g) that declined slowly after 20 days post-exposure (654.7±269.2 ng/g) but did not further decreased after 30 days post-exposure. The levels of norepinephrine and 5-hydroxyindole acetic acid increased significantly after exposure to 80- and 155-nm-sized TiO_2 nanoparticles, while decreases in the levels of 3,4-dihydroxyphenylacetic acid, dopamine, homovanillic acid and 5-hydroxyindole acetic acid were observed due to the deposition of TiO_2 nanoparticles in the brain. Thus, inhaled TiO_2 nanoparticles may translocate and deposit in the brain after absorption through the nasal mucosa and may affect the release and metabolism of monoaminergic neurotransmitters in the brain.

Oral Exposure

The gastrointestinal tract (GIT) may be an important route for the absorption of TiO_2 nanoparticles because food products, water and liquid beverages and drug carriers may contain TiO_2 nanoparticles [87, 88]. The gastrointestinal absorption of nanomaterials has been the subject of the recent efforts in the field of nanomedicine to develop effective carriers which increase the oral uptake of drugs and vaccines [89]. TiO_2 nanoparticles produced a marked harmful effect on male fertility and biochemical parameters as well as produced histopathological changes. Orally administered TiO_2 nanoparticles affected the liver, testes, serum and seminal vesicle [90–92].

Orally administered TiO_2 nanoparticles caused high coefficients of the liver in rats [91]. Alterations of serum biochemical parameters such as ALT, AST, lactate dehydrogenase and pathology, i.e. spotty necrosis of hepatocytes and the hydropic degeneration around the central vein of the liver [91], and a significant increase in serum nitric oxide, hepatic SOD, glutathione reductase (GR) enzyme activities and MDA concentration [90] were observed, which indicate the hepatic injury by the nanoparticles. The liver showed vacuolar and hydropic degeneration and cell death of some hepatic cells [90]. Oral exposure to TiO_2 nanoparticles produced a significant oxidative stress in red blood cells, liver and brain of male mice, which is clear from the higher levels of reactive oxygen species, and changed antioxidant enzymes activities. There was also a substantial increase in the levels of dopamine and norepinephrine in the brain cerebral cortex. It suggests that the TiO_2 nanoparticles have neurotoxic potential. The presence of these nanoparticles within the cytoplasm and nucleus was detected by transmission electron microscopy [93].

Fadda et al. [94] observed the level of lipid peroxidation enhanced in rat liver and a dramatic increase in serum glucose level (marker of metabolic disorder) and the pro-inflammatory biomarkers including tumour necrosis factor-α, interleukin-6, C-reactive protein and immunoglobin G as well as the vascular endothelial growth factor (VEGF) (angiogenic factor) and NO, oxidative (DNA) damage, the alteration in the apoptosis marker, caspase-3, and the drug metabolizing enzymes, cytochrome P450 (CYP450), in rat livers.

A number of studies have shown that the orally administered TiO_2 nanoparticles with size less than 100 nm may cause hepatic and renal toxicity in rats. Vasantharaja et al. [95] orally administered a dose of 50 and 100 mg/kg body weight TiO_2 nanoparticles to adult male Wistar rats. These nanoparticles mainly affected the liver and kidney as shown by the changes in the serum parameters. The altered levels of total protein, glucose, AST, ALT and ALP indicated that TiO_2 nanoparticles induced hepatic damage. A significant increase in blood urea nitrogen and uric acid points out that TiO_2 nanoparticles damage the kidney.

Bu et al. [92] observed disturbances in energy and amino acid metabolism and the gut microbes in Wistar rats orally administered with different doses of TiO_2 nanoparticles that may be attributable to some injury to the liver and heart due to TiO_2 nanoparticles. The metabonomics analysis of serum showed altered levels of amino acids in rats treated with TiO_2 nanoparticles. TiO_2 nanoparticles raised AST, creatine kinase and lactate dehydrogenase and caused mitochondrial swelling in heart tissues.

The significant change of serum alpha-HBDH and lactate dehydrogenase in TiO_2 nanoparticle-exposed groups showed

myocardial damage. The nephrotoxicity and pathology alteration of the kidneys was also observed. TiO_2 mainly accumulated in the liver, spleen, lungs and kidneys. This indicates the transportation of TiO_2 particles to other tissues and organs after absorption by the gastrointestinal tract [91].

There was a significant decrease in body weight gain, sperm motility percentage, sperm cell concentration, sperm viability and serum testosterone level, while there was a significant increase in sperm abnormalities and a reduction in the number and size of the epithelial lining of the tubuloalveolar gland and hyperplastic glandular epithelium of the seminal vesicle. The testes showed mild spermatogenesis besides congested testicular blood vessels [90].

The exposure to TiO_2 nanoparticles induced major degenerative changes in the albino rat visual cortex. The cytoplasm showed some inclusion bodies, swollen mitochondria, dilated rough endoplasmic reticulum and swollen Golgi apparatus. The dendrites and axonal bundles showed thinning and disintegration of the myelin sheath. The oligodendroglial cell showed small shrunken nucleus with peripherally clumping chromatin and dilated rough endoplasmic reticulum [96].

The effects on serum and heart tissue of adult rats after oral exposure to TiO_2 nanoparticles showed increases in serum tumour necrosis factor-α, immunoglobulin G, interleukin 6, C-reactive protein, vascular endothelial growth factor, myoglobin, troponin, creatine kinase-MB and nitrite levels and DNA damage. TiO_2 nanoparticles caused an increase in all serum and tissue parameters in a dose-dependent manner [97]. The Morris water maze and the passive avoidance tests on lactating Wistar rats showed that exposure to TiO_2 nanoparticles by gavage can significantly harm the memory and learning in the offspring [98].

TiO_2 nanoparticles can cause neuroinflammation that may cause changes in the cytokine expression in mouse hippocampus. Ze et al. [99] treated mice orally with 2.5, 5 and 10 mg/kg body weight with TiO_2 nanoparticles for 90 consecutive days. Titanium accumulated in the hippocampus and caused neuroinflammation and spatial memory damage. Also, the expression of Toll-like receptors, e.g. TLR-2 and TLR-4, tumour necrosis factor-α, NF-κB-inducible kinase, NF-κB2(p52), nucleic IκB kinase, nucleic factor-κB and RelA(p65) was significantly activated, while the expression of IκB and interleukin-2 was significantly suppressed.

However, Geraets et al. [100] observed that titanium content levels in the liver and spleen above the detection limit were found only in some rats. Low levels of titanium could be detected in mesenteric lymph nodes. From these results, it may be concluded that some minor absorption, but to a very limited extent, may take place in the gastrointestinal tract. This very low oral bioavailability (limited uptake) and slow tissue clearance might result in potential accumulation in the tissues in the long run.

Warheit et al. [20] conducted acute oral toxicity studies in rabbits. Treatment with 129.4 nm fine particles and anatase/rutile (80 nm/20 nm) TiO_2 nanoparticles in H_2O at the dose of 175, 550, 1750 or 5000 mg/kg at 48 h intervals for 14 days caused very low toxicity and short-term and reversible ocular conjunctiva redness. Another study investigated the acute toxicity in mice after a single oral administration of 25, 80 and 155 nm TiO_2 particles at the dose of 5 g/kg body weight [91]. After 2 weeks of exposure, there was no noticeable acute toxicity. However, there were increased hepatic coefficients in the female mice treated with 25 and 80 nm TiO_2 nanoparticles. Hepatic and renal injury was apparent from serum biochemical parameters (lactate dehydrogenase, BUN, AST, ALT) and the pathology of the kidney and liver. Significant alterations of serum LDH in 25 and 80 nm TiO_2 nanoparticle-treated organisms indicated myocardial damage. These studies showed biochemical changes due to oral exposure but did not confirm systemic toxicity. Bu et al. [92] treated Wistar rats with TiO_2 nanoparticles at the dose of 0.16, 0.4 and 1 g/kg. The urine and serum analysis by HNMR showed enhanced levels of citrate, taurine, hippurate, histidine, citrulline, trimethylamine-N-oxide, alphaketoglutarate, acetate and phenylacetylglycine.

The use of engineered nanoparticles in consumer products results in the presence of nanoparticles in drinking water sources, although the concentration of Ti compounds in drinking water is mostly low [8]. Although the majority of aggregated or stable nanoparticles were removed by simulated conventional and advanced treatment using bench-scale coagulation/flocculation/sedimentation-simulated conventional treatment, as well as using microfiltration and ultrafiltration-simulated advanced treatment, nanoparticle metals were detectable in 2–20, 3–8, and 48–99 % of Ag, TiO_2, and ZnO nanoparticles or their dissolved ions, respectively, that remained in finished water. So the consequent nanoparticle increase into treated drinking water is a potential route for the exposure and threat for human health [101]. Trouiller et al. [102] demonstrated in vivo in mice the genotoxicity, oxidative DNA damage and inflammation by TiO_2 nanoparticles in drinking water. The comet assay, the micronuclei assay, and the γ-H2AX immunostaining assay showed induced 8-hydroxy-2′-deoxyguanosine, γ-H2AX foci (indicative of DNA double-strand breaks), micronuclei and DNA deletions, a moderate inflammatory response (mRNA levels of inflammatory cytokines in the peripheral blood).

Intraperitoneal Injection

Intraperitoneal (IP) injection is the injection of a substance into a body cavity (the peritoneum). The method is widely used in humans to administer chemotherapy drugs for the treatment of some cancers, especially ovarian cancer.

Although this method is controversial, but still this particular use has been recommended as a standard of care [103]. Intraperitoneal studies may be done to address the effects of possible TiO_2 nanoparticles use in nanomedicine [8]. Intraperitoneal injection of TiO_2 nanoparticles has been shown to affect blood. The nanoparticles did not activate platelets in vitro but caused prothrombotic effects in the microcirculation in vivo [104], while Younes et al. [105] reported that blood cell count remained unchanged with the exception of the platelet count that increases after the intraperitoneal injection of 20 mg/kg body weight TiO_2 nanoparticles every 2 days for 20 days into rats. Intraperitoneal injection affects the spleen [106–109], lung [106, 110], serum, heart, kidney, liver [106, 107], brain [111], inflammatory response [110, 111] and reproductive system [107]. Guo et al. [107] treated male ICR mice with 200 and 500 mg/kg body weight TiO_2 nanoparticles every other day for 5 times by intraperitoneal injections as a result of which ALT and AST/ALT as well as blood urea nitrogen were increased showing the effect on the liver and renal system. Reduced sperm density and motility, increased sperm abnormality and germ cell apoptosis were also observed. Intraperitoneally injected anatase TiO_2 nanoparticles interfere with antioxidant defence mechanisms that results in oxidative stress in hepatocytes due to production of reactive oxygen species promoting apoptosis and necrosis [112].

Symptoms of acute toxicity, e.g. passive behaviour, appetite loss, tremor and lethargy, were also induced. Slightly elevated levels of the serum enzymes ALP and AST were found from the biochemical tests, whereas blood urea nitrogen was not significantly changed [106]. The highest accumulation of TiO_2 was in the spleen and resulted in the lesion and congestion and proliferation of lymph nodule of spleen tissue and caused apoptosis of the splenocyte [106, 108]. TiO_2 nanoparticles can induce the spleen pathological changes, apoptosis, leading to the reduction of immunity of mice [108]. TiO_2 was also deposited in the lung, liver and kidney of adult male and female ICR mice when treated with 324, 648, 972, 1296, 1944 or 2592 mg/kg of 80 and 110 nm, mostly being 100 nm anatase TiO_2 nanoparticles. Histopathological examinations showed the entrance of some TiO_2 particles into the spleen which cause severe lesions and neutrophil infiltration. These nanoparticles also caused thrombosis in the pulmonary vascular system that may be attributed to blocking of blood vessels. Moreover, hepatocellular necrosis and apoptosis, hepatic fibrosis, hydropic degeneration, swelling of renal glomerulus, proteinic liquids in renal tubules and interstitial pneumonia were also observed in the high-dose groups that may be associated with the alveolar septal thickening [106]. Intraperitoneally injected anatase TiO_2 nanoparticles caused hydropic degeneration and fatty degeneration of hepatocytes, portal and lobular infiltration by inflammatory cells, cloudy swelling and congested dilated central veins, cytoplasmic degeneration and damaged nuclei of hepatocytes [112].

There were also a decrease in the coefficients of the liver, heart and kidneys and a significant increase in serum biochemical parameters such as the ALT, ALT/AST and BUN, as well as significantly reduced sperm density and motility, increased sperm abnormality and germ cell apoptosis. But damage on the liver and kidney function is slight in a dose-dependent manner [107]. Intraperitoneally injected anatase TiO_2 nanoparticles changed serum alkaline phosphatase and glutamate oxaloacetate transaminase activity [112]. Jeon et al. [113] intraperitoneally injected anatase TiO_2 nanoparticles with the average diameter <25 nm for 7 days. Glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and alkaline phosphatase were enhanced approximately 18, 35 and 69 %, accumulated in the periphery of sinusoid in the liver. Enzymes, such as superoxide dismutase, catalase and aldehyde dehydrogenase, were significantly inhibited by 22, 38 and 15 %, respectively, and glutathione peroxidase was constant. An increase in the AST/ALT enzyme ratio and activity of lactate dehydrogenase was observed after sub-acute exposure to TiO_2 nanoparticles as IP injection of 20 mg/kg body weight every 2 days for 20 days [105].

Moon et al. [110] found that intraperitoneal administration of TiO_2 nanoparticles induces acute lung inflammation and exhibits additive or synergistic effects with lipopolysaccharide, to some extent, at least, through activation of oxidant-dependent inflammatory signalling and the nuclear factor NF-κB pathway, that results in the increase of pro-inflammatory mediators, e.g. tumour necrosis factor-α, interleukin-1β, and macrophage inflammatory protein-2 in bronchoalveolar lavage fluid and mRNA expression of tumour necrosis factor-α and interleukin. They treated mice with 40 mg/kg of P25 TiO_2 (21 nm) through intraperitoneal injections and observed inflammatory action on neutrophils, total protein content, tumour necrosis factor-α, interleukin-1β and macrophage inflammatory protein-2 increased by TiO_2 or TiO_2+lipopolysaccharide. Another research shows that intraperitoneal injection of TiO_2 nanoparticles in mouse effectively activated caspase-3 and caspase-9, decreased the Bcl-2 levels of gene and protein, increased the levels of Bax, cytochrome c genes and their protein expression and promoted ROS accumulation [108].

Histological examination by Younes et al. [105] showed that intraperitoneal injection of 20 mg/kg body weight TiO_2 nanoparticles every 2 days for 20 days induced a little inflammation overall. Furthermore, pathological changes in the liver of rats were induced by the TiO_2 nanoparticles. Titanium accumulated in the lung, liver and brain.

Nanosized TiO_2 promotes increased neuro-inflammatory responses that may be due to an increase in microglial activation in pre-inflamed brain. When male mice were treated with <1 μm (rutile) or 21 nm (P25) at the dose of 40 mg/kg for

30 min after 5 mg/kg lipopolysaccharide through intraperitoneal injections, the inflammatory action was increased by TiO_2 nanoparticles as expressed by increased interleukin-1β, tumour necrosis factor-α and inducible nitric oxide synthase and induced microglial activation, as well as they showed enhanced ROS that represent oxidative stress [111]. TiO_2 nanoparticles when injected intraperitoneally may damage the development and proliferation of B and T lymphocytes, reduce the activity of macrophages and decrease natural killer (NK) cell population levels, outcomes that appear to lead to an increase in tumour growth in situ. This suggested that TiO_2 nanoparticles might have the potential to enhance tumour growth through immunomodulation of B and T lymphocytes, macrophages and NK cells. TiO_2 nanoparticles (<25 or <100 nm) intraperitoneally injected once a day for 7 days into mice decreased splenocytes, CD4+ and lipopolysaccharide-stimulated natural killer cells CD8+, while B-lymphocyte development and lipopolysaccharide-stimulated spleen cell proliferation were retarded [109]. TiO_2 nanoparticles can change the neurobehavioral performance of adult Wistar rats. Younes et al. [105] reported significantly increased anxious index in rats shown by the elevated plus-maze test after sub-acute treatment of TiO_2 nanoparticles at 20 mg/kg body weight every 2 days for 20 days injected intraperitoneally.

At the higher doses of an intraperitoneal exposure study in mice, 5 nm anatase TiO_2 nanoparticles intraperitoneally injected to mice at the concentration of 5, 10, 50, 100 and 150 mg/kg body weight daily for 14 days caused serious damage to the kidneys, liver and myocardium and altered blood sugar and lipid levels. On treating female CD-1 (ICR) mice with anatase TiO_2 (5 nm), bulk rutile TiO_2 (10–15 μm) at the dose of 5–150 mg/kg body weight anatase TiO_2 nanoparticles and 150 mg/kg bulk TiO_2 every day for 14 days by intra-abdominal injections, biochemical parameters, such as creatine kinase, lactate dehydrogenase, aspartate aminotransferase and alpha-hydroxybutyrate dehydrogenase, were increased by both TiO_2 [114]. Furthermore, with increasing doses of TiO_2 nanoparticles, liver function indicators, e.g. ALT, total protein, leucine acid peptide, albumin levels and pseudocholinesterase, were enhanced significantly; the kidney function indicators, e.g. BUN and uric acid, were decreased; and the myocardium function indicators, e.g. LDH, alpha-hydroxybutyrate dehydrogenase, triglycerides, glucose and high-density lipoprotein cholesterol levels, and the activities of AST and creatine kinase were increased. The accumulation of TiO_2 nanoparticles in the organs was related to the inflammatory responses and the differences in the coefficients of organs of mice. The LD_{50} value of intraperitoneally injected TiO_2 nanoparticles in mice was found to be 150 mg/kg body weight. Signs of acute toxicity, like passive behaviour, tremor, loss of appetite and lethargy, were observed in mice intraperitoneally injected with 50-nm-sized TiO_2 nanoparticles at the dose of 324, 648, 972, 1296, 1944 or 2592 mg/kg for 24 and 48 h and 7 and 14 days. ALT and AST levels were slightly elevated. Some TiO_2 nanoparticles entered and caused lesions in the spleen. Thrombosis was observed in the pulmonary vascular system. Furthermore, the high-dose group showed hepatic fibrosis, renal glomerular swelling, hepatocellular necrosis and apoptosis and interstitial pneumonia related to alveolar septal thickening. Ma et al. [115] observed inflammatory responses and liver injury by 5-nm-sized TiO_2 nanoparticles intraperitoneally injected at the dose of 5, 10, 50, 100 and 150 mg/kg body weight daily for 14 days. TiO_2 nanoparticles significantly altered the mRNA and protein expression of numerous inflammatory pathways, together with NF-κB, macrophage migration inhibitory factor, tumour necrosis factor-α, interleukin-6, interleukin-1β, cross-reaction protein, interleukin-4 and interleukin-10. Neurons turned into filamentous shapes or inflammatory cells after translocation of TiO_2 nanoparticles from the abdominal cavity [116]. A cascade of reactions was triggered by oxidative stress and the injury of the brain, like lipid peroxidation, decreases in the total antioxidation capability and antioxidative enzyme activities, the too much release of NO, reduced glutamic acid and down-regulation of acetylcholinesterase activity. Intraperitoneal injection of TiO_2 nanoparticles causes acute systemic toxicity, involving pathological as well as biochemical effects on the kidney, liver, heart and brain.

Another study investigated an adjuvant effect of photocatalytic 28-nm-sized rutile TiO_2 nanoparticles administered through intraperitoneal injection at the dose of 2, 10, 50 and 250 μg, in combination with ovalbumin (OVA) in mice [117]. The inbred female mice in this study were immunized with 1 μg OVA alone or in combination with either 2, 10, 50 or 250 μg TiO_2 by intraperitoneal injections. The TiO_2 nanoparticles promoted an immune response with high serum levels of ovalbumin-specific IgE and IgG1 and influx of neutrophils, eosinophils and lymphocytes in bronchoalveolar lavage fluid.

Subcutaneous and Intramuscular Injection

A subcutaneous injection is given in the fatty layer of tissue just under the skin. There is little blood flow to fatty tissue, and the injected medication is generally absorbed more slowly, sometimes over 24 h. Growth hormone, insulin, epinephrine and other substances are injected subcutaneously. Subcutaneous injection of TiO_2 nanoparticles causes various functional and pathologic disorders, for example reduction in sperm production and olfactory bulb of the brain, also in the next generation. When pregnant Slc:ICR mice were treated

with 100 μL at 1 mg/mL of 25–70 nm anatase TiO_2 nanoparticles at 3, 7, 10 and 14 days post-coitum by subcutaneous injections, TiO_2 nanoparticles were found in the cortex and olfactory bulb of offspring brain, and markers for features of apoptosis were seen in olfactory cells. The reproductive system was also affected by the nanoparticles as expressed by disrupted and disorganised seminiferous tubules, decreased sperm production, few mature sperm, number of Sertoli cells and epididdymal sperm motility [118]. Similarly, Umezawa et al. [119] demonstrated that prenatal exposure by subcutaneous injections may affect the brain. Differential expression of the genes associated with the striatum during the prenatal period, dysregulation of the gene expression for the regions related to dopamine neuron system and the prefrontal region have been observed by Umezawa et al. [119].

The gene expression involved in the development and function of the foetal central nervous system in offsprings may be affected after maternal exposure of mice to TiO_2 nanoparticles. Shimizu et al. [120] injected TiO_2 subcutaneously into pregnant mice on gestational days 6, 9, 12 and 15. Brain tissues from male foetuses on embryonic day 16 and from male pups on postnatal days 2, 7 14, and 21 were analysed by complementary DNA (cDNA) microarray analysis. The expression level of the genes related with apoptosis as well as brain development was changed in the offspring. The genes responsible for oxidative stress in the brain were changed in mice age 2–3 weeks. The expression of genes for neurotransmitters and psychiatric diseases was also changed [120].

Takahashi et al. [121] demonstrated in mice that maternal exposure to TiO_2 nanoparticles subcutaneously may affect the development of the central dopaminergic system in the next generation. In pregnant Slc:ICR mice subcutaneously injected with 100 μL at 1 mg/mL of 25–70 nm anatase TiO_2 nanoparticles at gestational days 6, 9, 12, 15 and 18, monoamine levels (dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, 3-methoxytyramine-hydrochloride) were increased in the prefrontal cortex and neostriatum.

Hansen et al. [122] treated male Sprague-Dawley rats with TiO_2 (70 nm) and bulk TiO_2 (diameter 0.9 mm; height 1 mm) in the back of rats for 6 or 12 months by subcutaneous (bulk) and intramuscular (Nanoparticles) implantation. This induced local reaction such as the presence of granulomas (nanoparticles) and inflammatory infiltrates (bulk) and no local intolerance (both). There is another similar study by Gatti et al. [123] who treated male Sprague-Dawley rats with TiO_2 (70 nm) and bulk TiO_2 (diameter 6–12 mm; height 2 mm) in the back of rats for 6 or 12 months by subcutaneous (bulk) and intramuscular (nanoparticles) implantation and observed local reaction in the form of granulomas (nanoparticles) and inflammatory infiltrates (bulk).

Onuma et al. [124] treated female C57BL/6 mice by subcutaneous injections with hydrophilic rutile TiO_2 (minor axis 40–70 nm; major axis 200–300 nm) treated with $ZrO_2Al(OH)_3$ and with hydrophobic rutile TiO_2 (minor axis 40–70 nm; major axis 200–300 nm) treated with $ZrO_2Al(OH)_3$ and steric acid (only QR-32 cells or QR-32 cells mixed with 5 mg/0.1 mL TiO_2; or TiO_2 30 and 70 days before injecting QR-32 cells) and observed the carcinogenicity and metastatic ability in tumour cell lines resulting from QR-32 cells injected in site pre-implanted with both the TiO_2. QR-32 cells turned into tumorigenic after the injection in sites implanted with hydrophilic TiO_2 for 30 or 70 days

Intravenous Injection

Intravenous treatment is the infusion of liquid substances directly into a vein. In recent days, in nanomedicine, TiO_2 nanoparticulate carriers are intravenously injected directly into the blood and it has elevated public concerns in regard to their toxicity to humans [4, 125]. Scown et al. [126] quantified the content of titanium in the blood, brain, gills, liver and kidney of rainbow trout (*Oncorhynchus mykiss*) at time points. Upon a single high-dose exposure to TiO_2 nanoparticles via the bloodstream injected intravenously, TiO_2 accumulates in the kidneys but has a minimal effect on kidney function such as urine production or glomerular filtration rate [126].

High doses of titanium dioxide nanoparticles intravenously injected to mice caused death on the second day of injection of TiO_2 nanoparticles, while the lower doses caused acute toxicity symptoms like decreased physical activity, food and water intake and increased white blood cell count. The spleen of the TiO_2 nanoparticle-treated mice showed higher tissue weight/body weight coefficients and lower liver and kidney coefficients. The TiO_2 nanoparticle treatment induced damage in the liver, spleen, brain, lung and kidneys; however, the heart showed no pathological effects [125]. Yamashita et al. [127] showed that intravenously injected 0.8 mg of 35 nm TiO_2 nanoparticles for two consecutive gestational days to pregnant mice showed pregnancy complications, e.g. the accumulation of TiO_2 nanoparticles in the placenta, brain and liver of the foetus and smaller uteri and foetuses.

Geraets et al. [100] observed the rapid distribution of titanium through the systemic circulation in the liver, spleen, kidney, brain, heart, lung and thymus and reproductive organs after single and repeated intravenous exposure. The main targets of titanium were the liver, spleen and lung. Some decrease in the levels of titanium was also observed during the 90-day post-exposure period. The maximum relative decrease was 26 %. Slight variations in kinetic profile were observed among the various particles; however, these could not be clearly related to

primary particle size differences or the hydrophobicity. Some clues were found about the crystalline form (anatase or rutile) on total titanium recovery. TiO_2 nanoparticles caused liver damage and induced significantly the glutathione reductase expression in male Wistar rats intravenously injected with 5 mg/kg for 5 days [128].

van Ravenzwaay et al. [27] intravenously injected male Wistar rats with 5 mg/kg body weight of anatase-rutile TiO_2 mixture (20–30 nm) and rutile TiO_2 (200 nm) and observed TiO_2 distribution mostly in the liver and spleen after injection as well as inflammatory action as both TiO_2 formulations increased total cell count, polymorphonuclears, total protein content, ALP, lactate dehydrogenase, Γ-glutamyl transpeptidase and N-acetyl-glucosaminidase in bronchoalveolar lavage.

There is another study by Onuma et al. [124] who treated female C57BL/6 mice by intravenous injections with hydrophilic rutile TiO_2 (minor axis 40–70 nm; major axis 200–300 nm) treated with $ZrO_2Al(OH)_3$; hydrophobic rutile TiO_2 (minor axis: 40–70 nm; major axis 200–300 nm) treated with $ZrO_2Al(OH)_3$ and steric acid (tumour cell lines into mice (5/line)) and observed the carcinogenicity and metastatic ability due to tumour cell lines obtained from QR-32 cells injected in site pre-implanted with both TiO_2. QR-32 cells were converted tumorigenic after injection in sites implanted with hydrophilic TiO_2 for 30 or 70 days.

Intra-articular Injection

A joint injection or the intra-articular injection is used in medicine for the treatment of joint inflammations, such as gout, rheumatoid and psoriatic arthritis, carpal tunnel syndrome, tendinitis, bursitis and sometimes osteoarthritis. Anti-inflammatory agents, such as corticosteroids, are injected into the affected joint using a hypodermic needle. Sometimes, hyaluronic acid is used to replace bursa fluids due to its high viscosity. In recent times, nanomaterial coatings are being used in orthopaedic implantations, for example bone, cartilage, joint, etc. Nanoscale wear particles may generate from orthopaedic implants with nanoscale surface features because of leftover stress. Intra-articularly injected Sprague-Dawley rats with anatase (diameter 45.87±7.75 nm; thickness 10–15 nm) TiO_2 nanoparticles at the dose of 0.2–20 mg/kg in the knee joints on alternate days 4 times showed histopathological changes in the knee joint, and the aggregated TiO_2 nanoparticles deposited and resulted in synovium hypotrophy, lymphocyte and plasma cell infiltration and fibroblast proliferation. In the TiO_2-exposed synovium, oxidative damage was induced, expressed by glutathione peroxidase, reduced glutathione, oxidized glutathione, MDA and increased SOD. Further, lipid peroxidation was detected in the synovium through decreased ALP and increased AST/ALT, lactate dehydrogenase, fatty degeneration, inflammatory cell infiltration, and proteinic liquids in renal tubules and decreased blood urea nitrogen and creatinine, which were the biochemical and histological parameters showing the effect of TiO_2 nanoparticles in the liver and renal system [129].

In another study, intra-articular injection of TiO_2 nanoparticles in rats decreased the thickness of articular cartilage in distal femur at 1, 7, 14 and 30 days post-exposure. There was a strong linear correlation. TiO_2 nanoparticles decreased cartilage thickness and cartilage volume in a time-dependent manner. Oedema chondrocyte and shrunk nucleus were also observed in the radial and calcified regions of the cartilage. The degenerated chondrocytes, the condensed chromatin, the rich mitochondria, the dilated endoplasmic reticulum and the fragments of ruptured endoplasmic reticulum in the cytoplasm of chondrocytes at day 30 post-exposure were seen. These results point out the potential damage of articular cartilage caused by the particles in knee joint and suggested that bio-monitoring should be given importance in patients with prostheses replacement [130].

Prenatal Exposure

The gene expression involved in the development and function of the foetal central nervous system in offspring may be affected after maternal exposure of mice to TiO_2 nanoparticles. Shimizu et al. [120] injected TiO_2 subcutaneously into pregnant mice on gestational days 6, 9, 12 and 15 with 100 μL at 1 μg/μL of 25–70 nm anatase TiO_2 nanoparticles. Brain tissues obtained from male foetuses on embryonic day 16 and from male pups on postnatal days 2, 7, 14 and 21 were analysed by cDNA microarray analysis. The expression level of the genes related with apoptosis as well as brain development was changed in the offspring. The genes responsible for oxidative stress in the brain were changed in the mice age 2–3 weeks. The expression of genes for neurotransmitters and psychiatric diseases also changed [120].

Prenatal exposure to TiO_2 nanoparticles can result in modifications in the olfactory bulb, cerebral cortex and some regions closely related to dopamine systems of offspring mice [119]. Subcutaneous injection of 0.4 mg TiO_2 into pregnant mice on gestational days 6–15 caused the genes related to the striatum to be differentially expressed during the perinatal period as well as the dysregulation of the genes associated with the dopamine neuron system and prefrontal region in the later infantile period.

Takahashi et al. [121] demonstrated in mice that maternal exposure to TiO_2 nanoparticles might influence the development of the central dopaminergic system in offspring. The stress during foetal life induced by prenatal exposure to TiO_2 nanoparticles could be implicated in depressive-like behaviours in adulthood. In mice, maternal exposure to TiO_2

nanoparticles disturbed the antioxidant status and caused a substantial oxidative damage to the nucleic acids and lipids within the brain of newborn mice. Depressive-like behaviours during adulthood were also observed in the sucrose preference test and the force-swimming test [131].

Intragastric Administration

Cui et al. [132] found in their experiments that intragastric administration of anatase (6.9 nm) TiO_2 nanoparticles to female CD-1 (ICR) mice for 60 consecutive days caused hepatocyte apoptosis in the liver, followed by increased accumulation of reactive oxygen species (O^{2-}, H_2O_2, MDA, NO) and a decrease in stress-related gene expression levels that resulted in increased SOD, catalase activity, glutathione peroxidase, methoxytyramine-hydrochloride, glutathione S-transferase, heat shock protein 70 and p53 and decreased transferrin and cytochrome p450 1A. Intragastric administration of TiO_2 nanoparticles induced liver toxicity (inflammation) in mice in terms of hepatocyte apoptosis, histopathological changes, accumulation of titanium and damage in the function of the mice liver. A significant increase in the mRNA and protein expression of Toll-like receptor-2 and TLR-4 and several inflammatory cytokines and a significant decrease in the mRNA and protein expression of IκB and interleukin-2 were observed [133]. When female CD-1 (ICR) mice were treated with 5, 10 and 50 mg/kg of anatase (5 nm) nanoparticles for 60 consecutive days by intragastric administration, ALT, AST, ALP, lactate dehydrogenase, pseudocholinesterase, leucine acid peptide, IKK1, IKK2, NF-κB, NF-κBP52–65, tumour necrosis factor-α, NF-κB-inducible kinase, Toll-like receptor-2 and Toll-like receptor-4 were increased and IkB and interleukin-2 were decreased. Fatty degeneration, necrosis, apoptosis and inflammation were observed in the liver.

Wang et al. [91] treated male and female CD-1 (ICR) mice with 5 g/kg body weight of 25, 80 and 155 nm TiO_2 nanoparticles with intragastric administration (oral gavage). Blood urea nitrogen and creatinine increased by 25 and 80 nm TiO_2. These also caused proteinic liquids in renal tubules and glomerulus swelling.

Bu et al. [92] treated male and female Wistar rats with 0.16, 0.4 and 1 g/kg of rutile-anatase TiO_2 mixture (<50 nm) once a day for 14 consecutive days by intragastric administration. High nuclear magnetic resonance urine analysis showed an increase in α-ketoglutarate, hippurate, histidine, trimethylamine-N-oxide, taurine, citrulline, acetate, phenylacetylglycine and citrate levels and a decrease in methionine and 3-D-hydroxybutyrate levels. 1H nuclear magnetic resonance serum analysis showed an increase in trimethylamine-N-oxide, choline creatine, 3-D-hydroxybutyrate and phosphocholine and a decrease in glutamate, acetoacetate, glutathione, methionine, glutamine and pyruvate. WBC, lymphocytes, monocytes and eosinophils were increased. However, no alterations in histology were observed in the liver and spleen.

Intragastric administration for 60 consecutive days with TiO_2 nanoparticles resulted in the accumulation of reactive oxygen species in the hippocampus of mouse [134]. Intragastric administration of anatase TiO_2 nanoparticles for 30 days resulted in significant increases in the accumulation of reactive oxygen species in the mouse spleen, consequently leading to increased lipid peroxidation and the significant expression of heme oxygenase-1 via the p38-Nrf-2 signalling pathway [135].

Gui et al. [136] found that intragastric exposure in mice caused modifications in the expression of genes. Most of the genes were related to immune or inflammatory responses, cell structure, apoptosis, oxidative stress, biological and metabolic processes, cell cycle, ion transport, cell differentiation, signal transduction, transcription and translation.

Intragastric treatment of 5 nm anatase TiO_2 nanoparticle caused damage in the function of the liver as a result of the damage of the immune response and haemostasis blood system in mice treated with a higher dose, while this effect was very little in low-dose-treated mice. Liver functions were disrupted in terms of increased activities of ALT, ALP, AST, cholinesterase and lactate dehydrogenase and total protein; the reduction of albumin to globulin ratio, triglycerides, total bilirubin and total cholesterol levels; the reduction in body weight; the increased coefficients of the liver and histopathological changes in the liver. There were a decrease in red blood cells, haemoglobin, interleukin-2 activity, white blood cells, thrombocytes, reticulocytes, B lymphocytes, T lymphocytes and natural killer lymphocytes and an increase in red cell distribution width, platelets, haematocrit, NO level, mean corpuscular volume, mean corpuscular haemoglobin, mean platelet volume. Histology showed blurred hepatocytes and congested vessels [137].

Hu et al. [138] investigated that intragastric exposure to TiO_2 nanoparticles (5 nm anatase; 0, 5, 10 and 50 mg/kg BW; every day for 60 days) in ICR mice can potentially impair the spatial recognition memory, which may be due to the disturbance in trace element homeostasis, neurotransmitter and enzymes in the brain. Their aim was to determine whether exposure to TiO_2 nanoparticles results in significant changes in the function of the nervous system. Sixty days of exposure on a daily basis caused a significant alteration in the ion concentrations and activities in different enzymes in the brain. The Y-maze test showed that exposure to TiO_2 nanoparticles can considerably harm the spatial recognition memory. TiO_2 nanoparticles also impaired homeostasis of trace elements, neurotransmitter systems and enzymes in the mouse brain. There were significant changes in the contents of Mg, Na, Ca, Fe, K and Zn in the brain. TiO_2 nanoparticles also considerably inhibited the activities of

Ca^{+}_{2}-ATPase, Ca^{+}_{2}/Mg^{+}_{2}ATPase, Na^{+}/K^{+}-ATPase, nitric oxide synthase and acetylcholine esterase. Some monoamine neurotransmitters, e.g., norepinephrine, dopamine, 5-HT and its metabolite 5-HIAA, significantly decreased, whereas glutamate, acetylcholine and NO were significantly increased. Another study also revealed that TiO_2 nanoparticles accumulate and induce reactive oxygen species in mouse hippocampus, cause hippocampal apoptosis and impair spatial recognition memory in mice. Caspase-3 and caspase-9 were activated, Bcl-2 was inhibited and the levels of Bax and cytochrome c were promoted significantly [134].

The findings of Sang et al. [139] suggest that long-term (90 days) intragastric exposure to low-dose TiO_2 nanoparticles may result in spleen injury, significant increase in the spleen indices, histopathological changes, splenocyte apoptosis and reduction of immune capacity, due to alteration of inflammatory and apoptotic cytokine expression and decreased immunoglobulin, blood cells, platelets, haemoglobin and lymphocyte. Intragastric exposure to 5–6-nm-sized TiO_2 nanoparticles at the dose of 2.5, 5 and 10 mg/kg every day for 90 days caused chronic spleen injury in ICR mice [139]. This also led to decreased blood cells, platelets, lymphocyte subsets (e.g. CD3, CD4, CD8, B cell, natural killer cell), haemoglobin, immunoglobulin and Bcl-2 and heat shock protein 70 expression and increased levels of necrosis factor-κB, tumour necrosis factor-α, interleukin-2, IL-4, IL-6, IL-8, IL-10, IL-18, IL-1β, cross-reaction protein, transforming growth factor-β, interferon-γ, Bax and CYP1A1 expression. Long-term exposure to low-dose TiO_2 nanoparticles may cause spleen injury, due to change in the expression of inflammatory and apoptotic cytokines and reduced immune capacity.

Li et al. [144] treated female CD-1 (ICR) mice with anatase TiO_2 at the dose of 5, 10, 50, 100 and 150 mg/kg for 14 consecutive days by intra-abdominal injections and observed a dose-dependent increase in Ti liver content and TiO_2 bound on DNA, causing changes in DNA conformation and induced DNA cleavage.

Gui et al. [136] evaluated the nephrotoxicity, distribution, oxidative stress and gene expression profile in kidney using whole-genome microarray analysis technique in mice with intragastric exposure and found a significant decrease in the number of renal glomerulus, infiltration of inflammatory cells, tissue apoptosis and necrosis of renal tubules, reduction in body weight, unbalanced element distribution, increased kidney indices, reactive oxygen species production and lipid peroxidation, and protein and DNA peroxidation in the renal tissue of mouse. Most of the genes were related to inflammatory or immune responses, apoptosis, cell structure, biological and metabolic processes, oxidative stress, ion transport, cell cycle, signal transduction, transcription, translation and cell differentiation [136].

Intragastric administration of TiO_2 nanoparticles reduces fertility and causes injury of mouse ovaries. TiO_2 nanoparticles deposited in the ovary result in a significant reduction in body weight, relative weights of the ovaries and fertility, alterations in haematological and serum parameters and levels of sex hormones, increases in atretic follicles, inflammation and necrosis. In addition, exposure to TiO_2 nanoparticles resulted in marked alterations in the expressions of proteins and enzymes [140].

Abdominal/Intra-abdominal Exposure

Abdominal exposures of mouse to anatase TiO_2 nanoparticles caused oxidative stress and nephritis to the kidney. Coefficients of the kidney increased and titanium accumulated in the kidney. Histopathological changes in the kidney and an increase in the generation of reactive oxygen species and lipid peroxidation were observed. Antioxidants such as glutathione and ascorbic acid decreased. Activities of superoxide dismutase, catalase, ascorbate peroxidase and total antioxidant capacity were also decreased. In addition, the increase of creatinine, calcium and phosphonium and the reduction of uric acid and blood urea nitrogen represented the disrupted kidney functions [141].

Ma et al. [115] treated female CD-1 (ICR) mice with anatase TiO_2 (5 nm) and bulk rutile TiO_2 (10–15 μm) at the dose of 5–150 mg/kg body weight anatase TiO_2 nanoparticles and 150 mg/kg bulk TiO_2 every day for 14 days by intra-abdominal injections. Biochemical serum parameters showed that ALT, ALP, AST, lactate dehydrogenase, pseudocolinesterase, leucine acid peptide, total cholesterol and high-density lipoprotein cholesterol were increased by both particles, while albumin, globulin and triglycerides were increased and low-density lipoprotein cholesterol decreased by anatase TiO_2. Histology revealed basophilia, ischemia and vein congestion (both TiO_2) and apoptosis induced by anatase TiO_2. Inflammatory action was obvious from the increased NF-κB, migration inhibitory factor, tumour necrosis factor-α, interleukin-6, interleukin-1β, cross-reaction protein, interleukin-4 and interleukin-10 by anatase TiO_2. Liu et al. [114] treated female CD-1 (ICR) mice with anatase nanoparticles (5 nm) and bulk rutile (10–15 μm) at the dose of 5–150 mg/kg anatase TiO_2 and 150 mg/kg bulk TiO_2 every day for 14 days using intra-abdominal injections. Uric acid and blood urea nitrogen were decreased by both TiO_2. Zhao et al. [140] treated mice with anatase TiO_2 nanoparticles by intra-abdominal injections. Decreased creatinine and Ca^{2+}; increased phosphonium, blood urea nitrogen and uric acid; increased ROS and lipid peroxidation; and decreased SOD, catalase, ascorbate peroxidase, total antioxidant capacity, glutathione and ascorbic acid content were observed.

Similarly in another study, Ma et al. [116] found that 5 nm anatase TiO_2 nanoparticles for 14 days injected to the abdominal cavity translocate to and accumulate in the brain and

Table 1 TiO_2 exposure through the respiratory system

Reference	Model animal/sex/age	NP size/type	Dose	Exposure (organs/ tissues)	Observations/results/inference
Gustafsson et al. [67]	Male Dark Agouti rats	P25 Degussa TiO_2	1, 5 and 7.5 mg/kg body weight	Intratracheal instillation	Inflammatory action: transient increase in EOSs and NEUs in BAL, followed by a recruitment of DCs and NKs. Elevated levels of IL-1, IL-2, IL-6, CINC-1 and GM-CSF
Hussain et al. [75]	Male mice	Anatase TiO_2 (15 mm)	40 μL of a NP suspension (0.4 mg/mL (~0.8 mg/kg)	Oropharyngeal aspiration	Airway reactivity: increased by TiO_2 in TDI-sensitized mice Inflammatory action: TiO_2 increased NEUs and AMs in BAL of TDI-sensitized mice
Nemmar et al. [61]	Male Wistar rats	Rutile Fe-doped nanorod TiO_2 (length 80; diameter 7)	1.5 mg/kg	Intratracheal instillation	Inflammatory action: NEUs and IL-6 increased; SOD activity decreased in BAL Histology: inflammatory cell infiltration
Halappanavar et al. [37]	Female time-mated C57BL/6BomTac mice		1 h daily to 42.4±2.9 (SEM) mg surface-coated nano-TiO_2/m^3 for 11 consecutive days		Inflammatory action: increased NEUs and LYMs; decreased AMs in BAL
Hougaard et al. [36]	Female time-mated C57BL/6BomTac mice	21 nm, average crystallite size aerosolized powder 97 nm (peak size) rutile elongated modified with Al, Si and Zr and coated with polyalcohols	42.4±2.9 TiO_2 mg/m^3 for 1 h a day on gestation days 8–18	Inhalation	Inflammatory action: increased NEUs and LYMs; decreased AMs in BAL
Morimoto et al. [44]	Male Wistar rats	Rutile TiO_2 51±9 nm	2.8E+05/cm^3 for 4 weeks (6 h/day)	Inhalation	Inflammatory action: no effect
Leppänen et al. [45]	Male mice	Primary particle size 20 nm; geometric mean diameters of 91, 113 and 130 nm anatase + brookite (3:1)	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)	Inhalation	Airflow limitation effect: reduction in expiratory flow in all the exposure situations Inflammatory action: no effect
Cho et al. [71]	Female Wistar rats	TiO_2 (30–40)	50 and 150 cm^2/rat	Intratracheal instillation	Inflammatory action in BAL and histology of the lung: no effect
Tang et al. [66]	Male Sprague-Dawley rats	Anatase TiO_2 (5±1)	0.8, 4 and 20 mg/kg	Intratracheal instillation	Histology: AM increase, lung gaps expanded, hyperemia, alveolar thickness
Tang et al. [59]	Male Sprague-Dawley rats	TiO_2 (5)	0.8, 4 and 20 mg/kg	Intratracheal instillation	TiO_2 NP aggregation: present in lung at the lowest doses Histology: lung gaps expanded, hyperemia
Liu et al. [68]	3 male and 3 female Sprague-Dawley rats per group	5 and 200 nm	0.5, 5 or 50 mg/kg	Intratracheal instillation	AM phagocytic and chemotactic ability: reduced by TiO_2 NPs

Table 1 (continued)

Reference	Model animal/sex/age	NP size/type	Dose	Exposure (organs/ tissues)	Observations/results/inference
Li et al. [54]	Male Kunming mice	Anatase TiO_2 (3)	3.3 mg/kg TiO_2 once a week for 4 weeks	Intratracheal instillation	Inflammatory action: ACP and ALP increased in BAL Histology: destroyed alveolar walls
Scuri et al. [35]	Weanling (2-week-old), newborn (2-day-old), adult (12-week-old) male and female Fischer 344 rats	P25 Degussa TiO_2 (21)	12 mg/m^3; 5.6 h/day for 3 days	Inhalation	Neurotrophin expression: NGF, BDNF and their receptors increased in 2-day-old and 2-week-old rats Airway resistance: increased in 2-week-old mice
Rossi et al. [43]	Female BALB/c/Sca mice	Silica-coated rutile TiO_2 (~10× 40) Rutile TiO_2 (<5 μm)	10±2 mg/m^3 TiO_2 for 2 h a day, 3 days a week, for 4 weeks	Inhalation	Inflammatory action: EOSs, LYMs, AMs, PAS+ goblet cells, IL-1β, TNF-α, IL-4, IL-13 and IL-10 decreased by silica TiO_2 Airway reactivity: decreased by silica TiO_2; increased by fine TiO_2
Moon et al. [110]	BALB/c mice	P25 TiO_2 (21)	40 mg/kg	Intraperitoneal injections	Inflammatory action: NEUs, TPC; TNF-α, IL-1β, MIP2 increased by TiO_2 or TiO_2+LPS
Chen et al. [106]	Male and female ICR mice adult	80 and 110 nm, mostly being 100 nm; anatase	0, 324, 648, 972, 1296, 1944 or 2592 mg/kg	Intraperitoneal injected	Histology: alveolar septal thickening and interstitial pneumonia
Park et al. [55]	ICR mice	P25 TiO_2 (21)	5, 20 and 50 mg/kg) by a single intratracheal instillation	Intratracheal instillation	Inflammatory action: IL-1, TNF-α, IL-6, IL-12, IFN-γ, IL4, IL-5, IL-10 and IgE increased in BAL Histology: inflammatory proteins, granulomas Gene expression: up-regulation of genes involved in antigen presentation and immune cell chemotaxis
Sager and Castranova [58]	Male Fischer CDF (F344/DuCrl) rats	P25 TiO_2 (21)	0.26–1.04 mg/rat	Intratracheal instillation	Inflammatory action: increase in PMNs, ALB and LDH in BAL
Liu et al. [57]	Male and female Sprague-Dawley rats	5, 21 and 50 nm TiO_2 primary particles	0.5, 5 or 50 mg/kg	Intratracheal instillation	Inflammatory action: LDH and ALP increased in the lung by 5 and 50 nm TiO_2 Histology: inflammatory infiltration, alveolar wall thickening AM phagocytic ability: altered by 5 and 50 nm TiO_2
Ma-Hock et al. [39]	Male Wistar rats	Rutile-anatase TiO_2 mixture (25.1)	Aerosols of 2, 10 and 50 mg/m^3 for 6 h/day for 5 days	Inhalation	Inflammatory action: PMNs, GGT, TPC, LDH, ALP and NAG increased in BAL Cell replication: increased in bronchi and bronchioles
van Ravenzwaay et al. [27]	Male Wistar rats	Anatase-rutile TiO_2 mixture (20–30) Rutile TiO_2 (200)	Inhalation: aerosols of 100 and 250 mg/m^3 uncoated	Inhalation Intravenous injection	

Table 1 (continued)

Reference	Model animal/sex/age	NP size/type	Dose	Exposure (organs/ tissues)	Observations/results/inference
			and pigmentary TiO_2, respectively, for 6 h/day on 5 consecutive days. Intravenous injections: 5 mg/kg		TiO_2 distribution: lung and mediastinal lymph node after inhalation; mostly liver and spleen after injection Inflammatory action: both TiO_2 increased TCC, PMNs, TPC, ALP, LDH, GGT and NAG in BAL
Kobayashi et al. [56]	Male Crl:CD (SD) rats	Anatase TiO_2 (4.9) (1st and 2nd experiments) Anatase TiO_2 (23.4) Anatase TiO_2 (154.2)	5 mg/kg	Intratracheal instillation	Inflammatory action: 1st: 4.9 and 23.4 nm TiO_2 NPs increased TCC, NEUs, LDH; 2nd: agglomerated TiO_2 increased TCC, NEUs, LDH Histology: epithelium hypertrophy in all treated groups
Larsen et al. [117]	Inbred female BALB/cJ mice	TiO_2 (28)	Immunization with 1 μg OVA alone or in combination with either 2, 10, 50 or 250 μg TiO_2	Intraperitoneal injections	OVA-specific antibodies in serum: TiO_2 increased IgE and IgG1 levels compared to the OVA controls Inflammatory action: EOSs, NEUs, LYMs, IL-4 and IL-5 increased in BAL
Mühlfeld et al. [26]	Male WKY/NCrl BR rats		10 rats were exposed to 0.11 mg/m^3 TiO_2 aerosols for 1 h	Inhalation	TiO_2 distribution: connective tissue and the capillary lumen were the preferential target of NPs at 1 and 24 h, respectively

Table 2 TiO_2 exposure through the nervous system

Reference	Model animal/sex/age	NP size/type	Dose	Exposure (organs/tissues)	Observations/results/inference
Jeon et al. [143]	Mouse	TiO_2		(Brain)	Proteomic analysis: altered protein expression Oxidative stress: antioxidant and AchE activities reduced
Yamashita et al. [127]	Pregnant mice	35 nm	0.8 mg TiO_2 for 2 consecutive gestational days	Intravenous injection	Ti distribution: TiO_2 detected in foetal brain
Li et al. [54]	Male Kunming mice	Anatase TiO_2 (3)	3.3 mg/kg TiO_2 once a week for 4 weeks	Intratracheal instillation	Ti brain content: increased oxidative stress: O_2^-, OH^-, H_2O_2, MDA increased in the brain Histology: exudates, inflammatory infiltration and necrosis
Hu et al. [138]	Female mice	Anatase TiO_2 (5)	0, 5, 10 and 50 mg/kg body weight every day for 60 days	Intragastric administration	Neurotransmitters: ACh, Glu, and NO increased; NE, DA, DOPAC, 5-HT and 5-HIAA decreased Enzyme activity: decreased Na^+/K^+, Ca^{2+}, Ca^{2+}/Mg^{2+} ATPase; promoted AchE and iNOS
Shin et al. [111]	Male C57BL/6 mice	<1 μm rutile, 21 nm (P25)	40 mg/kg TiO_2 30 min after vehicle or 5 mg/kg LPS	Intraperitoneal injections	Inflammatory action: after LPS, TiO_2 NPs increased IL-1β, TNF-α and iNOS and induced microglial activation Oxidative stress: after LPS TiO_2 NPs enhanced ROS
Ma et al. [116]	Female ICR mice	5–15 nm anatase	5–150 mg/kg nano-TiO_2 and 150 mg/kg bulk TiO_2 every day for 14 days, respectively	Abdominal cavity injection	Ti brain content: higher increase with nano-TiO_2 Oxidative stress: O_2^-, H_2O_2, MDA, NOS, iNOS and NO increased; antioxidative enzymes, GLU and AchE decreased Histology: filamentous-shaped neurons and inflammatory cells
Takahashi et al. [121]	Pregnant Slc:ICR mice	Anatase TiO_2 (25–70)	100 μL of TiO_2 at 1 mg/mL at gestational days 6, 9, 12, 15 and 18	Subcutaneous injections	Monoamine levels: DA, DOPAc, HVA and 3-MT increased in the prefrontal cortex and neostriatum
Takeda et al. [118]	Pregnant Slc:ICR mice	Anatase TiO_2 (25–70)	100 μL of TiO_2 at 1 mg/mL at 3, 7, 10 and 14 days post-coitum	Subcutaneous injections	TiO_2 offspring brain distribution: cortex and olfactory bulb Apoptosis: presence of markers and features in olfactory cells
Shimizu et al. [120]	Pregnant ICR mice, male foetuses and pups	Anatase TiO_2 (25–70)	100 μL of TiO_2 at 1 μg/μL on gestational days 6, 9, 12 and 15	Subcutaneous injections	Gene expression: up-regulated cell death, apoptosis, brain development, oxidative stress, apoptosis, neurotransmitter genes
Wang et al. [86]	Female CD mice	25, 80 and 155 nm TiO_2	50 mg/kg TiO_2 BW every other day for 20 days	Intranasal instillation	Ti brain content: increased. Neurotransmitters: NE and 5-HT increased; DA, DOPAC, HVA, and 5-HIAA decreased
Wang et al. [81]	Female CD-1 (ICR) mice	Rutile TiO_2 (80) Anatase TiO_2 (155)	500 μg TiO_2 every other day for 15 times	Intranasal instillation	Ti brain distribution: mainly in the olfactory bulb and hippocampus Oxidative stress: CAT, MDA, Pr. carb. increased; SOD decreased Neurotransmitters: AchE, Glu and NO increased
Wang et al. [82]	Female CD-1 (ICR) mice	Rutile TiO_2 (80) Anatase TiO_2 (155)	500 μg TiO_2 every other day for 15 times	Intranasal instillation	Ti brain distribution: mainly in the hippocampus Oxidative stress: GSH-Px, GST, SOD, GSH and MDA increased Histology: irregular neuronal arrangement, condensated chromatin Inflammatory action: increased TNF-α and IL-1β levels

Table 3 TiO_2 exposure through the dermal and mucosal system

Reference	Model animal/sex/age	NP size/type	Dose	Exposure (organs/tissues)	Observations/results/ inference
Furukawa et al. [16]	CD-1 (ICR) female mouse	Coated TiO_2 (long axis 50–100; short axis 10–20)	5, 10 and 20 mg/animal in the post-initiation phase in a skin carcinogenesis model	Cutaneous application	Carcinogenicity: no increased development of skin nodules
Moon et al. [109]	Mice	TiO_2 (<25 or <100 nm)	Once a day for 28 consecutive days before subcutaneous implantation with B16F10 melanoma cells	Intraperitoneal injections	Tumour growth: increased
Adachi et al. [149]	Male hairless Wistar Yagi rats	W/O emulsion containing anatase TiO_2 (26.4±9.5)	4 mg/cm^2 TiO_2 on dorsal skin	Cutaneous application	TiO_2 absorption: TiO_2 detected in the horny layer of the interfollicular epidermis
Sadrieh et al. [11]	Female Yucatan minipigs	P25 Degussa TiO_2 (30–50) Rutile TiO_2 coated with aluminium hydroxide/ dimethicone copolymer (diameter 20–30, length 50–150) Submicron TiO_2 (300–500)	2 mg cream/cm^2 skin (4 applications/day, 5 days/ week, 4 weeks)	Cutaneous application	TiO_2 absorption: TiO_2 detected in the upper stratum corneum and follicular lumen, with few particles observed in dermal layers as contamination results
Wu et al. [19]	Male reared pigs, hairless mice (BALB/c/nu/nu)	Anatase TiO_2 (5±1, 10±1) Rutile TiO_2 (25±5, 60±10) P25 Degussa TiO_2 (~21) TiO_2 (0.3–0.5 μm)	24 mg TiO_2 formulations on the pig ear for 30 days and on the mouse interscapular skin for 60 consecutive days	Cutaneous application	TiO_2 absorption in pigs: TiO_2 detected in the stratum corneum, granulosum, prickle and basal cell layer, not in the dermis TiO_2 absorption in mice: increased MDA, reduced HYP content and excessive keratinisation in skin
Yanagisawa et al. [23]	Male C/NgaTndCrj (NC/Nga) mice	15, 50 or 100 nm rutile	20 μg TiO_2	Intradermal injections	Atopic dermatitis: allergen+TiO_2 enhanced ear thickening Inflammatory action: allergen+TiO_2 increased EOSs; IL-4, MCs and decreased IFN-γ; TiO_2 increased IL-13

Table 4 TiO_2 exposure through the cardiovascular system

Reference	Model animal/sex/age	NP size/type	Dose	Exposure (organs/tissues)	Observations/results/inference
Nemmaret al. [61]	Male Wistar rats	Rutile Fe-doped (9 %) pure (TiO_2) nanorods (length 80; diameter 7)	1 and 5 mg/kg	Intratracheal instillation	Cardiovascular parameter: HR and SBP increased
Courtois et al. [73]	Male Wistar or Sprague-Dawley rat	P25 Degussa TiO_2 (15)	100 μg TiO_2 in 0.5 mL saline	Intratracheal instillation	Intralobar arteries vasomotor responses to PGF2α, KCl, Ach: not altered
LeBlanc et al. [33]	Male Sprague-Dawley rats	P25 anatase-rutile TiO_2 (21)	6 mg/m^3 TiO_2 for 240 min	Inhalation	Coronary arteriolar endothelium dilation: impaired by TiO_2 Oxidative stress: ROS increased in coronary microvascular walls
LeBlanc et al. [31]	Male Sprague-Dawley rats	P25 anatase-rutile TiO_2 (21)	6 mg/m^3 TiO_2 for 240 min	Inhalation	Coronary arteriolar endothelium: TiO_2 increased spontaneous arteriolar tone and impaired flow and vasodilator induced dilation
Nurkiewicz et al. [30]	Male Sprague-Dawley rats	P25 anatase-rutile TiO_2 (21) Rutile TiO_2 (1 μm)	1.5–16 mg/m^3 TiO_2 for 240–720 min	Inhalation	Spinotrapezious arteriolar endothelium dilation: impaired by both TiO_2
Bihari et al. [104]	Male C57BL/6Ncrl mice	Rutile TiO_2 (~10× 40)	1 mg/kg TiO_2 10 min before thrombosis induction	Intravenous administration	Mesenteric and cremasteric thrombosis: not determined
Bu et al. [92]	Male and female Wistar rats	Rutile-anatase TiO_2 mixture (<50)	0.16, 0.4 and 1 g/kg per day for 14 days	Intragastric administration	Biochemical parameters: increased CK and LDH
Liu et al. [114]	Female CD-1 (ICR) mice	Anatase TiO_2 (5) Bulk rutile TiO_2 (10–15 μm)	5–150 mg/kg BW anatase TiO_2 and 150 mg/kg bulk TiO_2 everyday for 14 days	Intra-abdominal injections	Biochemical parameters: CK, LDH, AST and alpha-HBDH were increased by both TiO_2
Chen et al. [106]	male and female ICR mice	Anatase TiO_2 80 and 110 nm, mostly being 100 nm;	0, 324, 648, 972, 1296, 1944 or 2592 mg kg–1	Intraperitoneal injection:	Vascular system: pulmonary thrombosis
Wang et al. [91]	Male and female CD-1 (ICR) mice	TiO_2 25, 80 and 155 nm	5 g/kg	Intragastric administration	Biochemical parameters: 80 and 25 nm TiO_2 increased LDH and alpha-HBDH compared to controls and the fine group

Table 5 TiO_2 exposure through the liver

Reference	Model animal/sex/age	NP size/type	Dose	Exposure (organs/tissues)	Observations/results/inference
Yamashita et al. [127]	Pregnant mice	TiO_2 (35)	0.8 mg TiO_2 for 2 consecutive gestational days	Intravenous injection	Ti distribution: TiO_2 detected in foetal liver
Nemmar et al. [61]	Male Wistar rats	Rutile Fe-doped (9 %) pure (TiO_2) nanorods (length 80; diameter 7)	1 and 5 mg/kg	Intratracheal instillation	Biochemical parameters: AST and ALT increased Histology: inflammatory cell infiltration, mainly LYMs
Tang et al. [66]	Male Sprague-Dawley rats	Anatase TiO_2 (5±1)	0.8, 4 and 20 mg/kg	Intratracheal instillation	Biochemical parameters: ALB and GLU increased 1H NMR serum analysis: ketone bodies, choline, LDL, alanine and GLU increased; lactate, creatine and pyruvate decreased TEM analysis: swollen hepatocytes; congested sinusoids
Tang et al. [59]	Male Sprague-Dawley rats	TiO_2 (5)	0.8, 4 and 20 mg/kg	Intratracheal instillation	Biochemical parameters: ALT increased 1H NMR urine analysis: increase in valine, lactate, acetate, succinate, 2-OG, creatinine, taurine, TMAO, allantoin and hippurate 1-2; decrease in citrate and DMA
Li et al. [144]	Female CD-1 (ICR) mice	Anatase TiO_2	5, 10, 50, 100 and 150 mg/kg for 14 consecutive days	Intra-abdominal injections	Ti liver content: dose-dependent increase Interaction with DNA: TiO_2 was bound on DNA, caused changes in DNA conformation, induced DNA cleavage
Bu et al. [92]	Male and female Wistar rats	Rutile-anatase TiO_2 mixture (<50)	0.16, 0.4 and 1 g/kg once a day for 14 consecutive days	Intragastric administration	1H NMR urine analysis: increase in α-ketoglutarate, hippurate, histidine, TMAO, taurine, citrulline, acetate, PAG and citrate levels; decrease in methionine and 3-D-HB levels 1H NMR serum analysis: increase in TMAO, choline creatine, 3-D-HB and phosphocholine; decrease in glutamate, acetoacetate, glutathione, methionine, glutamine and pyruvate Histology: no alterations
Cui et al. [132]	Female CD-1 (ICR) mice	Anatase TiO_2 (6.9)	5, 10 and 50 mg/kg TiO_2 for 60 consecutive days	Intragastric administration	Histology: hepatocyte apoptosis Oxidative stress: O_2^-, H_2O_2, MDA, NO increased Gene expression: SOD, CAT, GSH-Px, MT, GST, HSP70, p53 and TF decreased; CYP1A increased
Cui et al. [133]	Female CD-1 (ICR) mice	Anatase TiO_2 (5)	5, 10 and 50 mg/kg TiO_2 for 60 consecutive days	Intragastric administration	Biochemical parameters: ALT, AST, ALP, LDH, PChE and LAP increased Inflammatory action: IkB and IL-2 decreased; IKK1, IKK2, NF-κB, NF-κBP52–65, TNF-α, NIK, TLR-2 and TLR-4 increased Histology: fatty degeneration, necrosis, apoptosis, inflammation

Table 5 (continued)

Reference	Model animal/sex/age	NP size/type	Dose	Exposure (organs/tissues)	Observations/results/inference
Duan et al. [137]	Female CD-1 (ICR) mice	Anatase TiO_2 (5)	62.5–250 mg/kg TiO_2 for 30 consecutive days	Intragastric administration	Biochemical parameters: ALT, ALP, AST, LDH, ChE, TP, TG and TCHO increased; ALB/GLB and TBIL decreased Histology: blurred hepatocytes, congested vessels
Wang et al. [154]	Male Sprague-Dawley rats	Anatase TiO_2 (diameter 45.87±7.75; thickness 10–15)	0.2–20 mg/kg TiO_2 in the knee joints every other day for 4 times	Intra-articular injection	Biochemical parameters: ALP decreased; AST/ALT and LDH increased Histology: fatty degeneration, inflammatory cell infiltration
Wu et al. [19]	Hairless mice	Anatase TiO_2 (10±1) Rutile TiO_2 (25±5; 60±10) P25 Degussa TiO_2 (~21) TiO_2 (0.3–0.5 μm)	24 mg of 5 % TiO_2 test formulation on the dorsal interscapular skin	Cutaneous application	Liver histology: TiO_2 penetrated the skin inducing necrosis Oxidative stress: increased MDA activity in the liver
Ma et al. [39]	Female CD-1 (ICR) mice	Anatase TiO_2 (5) Bulk rutile TiO_2 (10–15 μm)	5–150 mg/kg BW anatase TiO_2 and 150 mg/kg bulk TiO_2 everyday for 14 days	Intra-abdominal injections	Biochemical serum parameters: ALT, ALP, AST, LDH, PChE, LAP, TCHO and HDL-C increased by both particles; ALB, GLB and TG increased and LDL-C decreased by anatase TiO_2 Histology: basophilia, ischemia and vein congestion (both TiO_2). Apoptosis induced by anatase TiO_2 Inflammatory action: NF-κB, MIF, TNF-α, IL-6, IL-1β, CRP, IL-4 and IL-10 increased by anatase TiO_2
Liu et al. [114]	Female CD-1 (ICR) mice	Anatase TiO_2 (5) Bulk rutile TiO_2 (10–15 μm)	5–150 mg/kg BW anatase TiO_2 and 150 mg/kg bulk TiO_2 every day for 14 days	Intra-abdominal injections	Biochemical parameters: ALT, ALP, ALB, glucose, TG, TCHO and HDL-C levels were increased by both particles; LAP, PChE and TP were increased and TBIL was reduced by anatase TiO_2 Oxidative stress: induced by anatase TiO_2 in the liver
Chen et al. [106]	Male and female ICR mice	Anatase TiO_2 (80–110)	324–2592 mg/kg	Intraperitoneal injections	Biochemical parameters: ALT and AST increased Histology: fibrosis, hydropic degeneration, necrotic and apoptotic cells and NEUs were detected
Guo et al. [107]	Male ICR mice		200 and 500 mg/kg TiO_2 every other day for 5 times	Intraperitoneal injections	Biochemical parameters: ALT and AST/ALT increased
Liang et al. [65]	Male and female Sprague-Dawley rats	TiO_2 (5, 21)	0.5–50 mg/kg TiO_2	Intratracheal instillation	Biochemical parameters: no changes in TP, ALB, ALT, AST Oxidative stress: decreased SOD and increased MDA activity
Wang et al. [86]	Male and female CD-1 (ICR) mice	TiO_2 (25, 80, 155)	5 g/kg	Intragastric administration	Biochemical parameters: ALT and ALT/AST increased Histology: hydropic degeneration and necrosis

Table 6 TiO_2 exposure through the haematopoietic and immunological systems

Reference	Model animal/sex/age	NP size/type	Dose	Exposure (organs/tissues)	Observations/results/inference
Wang et al. [135]	Female CD-1 (ICR) mice	Anatase TiO_2	5–150 mg/kg TiO_2 for 30 consecutive days	Intragastric administration	Spleen histology: congestion, lymph nodule proliferation Oxidative stress: O_2^-, H_2O_2 and MDA levels and p38, JNK, NF-κB, Nrf-2 and HO-1 expression increased in the spleen
Moon et al. [109]	Mice	<25 or <100 nm TiO_2	Once a day for 7 days	Intraperitoneal injections	Spleen cells: splenocytes, CD4+, LPS-stimulated NK cells, CD8+ decreased; B-lymphocyte development and LPS-stimulated spleen cell proliferation were retarded by TiO_2
Nemmar et al. [61]	Male Wistar rats	Rutile Fe-doped (9 %) pure (TiO_2) nanorods (length 80; diameter 7)	1 and 5 mg/kg	Intratracheal instillation	Blood parameters: WBC, IL-6, SOD, GSH and PLTs increased
Rossi et al. [43]	Female BALB/c/Sca mice	Silica-coated rutile TiO_2 (~10× 40) Rutile TiO_2 (<5 μm)	10±2 mg/m^3 TiO_2 for 2 h a day, 3 days a week, for 4 weeks	Inhalation	Inflammatory action: TiO_2 NPs decreased TNF-α and IL-13 expression in spleen cells
Bu et al. [92]	Male and female Wistar rats	Rutile-anatase TiO_2 mixture (<50)	0.16, 0.4 and 1 g kg-1 once a day for 14 days	Intragastric administration	Blood parameters: WBC, LYMs, MONs, EOS increased Spleen histology: no alterations
Duan et al. [137]	Female CD-1 (ICR) mice	5 nm anatase	62.5–250 mg/kg	Intragastric administration	Blood parameters: WBC, RBC, Hb, mean corpuscular Hb concentration, thrombocytes, reticulocytes decreased; mean corpuscular volume, mean corpuscular Hb, red cell distribution width, PLTs, HT and mean PLT volume increased Immunological parameters: CD3, CD4, CD8, CD4/CD8, B and NK cells decreased Inflammatory action: IL-2 decreased and NO increased by TiO_2
Liang et al. [65]	Male and female Sprague-Dawley rats	TiO_2 (5, 21)	0.5, 5 or 50 mg/kg body weight	Intratracheal instillation	Oxidative stress: decreased SOD activity in plasma
Li et al. [108]	Female CD-1 (ICR) mice	Anatase TiO_2 (~6–7)	5–150 mg/kg TiO_2 every day for 45 days	Intraperitoneal injections	Oxidative stress in spleen: increased ROS and MDA Spleen histology: congestion, lymph nodule proliferation, splenocyte apoptosis Apoptosis mechanism: TiO_2 activated caspase-3 and caspase-9, decreased Bcl-2, increased Bax and cytochrome c
Chen et al. [106]	Male and female ICR mice	80 and 110 nm, mostly being 100 nm	0, 324, 648, 972, 1296, 1944 or 2592 mg/kg	Intraperitoneal injections	Spleen histology: severe lesions; NEU infiltration
Wang et al. [86]	Male and female CD-1 (ICR) mice	25, 80 and 155 nm	5 g/kg body	Intragastric administration	Spleen histology: no alterations

Table 7 TiO_2 exposure through the renal system

Reference	Model animal/sex/age	NP size/type	Dose	Exposure (organs/tissues)	Observations/results/inference
Zhao et al. [140]	Mice	Anatase TiO_2		Intra-abdominal injections	Biochemical parameters: Cr, Ca^{2+} and phosphonium increased; BUN and UA decreased Oxidative stress: ROS and LPO increased; superoxide dismutase, catalase, ascorbate peroxidase, total antioxidant capacity, glutathione and ascorbic acid content decreased
Tang et al. [66]	Male Sprague-Dawley rats	Anatase TiO_2 (5±1)	0.8, 4 and 20 mg/kg	Intratracheal instillation	Biochemical parameters: BUN increased 1H NMR serum analysis: ketone, bodies, choline, LDL, alanine and GLU increased; lactate, creatine and pyruvate decreased TEM analysis: tubule epithelial cell damage, vascular deformity
Tang et al. [59]	Male Sprague-Dawley rats	TiO_2 (5)	0.8. 4 and 20 mg/kg	Intratracheal instillation	Biochemical parameters: BUN and Cr increased 1H NMR urine analysis: increase in valine, lactate, acetate, succinate, 2-OG, creatinine, taurine, TMAO, allantoin and hippurate 1-2; decrease in citrate and DMA
Wang et al. [154]	Male Sprague-Dawley rats	Anatase TiO_2 (diameter 45.87±7.75; thickness 10–15)	0.2–20 mg/kg TiO_2 in the knee joints every other day for 4 times	Intra-articular injection	Biochemical parameters: BUN and Cr decreased Histology: proteinic liquids in renal tubules
Guo et al. [106]	Male mice 6 weeks		200 and 500 mg/kg every other day for 5 times for a week	Intraperitoneal injections	Biochemical parameters: BUN increased
Liu et al. [114]	Female CD-1 (ICR) mice	Anatase TiO_2 (5) Bulk rutile TiO_2 (10–15 μm)	5–150 mg/kg BW anatase TiO_2 and 150 mg/kg bulk TiO_2 everyday for 14 days	Intra-abdominal injections	Biochemical parameters: UA and BUN decreased by both TiO_2
Chen et al. [106]	Male and female ICR mice	80 and 110 nm, mostly being 100 nm	0, 324, 648, 972, 1296, 1944 or 2592 mg/kg	Intraperitoneal injections	Biochemical parameters: no alterations in BUN Histology: glomerulus swelling, proteinic liquids in renal tubules
Liang et al. [65]	Male and female Sprague-Dawley rats	TiO_2 (5, 21)	0.5, 5 or 50 mg/kg body weight	Intratracheal instillation	Biochemical parameters: no alterations in BUN and Cr Oxidative stress: decreased SOD and GSH-PX; increased MDA renal activity (5 nm TiO_2)
Wang et al. [86]	Male and female CD-1 (ICR) mice	25, 80 and 155 nm	5 g/kg TiO_2	Intragastric administration (oral gavage)	Biochemical parameters: BUN and Cr increased by 25, 80 nm TiO_2 Histology: proteinic liquids in renal tubules, glomerulus swelling

Table 8 TiO_2 exposure through the muscular and skeletal system

Reference	Model animal/sex/age	NP size/type	Dose	Exposure (organs/tissues)	Observations/results/inference
Wang et al. [154]	Male Sprague-Dawley rats	Anatase TiO_2 (diameter 45.87±7.75; thickness 10–15)	0.2, 2 and 20 mg/kg TiO_2 in the knee joints every other day for 4 times	Intra-articular injection	Oxidative stress in synovium: GSH-Px, GSH, GSSG, MDA and SOD increased Histology: synovium hypertrophy, LYMs, plasma cell infiltration and fibroblast proliferation
Hansen et al. [122]	Male Sprague-Dawley rats	TiO^2 (70) Bulk TiO_2 (diameter 0.9 mm; height 1 mm)	In the back of rats for 6 or 12 months	Subcutaneous (bulk) and intramuscular (NPs) implantation	Local reaction: presence of granulomas (NPs), inflammatory infiltrates (bulk); no local intolerance (both)
Gatti et al. [123]	Male Sprague-Dawley rats	TiO_2 (70) Bulk TiO_2 (diameter 6–12 mm; height 2 mm)	In the back of rats for 6 or 12 months	Subcutaneous (bulk) and intramuscular (NPs) implantation	Local reaction: granulomas (NPs), inflammatory infiltrates (bulk)
Onuma et al. [124]	Female C57BL/6 mice	Hydrophilic rutile TiO_2 treated with $ZrO_2Al(OH)_3$ (minor axis 40–70; major axis 200–300) Hydrophobic rutile TiO_2 treated with $ZrO_2Al(OH)_3$ and steric acid (minor axis 40–70; major axis 200–300)	Subcutaneous injections: QR-32 cells alone; QR-32 cells mixed with 5 mg/0.1 mL TiO_2; and 5 mg/0.1 mL TiO_2 30 and 70 days before QR-32 cell injection Intravenous injections: tumour cell lines into mice (5/line)	Subcutaneous injections, intravenous injections	Carcinogenicity: QR-32 cells became tumorigenic after injection in sites implanted for 30 or 70 days with hydrophilic TiO_2 Metastatic ability: acquired by tumour cell lines derived from QR-32 cells injected in the site pre-implanted with both TiO_2

cause brain injury in mice. Neurons may turn into filamentous shapes or inflammatory cells. The accumulation of nanoparticles was dose dependent. The oxidative stress and injury of the brain takes place as anatase TiO_2 nanoparticles can trigger a series of reactions like lipid peroxidation, decreases in total antioxidation capacity and antioxidative enzyme activities, reduction of glutamic acid, excessive release of nitric oxide and the down-regulated level of acetylcholinesterase activities.

Mohamed [142] injected 500, 1000 and 2000 mg/kg body weight suspension of nanoparticles for five consecutive days into the abdominal cavity and examined genotoxicity, mutagenicity and apoptosis in p53 exons (5–8) and myocardial cells. Comet assay showed highly significant tail length, %DNA in tail and tail moment in a dose-dependent manner. Smeared DNA and apoptotic fragmentized laddered also appeared on agarose gel. The heart of TiO_2 nanoparticle-treated groups had abnormal pathology increasing gradually with increasing titanium dose as shown by the appearance of diffuse muscle hyalinosis and congested blood vessel in the low TiO_2 group (500 mg/kg); diffuse muscle hyalinosis, diffuse muscular edema, haemorrhage and focal leucocytic infiltration in the medium TiO_2 group (1000 mg/kg); and Zenker's necrosis of muscles with mononuclear cell infiltration in the high TiO_2 group (2000 mg/kg).

Jeon et al. [143] demonstrated the protein profile alterations in mouse brain. TiO_2 nanoparticles changed the expression levels of 11 proteins by more than 2-fold (up-regulation of eight proteins and down-regulation of three portions) and reduced the activities of a number of antioxidative enzymes and acetylcholine esterase in the brain. However, no TiO_2 nanoparticles were identified in the brain.

Li et al. [144] injected 5, 10, 50, 100 and 150 mg/kg body weight anatase TiO_2 nanoparticles into the abdominal cavity of mice everyday for 14 days. The weights of the livers in the mice which were treated with higher concentrations of TiO_2 nanoparticles significantly increased. TiO_2 nanoparticles accumulated in the DNA of the liver and were placed into DNA base pairs or bound to DNA nucleotides with three atoms of oxygen or nitrogen and two atoms of phosphorous of the DNA. The bond length of the Ti–O(N) and Ti–P were 1.87 and 2.38 Å, respectively. The nanoparticles changed the conformation of the DNA. The gel electrophoresis revealed that a higher dose of TiO_2 nanoparticles caused liver DNA cleavage [144].

In Vitro Studies

Since in vitro studies may be helpful in the prediction of acute toxicity, a few are mentioned here. The detailed discussion on in vitro studies and genotoxicity and carcinogenicity of the TiO_2 nanoparticles will be studied in our separate review paper. The effect of TiO_2 nanoparticles on erythrocytes was

Table 9 TiO_2 exposure through the reproductive system

Reference	Model animal/sex/age	NP size/type	Dose	Exposure (organs/tissues)	Observations/results/inference
Takeda et al. [118]	Pregnant Slc:ICR mice	Anatase TiO_2 (25–70)	100 μL of TiO_2 at 1 mg/mL at 3, 7, 10 and 14 days post-coitum	Subcutaneous injections	Reproductive effects: disorganised and disrupted seminiferous tubules, few mature sperm, decreased sperm production, epididymal sperm motility, number of Sertoli cells
Guo et al. [107]	Male mice 6 weeks		200 and 500 mg/kg every other day for 5 times for a week	Intraperitoneal injections	Reproductive effects: reduced sperm density and motility, increased sperm abnormality and germ cell apoptosis

examined by Li et al. [145]. The TiO_2 nanoparticles caused abnormal sedimentation, haemagglutination and dose-dependent haemolysis of erythrocytes unlike TiO_2 fine particles. Another study on washed human erythrocytes after 37 °C incubation for 1 h reported haemolysis caused by TiO_2 fine particles 73 times greater than nanoparticles [146]. However, the haemolysis was inhibited by plasma, which indicated the prevention of haemolysis in vivo due to the presence of plasma. In another study on mouse macrophages (Ana-1 and MH-S cells) administered with anatase 5-, 10-, 25- and 100-nm-sized TiO_2 nanoparticles, low toxicity was induced to MH-S cells [147]. Another study found that 25- and 80-nm-sized TiO_2 nanoparticles at the concentrations of 10, 20, 40 and 80 mg/L for 24-h exposure inhibited intracellular communication via gap junction between lung fibroblasts [148]. Several in vitro studies also showed the toxicity of TiO_2 nanoparticles on circulatory system cells.

Adachi et al. [149] also found no penetration of TiO_2 nanoparticles into viable cell layers or any cellular changes biologically. They used skin exposed to water/oil emulsion containing 10 % by weight ultrafine TiO_2 particles. The skin showed no morphological and immunohistochemical alterations by light microscopy. Electron microscopy showed the localization of most TiO_2 nanoparticles in the keratinized layer of follicular infundibulum and the interfollicular stratum disjunctum. Energy-dispersive X-ray spectrometry confirmed no TiO_2 nanoparticles in the viable skin. Furthermore, light microscopy or low-vacuum scanning electron microscopy with EDX showed a specific affinity of TiO_2 nanoparticles to the follicular opening area.

In another in vitro study, similar results were found by Miquel-Jeanjean et al. [150] evaluating cutaneous penetration and localization of TiO_2 nanoparticles included in a sunscreen containing the nanoparticles with ≥20 nm primary size applied for 24 h. Ti was deposited 102.35±4.20 % in intact skin and 102.84±5.67 % in damaged/irradiated skin in the surface and stratum corneum. Only 0.19±0.15 % nanoparticles were found in the viable epidermis and 0.39±0.39 % in the dermis. No Ti was found in the receptor fluid. From this study, it may be concluded that TiO_2 nanoparticles incorporated in sunscreens stay in the topmost layers of the stratum corneum in intact skin and even in damaged or irradiated skin with simulated solar radiation.

Braydich-Stolle et al. [151] using a model of HEL-30 mouse keratinocyte cell line in vitro demonstrated that both crystal structure and size of the TiO_2 nanoparticles contributed to cytotoxicity. The crystal structure influences the mechanism of cell death. The results showed that pure anatase TiO_2 nanoparticles initiated cell necrosis regardless of size, and the rutile TiO_2 nanoparticles induced apoptosis by means of the production of ROS.

Nemmar et al. [61] have shown in vitro that intratracheal instillation dose-dependently reduced cellular viability of human lung cancer cells and human hepatoma cells. The rutile Fe-TiO_2 caused direct toxicity on human lung cancer cells and human hepatoma cells. Nemmar et al. [61] also showed in vitro in Wistar rats that intratracheal instillation significantly and dose-dependently induced platelet aggregation. It affects the liver and increases thrombotic potential, systolic blood pressure and heart rate. Male Wistar rats exposed to rutile Fe-doped (9 %) pure TiO_2 nanorods (length 80 nm; diameter 7 nm) at 1 and 5 mg/kg by intratracheal instillation displayed changes in cardiovascular parameters like increased heart rate and systolic blood pressure.

Savi et al. [152] showed both in vivo and in vitro that acute exposure to TiO_2 nanoparticles alters acutely cardiac excitability and increases the chances of arrhythmic events. In vivo, on a single intratracheal dose of 2 mg/kg of TiO_2 nanoparticles in saline solution, cardiac conduction velocity and tissue excitability increased, resulting in an increased tendency for inducible arrhythmias. Computational modelling of ventricular action potential pointed out that a membrane leakage can explain the effects induced by the nanoparticles measured on real cardiomyocytes.

In a study by Wang et al. [153], about 30 % of the TiO_2 nanoparticles entered into neural stem cells after 48 h of incubation with 50 and 30 nm and nanotubes 100 nm×4–6 nm regardless of the type and dose of the

nanoparticles. When the TiO_2 nanoparticles were removed from the culture medium, about 35 % 50 nm TiO_2 nanoparticles, 34.6 % 30 nm TiO_2 nanoparticles and 41.7 % of nanotubes were released by exocytosis from the cells within the first 24 h. The release was decreased over time and became negligible at 72 h. During cell division, exocytosis did not occur. Furthermore, the study also suggested that both endocytosis and exocytosis of TiO_2 nanoparticles were energy-dependent processes and that the serum proteins influenced the uptake of the nanoparticles by cells. In a similar study, Wang et al. [154] demonstrated that titania nanotubes (TiO_2-NTs) passed through the karyotheca entering the mouse neural stem cell nucleus after co-incubation for 48 h.

Conclusion

In summary, the acute toxicity of TiO_2 nanoparticles has been studied frequently in rat and mouse models through various exposure routes of administration (Tables 1, 2, 3, 4, 5, 6, 7, 8 and 9). The number of studies performed on the respiratory system prevails over the other exposure routes. Studies about the effects of TiO_2 nanoparticles on the pulmonary system showed both local and systemic effects and intensified the pre-existing symptoms. TiO_2 nanoparticles administered through the lung cause more inflammatory responses as compared to the fine particles of similar chemistry at equal dose concentrations. However, TiO_2 nanoparticles and TiO_2 fine particles induced similar pulmonary inflammation on the basis of equal particle surface area. The results from the other routes of exposure cannot be ignored. For example, research studies make evident the absorption of the TiO_2 nanoparticles from the lungs or gastrointestinal tract into systemic circulation and distribution to different organs like the kidneys, liver, spleen or even the brain and induce organ injuries and inflammations. However, most of the doses used in the studies are too high to be implied on occupational situations. A number of in vitro studies also demonstrate the effects of TiO_2 nanoparticles on the blood circulatory system.

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