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Review

The potential health risk of titania nanoparticles

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ABSTRACT

Widespread use of titania nanoparticles (TNPs) has caused a significant release of TNPs into the environment, increasing human exposure to TNPs. The potential toxicity of TNPs has become an urgent concern. Various models have been used to evaluate the toxic effects of TNPs, but the relationship between TNPs' toxicity and physicochemical properties is largely unknown. This review summarizes relevant reports to support the development of better predictive toxicological models and the safe future application of TNPs.

Contents

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1. Introduction

Titania (titanium dioxide) has three natural forms: rutile, anatase, and brookite [1,2]. Titania nanoparticle (TNP) is a widely used nanomaterial; cosmetics and sunscreen products alone account for 50% of TNP usage [3]. Because they are an n-type semiconductor [4], TNPs are also used as a photocell coating and as a photo-catalyst to reduce waterborne or airborne pollutants such as organic dyes, nitric oxide, organophosphorus insecticides, formaldehyde, and benzene [3,5,6]. TNPs have been added to self-cleaning sanitary ceramics, antimicrobial plastic packaging, and cement. Because light-mediated TiO_2 surface hydroxylation makes it fouling-resistant, it is used in window glass, pavement, and walls [7–9]. TNPs have also been used as additives in sugar, film, toothpaste, and capsules [10].

Although limited epidemiological studies in U.S. and European TNP production factories showed no significant respiratory carcinogenic risks [11–14]. TNP toxicity has been observed in diverse models, such as rodents [15–18], aquatic organisms [19–21], and human cells [22–24]. Female workers were reported to experience shortness of breath and pleural effusions after 5–13 months of exposure in a polyacrylic ester nanoparticle processing factory [25], raising significant doubt about the human safety of TNPs.

Because of widespread environmental exposure to TNPs, it is urgent to elucidate their effects on human health. Currently available reports cover a diverse range of nanoparticle properties, exposure schemes, and toxicological models, impeding clear interpretation. Here we summarize the likely scenarios of TNP release and human exposure and compare the potential *in vivo* and *in vitro* toxic effects of TNP in association with its properties. Rather than being exhaustive, this review seeks to promote scientific awareness of the potential risk of TNPs. Our hope is to stimulate the development of guidelines for the proper use and disposal of TNPs, thereby reducing its potential harm to human health and the environment [7].

2. TNP production and pollution

In 2006, 40,000 tons of TNPs were produced in the U.S.[26]. Due to ever-increasing market demand, the annual production of TNPs is predicted to reach 2.5 million tons by 2025 (Fig. 1) [26]. As a result, a significant amount of TNPs will be released into the environment [7,27] (Fig. 2).

TNPs distributed in the environment may affect the biosphere via food chain, air, and water [28]. Workers at TNP production factories and academic researchers encounter the highest exposure risks, possibly through skin penetration and inhalation. Material dispersal cannot be avoided during application, leading to free nanomaterial liberation. Data from a wastewater treatment plant showed raw sewage to contain 100–3000 μg Ti/L and effluent to contain 5–15 μg/L Ti [29]. Although many countries regulate solid waste disposal, none specifically addresses nanoparticle disposal [30]. Therefore, the nanoparticle pollution risk is eminent and cannot be ignored.

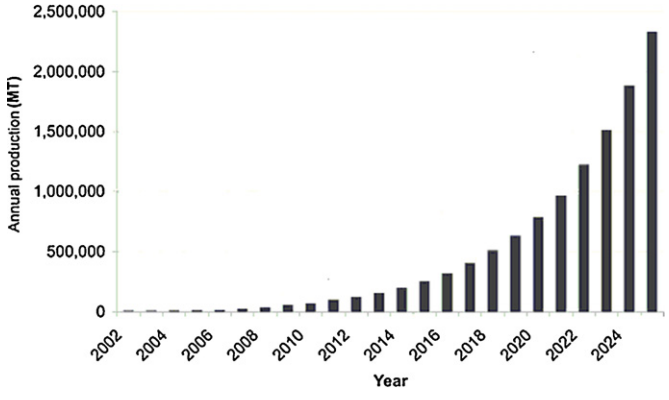

Fig. 1. Forecast of TNP production in the U.S. (MT = metric tons). Reproduced with permission from [26].

3. Potential toxicity of TNPs

Although TNP is classified as a suspected carcinogen [12], a retrospective cohort mortality study revealed no significant association between mortality risk and TNP exposure levels [13]. However, workers who manufacture polyacrylic ester nanoparticles developed symptoms such as shortness of breath, pleural effusions, and rash with intense itching on their faces, hands, and forearms. Some even experienced hypoxemia and pericardial effusions [25]. Because of the limited data on TNP toxicity to humans, the potential risk is still in doubt. Therefore, researchers should use various toxicological models, such as animals, aquatic organisms, and human cells, to generate the needed information.

3.1. Toxicity of TNPs to mammals

TNP's mammalian toxicity was investigated using rodent models under various exposure schemes and conditions. The endpoint assays included inflammation, oxidant stress, cell proliferation, and histopathological changes [30].

3.1.1. Biodistribution and systemic toxicity

After a single intravenous (iv) injection of TNP in male Wistar rats with TNP suspension (5 mg/kg body weight), various organs, biochemical indices, and antigen levels were analyzed after 1, 14, and 28 days. At day 28, TNPs had accumulated in liver, spleen, lung, and kidney, in descending order. No obvious abnormality was observed in immune response and organ function. Hence, brief, low-dose TNP exposure was relatively safe [15]. Toxicokinetics of TNPs at a higher dose was studied in Balb/c female mice treated (iv) with 560 mg/kg TNP. TNP microparticle aggregates were found in lung, liver, lymph node, spleen, and kidney [31]. In an investigation of the acute toxicity and biodistribution of fine and ultrafine TiO_2 at a larger dose (5 g/kg BW by oral gavage), TNPs were transported to other organs and tissues via the gastrointestinal tract and induced hepatic injury and myocardial damage within 2 weeks [32]. ICR mice were treated with anatase TNPs by intraperitoneal (ip) injection daily for 45 days. TNPs accumulated in the spleen and other organs, and reduced immunity and pathological changes were observed [18]. These studies show a general pattern of translocation and biodistribution of TNPs that is illustrated in Fig. 3 [33]. All of the different exposure routes lead to blood entrance and translocation into various organs. Systemic toxicity is therefore a possibility.

3.1.2. Respiratory system

Exogenous fine particles enter the body mainly via the respiratory system, after which phagocytosis by alveolar macrophages induces reactive oxygen species (ROS) [34]. Alveolar macrophages usually contain enzymatic and non-enzymatic antioxidants that scavenge ROS; however, these are insufficient to prevent oxidative stress and pulmonary damages [35,36]. TNPs have been found to cause pulmonary damage and inflammation [37,38]. Acute exposure to TNP rods and dots for 24 h caused pulmonary and cardiac edema, lung and systemic inflammation, and platelet aggregation [39,40]. Levels of inflammation and oxidative stress were exacerbated when TiO_2 was coated with Fe. The coated TNPs increased the risk of hepatic injury, thrombus, tachycardia, and systolic hypertension and induced splenic congestion, lymph nodule proliferation,

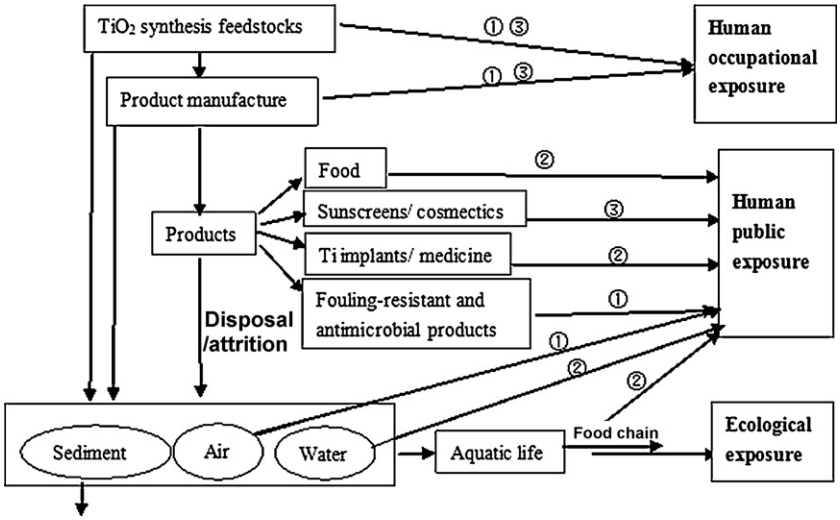

Fig. 2. Potential release, exposure, and uptake of TNPs in the ecosystem (1) inhalation; (2) ingestion; (3) deposition. Reproduced with permission from [27].

and splenocyte apoptosis [41,42]. In another study, treatment of female mice with TNPs (5 g/kg) by oral gavage caused hepatic injury, nephrotoxicity, and pathological changes in the kidneys [32].

3.1.3. Skin

TNPs are widely used in sunscreens, cosmetics, and even clothes to provide protection from harmful UV irradiation. These applications cause general skin exposure, posing the potential risk of percutaneous absorption and ROS-mediated skin aging [43–48]. Skin is the body's first line of defense from the outside world. Skin hair follicles are surrounded by a tight network of capillaries (Fig. 4), which facilitates transdermal drug delivery [49] but also provides a possible route for NP entry and a long-term NP reservoir. Although only 1% of applied TNPs were found in the orifices of hair follicles, these deposits were much harder to remove than those on the skin surface [45–48]. The stratum corneum (the upper layer of epidermis) shields against percutaneous penetration by most extraneous substances [50], but TNPs were shown to penetrate the stratum corneum and the stratum granulosum in pigs [44]. So far, there is no evidence that NP skin penetration leads to systemic exposure. Most studies have found that TNPs remain at the outmost layer of stratum corneum, possibly in skin furrows or the infundibulum; little TNP has been found in the living epidermis [49,51–56]. Moreover, acute dermal irritation studies and local lymph node assays in mice found no irritation or sensitization [57]. Recently, TNPs were found to induce ROS and skin aging after prolonged exposure, especially under illumination [43]. Treatment of human keratinocyte HaCaT cells with low-dose TNPs (<60 μg/mL) for 24 h altered cell-matrix adhesion but did not affect cell viability [58]. In contrast, ROS induced dose- and time-dependent apoptosis at a higher concentration of TNPs [59] and induced DNA damage and micronuclei in epidermal cells [60]. Photo-irradiation increased the level of ROS and decreased cell viability [61]. ROS-mediated protein

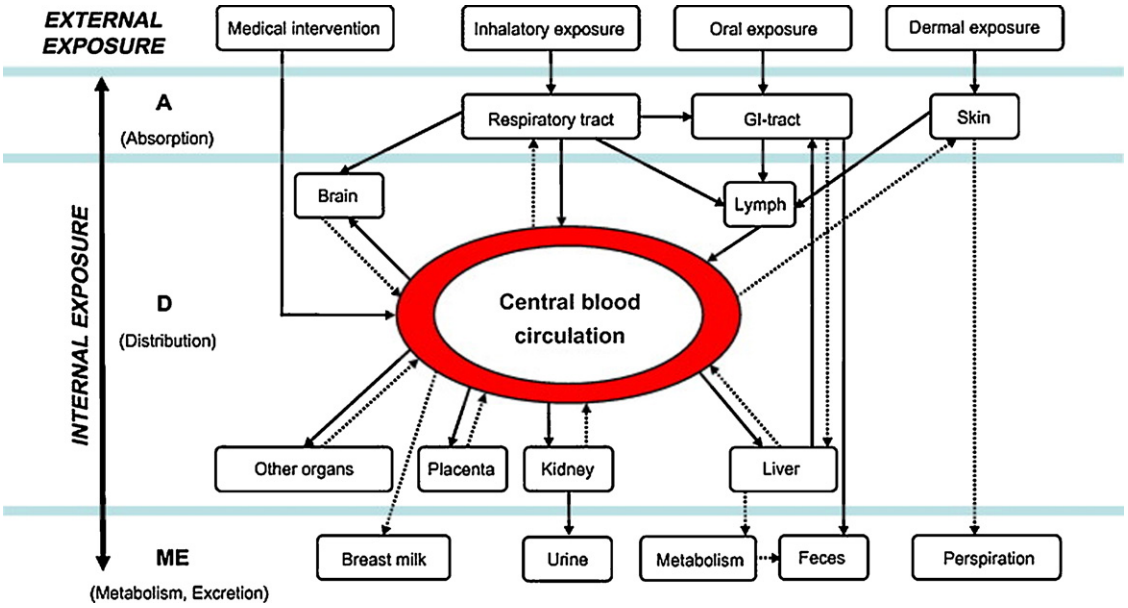

Fig. 3. Kinetic properties of NPs in the body. The internal exposure is the portion of the external dose that enters the systemic circulation. Black lines represent confirmed routes of nanoparticles; dashed lines represent hypothetical routes. (Other organs = spleen, heart, reproductive organs, etc.)
Reprinted with permission from [33].

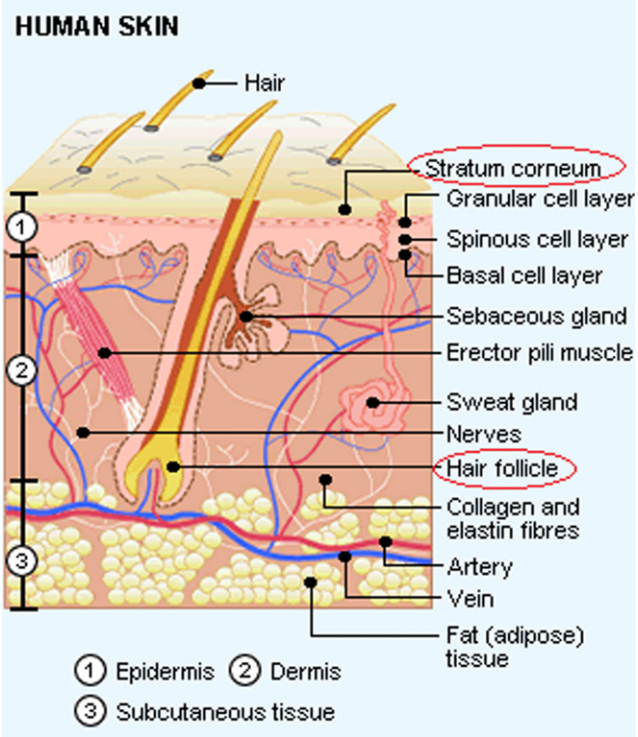

Fig. 4. Skin structure diagram.
Adapted from [125].

tyrosine nitration was also observed in mouse skin homogenate, although the relation of this finding to chronic cutaneous diseases needs further study [62]. Considering the aging risks posed by photo-induced oxidative stress and the lack of data on long-term effects on mammalian skin, further investigation of TNP-induced skin alteration is warranted.

3.1.4. Brain and CNS

Because of its high metabolic rate, low capacity for cellular regeneration, and numerous cellular ROS targets, brain is highly vulnerable to oxidative stress. The olfactory nerve is speculated to be the most likely pathway for the transport of intranasally instilled NPs to the brain [30,63,64]. High accumulation, oxidative stress, and obvious morphological alteration of hippocampal neurons and olfactory bulb were detected after nasal exposure to TNPs [65]. In contrast, TNPs induced only a slight brain lesion after oral gavage [32]. The hippocampus was recognized as the main target for TNPs introduced through the olfactory bulb route. TNPs exhibited a time-dependent translocation capacity in CNS after intranasal instillation [66]. Further studies indicated that TNPs could reduce the spatial recognition memory ability of mice. This finding was attributed to the disturbance of homeostasis of trace elements, enzymes, and neurotransmitter systems [67]. TNPs were also able to translocate from the abdominal cavity to the brain and then to cause brain injury and a cascade of reactions, such as lipid peroxidation, excessive release of nitric oxide, reduced antioxidative enzyme activity, reduction of glutamic acid, and down-regulation of acetylcholinesterase activity [68]. Exposure to TNPs enhanced the expression of tumor necrosis factor alpha (TNF-α), interleukin (IL-1β), factor-κB-inducible kinase, nucleic factor-κB (NF-κB), and IκB kinase in a dose-dependent manner, while expression of IκB was down-regulated [67–69]. Taken together, the evidence supports concern about the potential neurotoxicity of TNPs.

3.1.5. Developmental toxicity

During early fetal development, blood barriers are incomplete and hazardous substances can easily gain entry and cause injury [70]. In fact, TNPs were shown to be transferred from pregnant mice to their pups [70–73]. When pregnant mice were treated with TNPs via subcutaneous injection, the genital and cranial nerve systems of the male offspring were affected [70]. When time-mated female mice were treated with TNPs, their offspring showed moderate neurobehavioral alterations, although cognitive function was not affected [71]. This finding can be explained by changes in the expression of genes associated with CNS development and function [72]. Maternal intranasal instillation with respirable TNPs increased allergic susceptibility [73] and caused pathologic and functional disorders and reduced daily sperm production in offspring [70].

3.2. TNP toxicity to organisms in the ecosystem

Environmental release of TNPs may pose a significant risk to life within the ecosystem, such as algae, zooplankton, bacteria, and fish [74,75]. TNP aggregation status and abiotic factors, such as ionic strength and pH, affect TMP ecotoxicity [75]. The TNP EC_{50} for the green alga *Desmodesmus subspicatus* was 44 mg/L, showing TNP to be more toxic than bulk TiO_2 [74]. Concentrations of TNPs above 100 mg/L, which was considered nontoxic, were toxic to fish and bacteria [21]. The LC_{50} of filtered titania solution to the zooplankton *Daphnia magna* was 5.5 mg/L for 48 h of exposure [19], and the mortality was over 70% with chronic exposure [76]. In another study, the viability of *Daphnia magna* was reported to be significantly affected, while the crustacean *Thamnocephalus platyurus* was not affected under the same conditions [77]. Although TNPs showed only slight effects on *D. magna* mortality, its reproduction was severely affected by chronic exposure [78]. The ecotoxicity of TNP is ROS-mediated damage to organisms induced by TNP accumulation, disruption of membranes, and other oxidative stress responses [76,79]. TNP micelles or protein-coated TNPs were easily internalized by *Salmonella typhimurium* [80]. Moreover, TNP penetrated multilayered membranes of *Anabaena variabilis* and caused oxidative structural membrane damage [81,82]. Consistent with the protective enzymes inhibited, TNP induced oxidative stress, DNA damage and cytotoxicity in *Escherichia coli* [82] and illumination-enhanced ROS-mediated acute toxicity in daphnids [83].

In summary, TNP is classified as a harmful material that presents a low risk to the ecosystem [84]. However, despite low or moderate toxicity in short-term evaluations, long-term TNP exposure showed greater risks [76]. Rainbow trout treated with 1 mg/L of TNPs for 14 days showed pathologic alterations in the gills (edema and thickening of gill lamellae) as well as oxidative stress, dose/time-dependent inhibition of growth, and changes in Cu and Zn levels in tissues [85]. Further, TNPs have a stronger adsorptive capacity for metal ions (*e.g.*, Cu, Cd) than do sediment particles [86,87]. In carp, for example, the presence of TNPs significantly enhanced accumulation of toxic metal ions in the viscera and gills [87]. Furthermore, TNPs passed through the blood–heart/brain barriers [88].

3.3. Toxicity of TNPs to mammalian cells

Both cytotoxicity and animal models are used to evaluate the potential toxicity of nanomaterials. When human bronchial epithelial cells (BEAS-2B) were incubated with TNPs, the aggregated TNPs penetrated into the cytoplasm and were observed surrounding the nucleus. They induced an increase in ROS, expression of the oxidative stress-related genes, a decrease in reduced glutathione (GSH), and eventually, cell death [89]. TNPs were also reported to induce mitochondrial dysfunction [90]. Cellular uptake and cytotoxicity of TNPs of various shapes were studied in Hela cells. The zero-dimensional (0D) nanodots and 1D nanorods were

internalized after a 24-h incubation, while little of the 3D aggregates was taken up [61]. Cell viability was 80% after treatment with 0D TNPs at and 100% after treatment with 1D and 3D TNPs, all at 125 μg/mL. However, viability after treatment with 1D nanorods decreased by 40% under irradiation, indicating generation of ROS and increased apoptosis [61]. Anatase TNPs ($LC_{50} \sim 3.6$ μg/mL) were more toxic than rutile ($LC_{50} \sim 550$ μg/mL) TNPs to human dermal fibroblasts [23]. At a non-cytotoxic concentration (2.5–120 ppm) of P25, brain microglia (BV2) showed a rapid and sustained oxidative stress response and impaired mitochondrial energy production [24]. Human lymphoblastoid cells were affected by TNPs in a time- and dose-dependent manner. However, most of the cells recovered after removal of the nanoparticles [91].

TNPs were reported to induce cell apoptosis and necrosis [92]. Rutile TNPs induced apoptosis through ROS generation [59], while anatase TNPs caused necrosis by inducing membrane leakage [93]. TNPs induced mitotic perturbation, DNA damage, formation of micronuclei (MN), and eventually, apoptosis in Syrian hamster embryo cells [94]. In mitochondrially mediated splenocyte apoptosis, TNPs activated caspases 3 and 9, down-regulated expression of Bcl-2 member genes and proteins, and up-regulated expression of Bax and cytochrome c genes and proteins [18]. TNP altered cellular signaling pathways and induced cellular stress and inflammatory responses. In a study of TNP-induced neuroinflammatory responses, TNPs elevated TNF-α gene expression and activated IκB kinase (NIK), IKK1, and IKK2, eventually causing neuroinflammation and impairment of cognitive function and spatial memory [66,67,69]. TNPs also triggered both chronic inflammation and a change in autoimmunity. After intratracheal instillation of TNPs, mice showed inflammation and increased pro-inflammatory cytokines and proteins in the lung [95].

TNPs can potentially induce genotoxicity and carcinogenicity. Anatase TNPs cause dose-related oxidative DNA damage and micronucleus (MN) formation in A431 and BEAS 2B cells [60,96], as well as sister chromatid exchange at a higher dosage [97]. IMR-90 cells were more sensitive to TNP, showing genotoxicity (ROS-induced DNA adduct formation) [98]. In HepG2 cells, DNA breakage and oxidized purines were found, accompanied by activation of *p*53 and its downstream DNA damage response genes [99]. Mice given drinking water containing TNPs for five days experienced the formation of 8-hydroxy-2′-deoxyguanosine, γ-H2AX foci, micronuclei, DNA deletions, and DNA double-strand breaks [100]. TNPs inserted into DNA base pairs or directly bound to DNA nucleotides and altered DNA conformation [101]. Anatase TNPs at a dose of 150 mg/kg caused liver DNA cleavage in mice [101]. Furthermore, TNP exposure in utero increased the number of DNA deletions in the fetus [100]. Cellular ROS and apoptosis were not observed when human keratinocyte HaCaT cells were treated with TNPs. However, genes related to inflammatory response and cell adhesion were up-regulated [58].

Although TNPs cause inflammation, cell death, and genotoxicity via different signaling routes (as proposed in Fig. 5), they exert two basic mechanisms of toxicity: (1) toxicity induced by ROS-mediated inflammatory reaction and (2) direct toxicity caused by interaction with biomolecules, such as DNA and pepsin. Current studies focus on the former mechanism, while the latter is more fundamental and less well elucidated.

4. Challenges in evaluating TNP's nanotoxicity

The inconsistent reports about TNP toxicity reflect the overall complexity of evaluating nanotoxicity. First, the toxicity of NPs depends on a large number of NP-related properties, such as

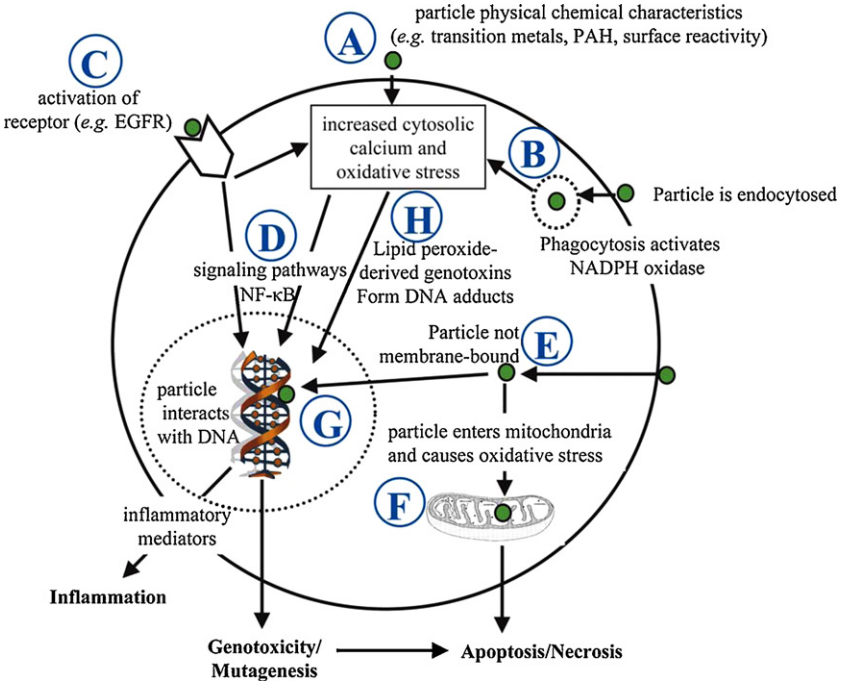

Fig. 5. Hypothetical cellular interactions of TNPs, with emphasis on potential oxidative stress-induced effects and their consequences. (A) Particle-associated characteristics induce lipid peroxidation, intracellular oxidative stress, and increased cytosolic calcium ion concentration. (B) NPs may undergo active endocytosis via different mechanisms (caveolae, clathrin-coated pits, or receptor-mediated mechanisms). In phagocytic cells, phagocytosis triggers activation of NADPH oxidase and generation of ROS. (C) Particles and their associated metals, as well as oxidative stress, can activate the EGF receptor. (D) Oxidative stress, receptor activation, and increased calcium ions activate transcription of pro-inflammatory genes via transcription factors such as NF-κB. NPs may enter the cell by passive diffusion, remain non-membrane bound, and then (E) enter mitochondria and (F) disrupt normal electron transport, leading to oxidative stress. (G) Free particles may also enter the nucleus via the nuclear pore complex and interact with the genetic material. (H) Lipid peroxide-derived products such as 4-hydroxy nonenal form DNA adducts that may cause genotoxicity and mutagenesis (EGFR, epidermal growth factor receptor).

Reproduced with permission from [126].

the material, crystal form, size, shape, surface area and properties, aggregation status, and impurities [102,103], which are not comprehensively characterized in most published reports. Second, toxicological animal models are crucial to such evaluations, but there may be inter-species differences in toxic effects, and none of the models can predict human toxicity with certainty. Cell lines are even more variable, resulting in inconsistent reports from different labs using the same cell line. Third, experimental conditions are highly variable among labs, and results cannot be meaningfully compared when parameters like dose, exposure time, endpoint assay, and evaluation model differ.

4.1. Evaluation model and experimental conditions

Contradictory results are often obtained from different experimental species. For example, when the subchronic pulmonary responses of different rodent species to inhaled TNPs were investigated, rats showed more severe and persistent inflammation than did mice and hamsters, although the lung burden was equal. Tumors developed in rats but not in mice [16,17,104].

Nanoparticles usually exert classic dose-dependent effects that are enhanced with exposure time [19,23,39]. Dose-dependent alveolar proteinosis, collagenized fibrosis, thickened alveolar walls impairing oxygen diffusion, cholesterol granuloma formation, and focal pleurisy have been observed in rats exposed to TNPs [40,105].

In toxicological evaluations, the effects of a single dose cannot truly recapitulate the effects of environmental exposure to nanomaterials. When ICR mice were intraperitoneally injected with anatase TNPs (5, 10, 50, 100, and 150 mg/kg body weight) for 14 consecutive days, the LD_{50} was 150 mg/kg and inflammatory responses were generated. TNPs accumulated in various organs in the order of liver > kidney > spleen > lung > brain > heart [18]. When mice were treated with a lower dose of TNPs, little pathological change was observed. However, when mice were treated with a higher dose, liver, kidney, and myocardium were seriously damaged. The balance of blood glucose and of lipids was also perturbed [106].

The route of administration also makes a difference. When TNPs were injected subcutaneously, NPs were found in liver, lymph nodes, and spleen. In contrast, when TNPs were injected intravenously, they were found in lung and kidney [31].

4.2. Effects of TNP physicochemical properties on its toxicity

4.2.1. Primary size

Size is widely considered a primary factor in nanotoxicity [39,107–110]. Exposure to NPs was reported to induce greater inflammation than exposure to larger particles with identical chemical composition and mass concentration [30,111–115]. ROS-mediated membrane breakdown and metabolic pathway alterations induced by TNPs were size-dependent [94]. In a pulmonary toxicity evaluation of instilled TNPs of different primary sizes, the smaller particles were found to induce more severe inflammation in the short term [110]. In lungs, the likelihood that NPs would escape alveolar macrophage phagocytosis was highly and inversely related to size. Consequently, smaller NPs may be substantially more likely to translocate from the alveolar epithelium to pulmonary interstitial sites, leading to inflammation [30,109,113].

Conflicting data showing that particle size is not related to inflammatory potential has also been reported [22,23,39,57,102]. It is likely that the factors involved in nanotoxicity are complex and interactive. These include pharmacokinetic properties and aggregation status, which are also important and cannot be ignored.

4.2.2. Crystal form

In a cytotoxicity study, anatase TiO_2 was found to be 100 times as toxic as an equivalent sample of rutile TiO_2, and the cytotoxic effects of mixed anatase/rutile TiO_2 were intermediate. The more cytotoxic crystal form tends to be more active in generating ROS [23]. In a study of the dependence of membrane LDH leakage and ROS generation on TNP crystal structure, LDH leakage was associated with cell necrosis and was induced by anatase TNPs without ROS generation. In contrast, the rutile TNPs stimulated a relatively high level of ROS, which eventually induced apoptosis. Hence, it was concluded that anatase TNPs mediated necrotic cell death, while rutile TNPs induced apoptosis [93]. Three forms of TNPs were intratracheally instilled in rats, with quartz used as control. The levels of lung inflammation, cytotoxicity, cell proliferation, and histopathological changes were presented as quartz > 80/20 anatase/rutile (uf-3) > fine rutile = ultrafine rutile (uf-1/uf-2). The different responses to uf-3 versus uf-1 and uf-2 TNPs can be attributed to their crystal forms [57]. Similar results have also been achieved by investigating three different crystal forms of TNPs with similar size distributions. The results showed that particle surface reactivity plays a more important role in toxicity than particle size or surface area [102]. In contrast, another study found that anatase TiO_2 did not induce greater ROS generation and that viability did not differ significantly in cells treated with anatase, rutile, and mixed crystal TNPs [93].

4.2.3. Aggregate size

NPs are often described by their primary sizes. Such descriptions become insufficient when discussing the biological effects of NPs, which also depend on the aggregate size of the NPs in various biological fluids. The aggregate size of NPs is influenced by the ionic strength of the solution. TNPs with a diameter of 100 nm can form aggregates of microsize in high-ionic strength solutions (*e.g.*, PBS). High ionic strength reduces the thickness of the electric double layer surrounding the charged NPs and shields the electrostatic repulsion between NPs, resulting in aggregation. In the absence of special modifications, TNPs would be expected to aggregate in body fluids, according to dynamic light scattering results [31]. For example, the average diameter of Degussa P25 measured by dynamic light scattering was 542 nm in de-ionized water and 3500 nm in DMEM, while its primary size was approximatly 26 nm [110]. There is no discernable relationship between agglomeration size, primary size, and crystalline structure. After cellular uptake, most TNPs are localized in endosomes [116,117] and lysosomes, while some are localized in cytoplasm [60,118] or in the peri-nuclear region [89,98] and may even penetrate into the nucleus after prolonged incubation [60,116]. The extent of TNP uptake can be diverse, depending on cell line species, TNP physicochemical properties, incubation time, and the assays used [119]. TNPs with the same aggregate size and crystal structure but with different primary sizes differed in their cytotoxicity [93]. This size-dependent cytotoxicity has also been observed in other NPs [112–114]. Therefore, both the particle aggregate size and the primary size should be considered in evaluating size-dependent toxicity. However, the size-dependent effects were not confirmed *in vivo* in groups of rats dosed with different agglomerations of TNPs of equal primary size [110].

4.2.4. Surface coating

Surface coating may play a significant role in determining cytotoxicity. Chitin–chitosan/nano TiO_2 composite scaffolds have good biocompatibility with bone tissue [120]. Nonetheless, surface coating on NPs can dramatically alter their risk potential. Treatment of human macrophages and fibroblasts with SiO_2-coated rutile TNPs caused increases in pulmonary neutrophilia, tumor necrosis factor-alpha (TNF-a), and neutrophil-attracting chemokines CXCL1 and CXCL8. However, these changes were minimal when cells were

Table 1
Factor analysis studies of toxicity based on the inherent physicochemical properities of TNPs.

Study type	Material/characteristics	Animal/cell	Treatment			Factors in TNPs induced toxicity							Ref.
			Route	Duration	Dose/ concentration	Primary size	Aggregation	Surface area	Crystal structure	Surface chemical reactivity	Coating	pH	
In vivo	TNPs (5 nm, 21 nm, 50 nm)	Sprague-Dawley rats	it	Single dose	0.5, 5, 50 mg/kg BW	+							[106]
In vivo	R-TNPs (uf-1, uf-2); R fine-TiO_2 (F-1); A/R ~ 80/20 P25 (uf-3)	IGS BR rats	it	Single dose	1 or 5 mg/kg BW				+	+		+	[57]
In vivo	Fine TiO_2 (382 nm); R-TNPs (149 nm); A/R TNPs (129 nm); fine quartz (534 nm); nano quartz (12 nm)	IGS BR rats	it	Single dose	1 or 5 mg/kg BW	–		–		+			[102]
In vivo	Fine TiO_2 (R, 300 nm); TNP rods (A, 200 nm × 35 nm); TNP dots (A, 10 nm)	IGS BR rats	it	Single dose	1 or 5 mg/kg BW	–		–					[39]
In vivo	TiO_2 particles (5, 23, and 154 nm)	CD (SD) rats	it	Single dose	5 mg/k BW	+							[110]
In vivo	TNP (3 nm, 20 nm)	Kunming mice	it	Single dose	0.4, 4, 40 mg/k BW	–	–	–				+	[124]
In vitro	A, A/R, R, Degussa TNPs (3–5 nm)	Human dermal fibroblasts and lung epithelial cells		24 h	0.1 μg/Ml–100 mg/mL	–		–	+				[23]
In vitro	100% A (6.3, 10, 50, 100 nm); 40% A and 60% R (39 nm); 61% A and R (39 nm); amporphous (40 nm); 100% R (51 nm); Degussa P25 75% A and 25% R (26 nm); Ruthenium TiO_2 100% A (40 nm)	HEL-30 mouse keratinocyte cell line		24 h	0, 10, 25, 50, 100, and 150 μg/mL	+	+		+				[93]
In vivo	R-TiO_2 (80 nm) and A-TiO_2 (155 nm)	CD-1(ICR) female mice	it	Every other day for 30 days	500 μg/mouse				+				[66]
In vivo	Commercial TiO_2 materials: R (initial size < 5 μm); nano (~30 nm) R/A; nano (<25 nm) A; silica-coated nano (~10 nm × 40 nm); needle-like A ($cnTiO_2$); A/B TiO_2 (21 nm); SiO_2 NPs	Balb/c mice	INH.	2 h on 4 consecutive days or 2 h on 4 consecutive days per week for 4 weeks	10 ± 2 mg/m^3	–	–	–			+		[22]

R, rutile; A, anatase; B, brookite; it, intranasal instillation; INH, inhalation; NPs, nanoparticles; BW, body weight; "–", independent factor; "+", dependent factor.

treated with uncoated rutile TNPs, anatase TNPs, and SiO_2 NPs [22]. UV Titan (rutile TiO2 modified with Al, Si, Zr and coated with polyalcohol) is reported to produce prolonged lung inflammation in dams and developmental neurotoxicity in pups [71]. In addition, it causes acute-phase immune and inflammatory responses by altering gene expression [121].

Investigations based on the inherent physicochemical properities of TNPs are summarized in Table 1. The toxicity of TNPs, like that of other NPs, is associated with physicochemical properties, such as size, crystal form, surface coating, and in-solution aggregation. As the data show, nanotoxicity may involve many factors, and much remains to be learned.

5. Concluding remarks

Nanotoxicology has developed through three stages: descriptive toxicology, mechanistic toxicology, and regulatory toxicology. Various toxic effects of TNPs have been widely investigated in animals and cells using different exposure routes, doses, duration, and endpoints. The molecular mechanisms of toxicity and the structure–toxicity relationships are just starting to emerge. Most studies show that TNP is toxic to various experimental models, especially when escalating doses are used. However, results obtained from research in cells and animal models cannot be assumed to apply directly to human beings. Therefore, efforts must be made to develop more predictive models and conduct more epidemiological investigations.

The ultimate goal of our efforts is to predict and control TNP toxicity in order to assure its safe application. However, this challenging goal cannot be achieved until the molecular mechanisms of TNP toxicity and the associated structure–toxicity relationships are elucidated. More comprehensive and quantitative models must be built to assist in evaluating the impact of TNPs on humans. Further, approaches to modulate potential TNP toxicity, such as surface coating modifications using nano-combinatorial chemistry methods [122,123], should be developed.

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References

[1] A. Menard, D. Drobne, A. Jemec, Ecotoxicity of nanosized TiO_2. Review of in vivo data, Environ. Pollut. 159 (2011) 677–684.
[2] D. Reyes-Coronado, G. Rodríguez-Gattorno, M. Espinosa-Pesqueira, C. Cab, R. De Coss, G. Oskam, Phase-pure TiO_2 nanoparticles: anatase, brookite and rutile, Nanotechnology 19 (2008) 145605.
[3] R. Hu, X. Gong, Y. Duan, N. Li, Y. Che, Y. Cui, M. Zhou, C. Liu, H. Wang, F. Hong, Neurotoxicological effects and the impairment of spatial recognition memory in mice caused by exposure to TiO_2 nanoparticles, Biomaterials (2010).
[4] A. Fujishima, X. Zhang, D. Tryk, Heterogeneous photocatalysis: from water photolysis to applications in environmental cleanup, Int. J. Hydrogen Energy 32 (2007) 2664–2672.
[5] C. Fotiadis, N.P. Xekoukoulotakis, D. Mantzavinos, Photocatalytic treatment of wastewater from cottonseed processing: effect of operating conditions, aerobic biodegradability and ecotoxicity, Catal. Today 124 (2007) 247–253.
[6] U. Singh, S. Verma, H. Ghosh, M. Rath, K. Priyadarsini, A. Sharma, K. Pushpa, S. Sarkar, T. Mukherjee, Photo-degradation of curcumin in the presence of TiO_2 nanoparticles: fundamentals and application, J. Mol. Catal. A: Chem. 318 (2010) 106–111.
[7] J. Lee, S. Mahendra, P.J.J. Alvarez, Nanomaterials in the construction industry: a review of their applications and environmental health and safety considerations, ACS Nano (2010).
[8] L. Raki, J. Beaudoin, R. Alizadeh, J. Makar, T. Sato, Cement and concrete nanoscience and nanotechnology, Materials 3 (2010) 918–942.
[9] Y. Kikuchi, K. Sunada, T. Iyoda, K. Hashimoto, A. Fujishima, Photocatalytic bactericidal effect of TiO_2 thin films: dynamic view of the active oxygen species responsible for the effect, J. Photochem. Photobiol. A 106 (1997) 51–56.
[10] U.S. Food and Drug Administration, Listing of color additives exempt from certification, in: Title 21 – Food and Drugs, Code of Federal Regulations 73.575, U.S., 1996.
[11] J.L. Chen, W.E. Fayerweather, Epidemiologic study of workers exposed to titanium dioxide, J. Occup. Environ. Med. 30 (1988) 937.
[12] P. Boffetta, V. Gaborieau, L. Nadon, M.E. Parent, E. Weiderpass, J. Siemiatycki, Exposure to titanium dioxide and risk of lung cancer in a population-based study from Montreal, Scand. J. Work. Environ. Health 27 (2001) 227–232.
[13] J.P. Fryzek, B. Chadda, D. Marano, K. White, S. Schweitzer, J.K. McLaughlin, W.J. Blot, A cohort mortality study among titanium dioxide manufacturing workers in the United States, J. Occup. Environ. Med. 45 (2003) 400.
[14] A.V. Ramanakumar, M. Parent, B. Latreille, J. Siemiatycki, Risk of lung cancer following exposure to carbon black, titanium dioxide and talc: results from two case-control studies in Montreal, Int. J. Cancer 122 (2008) 183–189.
[15] E. Fabian, R. Landsiedel, L. Ma-Hock, K. Wiench, W. Wohlleben, B. van Ravenzwaay, Tissue distribution and toxicity of intravenously administered titanium dioxide nanoparticles in rats, Arch. Toxicol. 82 (2008) 151–157.
[16] E. Bermudez, J.B. Mangum, B. Asgharian, B.A. Wong, E.E. Reverdy, D.B. Janszen, P.M. Hext, D.B. Warheit, J.I. Everitt, Long-term pulmonary responses of three laboratory rodent species to subchronic inhalation of pigmentary titanium dioxide particles, Toxicol. Sci. 70 (2002) 86.
[17] P.M. Hext, J.A. Tomenson, P. Thompson, Titanium dioxide: inhalation toxicology and epidemiology, Ann. Occup. Hyg. 49 (2005) 461.
[18] N. Li, Y. Duan, M. Hong, L. Zheng, M. Fei, X. Zhao, J. Wang, Y. Cui, H. Liu, J. Cai, Spleen injury and apoptotic pathway in mice caused by titanium dioxide nanoparticles, Toxicol. Lett. 195 (2010) 161–168.
[19] S.B. Lovern, R. Klaper, *Daphnia magna* mortality when exposed to titanium dioxide and fullerene (C60) nanoparticles, Environ. Toxicol. Chem. 25 (2006) 1132–1137.
[20] S.B. Lovern, J.R. Strickler, R. Klaper, Behavioral and physiological changes in *Daphnia magna* when exposed to nanoparticle suspensions (titanium dioxide, nano-C60, and C60HxC70Hx), Environ. Sci. Technol. 41 (2007) 4465–4470.
[21] C. Blaise, F. Gagne, J. Ferard, P. Eullaffroy, Ecotoxicity of selected nanomaterials to aquatic organisms, Environ. Toxicol. 23 (2008) 591–598.
[22] E.M. Rossi, L. Pylkk nen, A.J. Koivisto, M. Vippola, K.A. Jensen, M. Miettinen, K. Sirola, H. Nyk senoja, P. Karisola, T. Stjernvall, Airway exposure to silica-coated TiO_2 nanoparticles induces pulmonary neutrophilia in mice, Toxicol. Sci. 113 (2010) 422.
[23] C.M. Sayes, R. Wahi, P.A. Kurian, Y. Liu, J.L. West, K.D. Ausman, D.B. Warheit, V.L. Colvin, Correlating nanoscale titania structure with toxicity: a cytotoxicity and inflammatory response study with human dermal fibroblasts and human lung epithelial cells, Toxicol. Sci. 92 (2006) 174.
[24] C. Thomas, N. Saleh, R.D. Tilton, G.V. Lowry, B. Veronesi, Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): implications for nanoparticle neurotoxicity, Environ. Sci. Technol. 40 (2006) 4346–4352.
[25] Y. Song, X. Li, X. Du, Exposure to nanoparticles is related to pleural effusion, pulmonary fibrosis and granuloma, Eur. Respir. J. 34 (2009) 559.
[26] C.O. Robichaud, A.E. Uyar, M.R. Darby, L.G. Zucker, M.R. Wiesner, Estimates of upper bounds and trends in nano-TiO_2 production as a basis for exposure assessment, Environ. Sci. Technol. 43 (2009) 4227–4233.
[27] J.S. Tsuji, A.D. Maynard, P.C. Howard, J.T. James, C. Lam, D.B. Warheit, A.B. Santamaria, Research strategies for safety evaluation of nanomaterials, part IV: risk assessment of nanoparticles, Toxicol. Sci. 89 (2006) 42.
[28] F. Mowat, J. Tsuji, Nanotechnology and the water market: applications and health effects, in: Nanotech (Ed.) Technical Proceedings of the 2006 NSTI Nanotechnology Conference and Trade Show, U.S., 2006, pp 416–419.
[29] M. Kiser, P. Westerhoff, T. Benn, Y. Wang, J. Perez-Rivera, K. Hristovski, Titanium nanomaterial removal and release from wastewater treatment plants, Environ. Sci. Technol. 43 (2009) 6757–6763.
[30] G. Oberdörster, E. Oberdörster, J. Oberdörster, Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles, Environ. Health Perspect. 113 (2005) 823–839.
[31] A. Patri, T. Umbreit, J. Zheng, K. Nagashima, P. Goering, S. Francke-Carroll, E. Gordon, J. Weaver, T. Miller, N. Sadrieh, Energy dispersive X-ray analysis of titanium dioxide nanoparticle distribution after intravenous and subcutaneous injection in mice, J. Appl. Toxicol. 29 (2009) 662–672.
[32] J. Wang, G. Zhou, C. Chen, H. Yu, T. Wang, Y. Ma, G. Jia, Y. Gao, B. Li, J. Sun, Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration, Toxicol. Lett. 168 (2007) 176–185.
[33] W.I. Hagens, A.G. Oomen, W.H. de Jong, F.R. Cassee, A.J.A.M. Sips, What do we (need to) know about the kinetic properties of nanoparticles in the body? Regul. Toxicol. Pharmacol. 49 (2007) 217–229.
[34] P. Abidi, F. Afaq, J.M. Arif, M. Lohani, Q. Rahman, Chrysotile-mediated imbalance in the glutathione redox system in the development of pulmonary injury, Toxicol. Lett. 106 (1999) 31–39.
[35] J.E. Repine, A. Bast, I. Lankhorst, Oxidative stress in chronic obstructive pulmonary disease, Am. J. Respir. Crit. Care Med. 156 (1997) 341.
[36] C.W. White, J.E. Repine, Pulmonary antioxidant defense mechanisms, Exp. Lung Res. 8 (1985) 81.

[37] K. Lee, Y.S. Yang, S.J. Kwon, J.S. Lee, S.J. Choi, H.S. Seo, M.S. Kang, B.C. Lee, S.N. Kim, H.S. Yang, Lung injury study by 15 days inhalation exposure of titanium dioxide nanoparticles in rats, Toxicol. Lett. 189 (2009) S186.
[38] H.W. Chen, S.F. Su, C.T. Chien, W.H. Lin, S.L. Yu, C.C. Chou, J.J.W. Chen, P.C. Yang, Titanium dioxide nanoparticles induce emphysema-like lung injury in mice, FASEB J. 20 (2006) 2393.
[39] D.B. Warheit, T.R. Webb, C.M. Sayes, V.L. Colvin, K.L. Reed, Pulmonary instillation studies with nanoscale TiO_2 rods and dots in rats: toxicity is not dependent upon particle size and surface area, Toxicol. Sci. 91 (2006) 227.
[40] A. Nemmar, K. Melghit, B.H. Ali, The acute proinflammatory and prothrombotic effects of pulmonary exposure to rutile TiO_2 nanorods in rats, Exp. Biol. Med. 233 (2008) 610.
[41] A. Nemmar, K. Melghit, S. Al-Salam, S. Zia, S. Dhanasekaran, S. Attoub, I. Al-Amri, B.H. Ali, Acute respiratory and systemic toxicity of pulmonary exposure to rutile Fe-doped TiO_2 nanorods, Toxicology (2010).
[42] Y. Cui, X. Gong, Y. Duan, N. Li, R. Hu, H. Liu, M. Hong, M. Zhou, L. Wang, H. Wang, Hepatocyte apoptosis and its molecular mechanisms in mice caused by titanium dioxide nanoparticles, J. Hazard. Mater. (2010).
[43] J. Wu, W. Liu, C. Xue, S. Zhou, F. Lan, L. Bi, H. Xu, X. Yang, F.D. Zeng, Toxicity and penetration of TiO_2 nanoparticles in hairless mice and porcine skin after subchronic dermal exposure, Toxicol. Lett. 191 (2009) 1–8.
[44] F. Menzel, T. Reinert, J. Vogt, T. Butz, Investigations of percutaneous uptake of ultrafine TiO_2 particles at the high energy ion nanoprobe LIPSION, Nucl. Instrum. Methods Phys. Res. Sect. B 219 (2004) 82–86.
[45] J. Lademann, H.J. Weigmann, C. Rickmeyer, H. Barthelmes, H. Schaefer, G. Mueller, W. Sterry, Penetration of titanium dioxide microparticles in a sunscreen formulation into the horny layer and the follicular orifice, Skin Pharmacol. Physiol. 12 (2000) 247–256.
[46] J. Lademann, H. Richter, A. Teichmann, N. Otberg, U. Blume-Peytavi, J. Luengo, B. Wei, U.F. Schaefer, C.M. Lehr, R. Wepf, Nanoparticles – an efficient carrier for drug delivery into the hair follicles, Eur. J. Pharm. Biopharm. 66 (2007) 159–164.
[47] J. Lademann, F. Knorr, H. Richter, U. Blume-Peytavi, A. Vogt, C. Antoniou, W. Sterry, A. Patzelt, Hair follicles – an efficient storage and penetration pathway for topically applied substances, Skin Pharmacol. Physiol. 21 (2008) 150–155.
[48] J. Lademann, H. Richter, U. Schaefer, U. Blume-Peytavi, A. Teichmann, N. Otberg, W. Sterry, Hair follicles – a long-term reservoir for drug delivery, Skin Pharmacol. Physiol. 19 (2006) 232–236.
[49] G.J. Nohynek, J. Lademann, C. Ribaud, M.S. Roberts, Grey goo on the skin? Nanotechnology, cosmetic and sunscreen safety, Crit. Rev. Toxicol. 37 (2007) 251–277.
[50] M. Yaar, B. Gilchrest, Aging of skin, in: I.M. Freedberg, A.Z. Eisen, K. Wolff, K.F. Austen, L.A. Goldsmith, S.I. Katz (Eds.), Fitzpatrick's dermatology in general medicine, McGraw Hill, New York, 2003, pp. 1386–1398.
[51] A.S. Dussert, E. Gooris, J. Hemmerle, Characterization of the mineral content of a physical sunscreen emulsion and its distribution onto human stratum corneum, Int. J. Cosmet. Sci. 19 (1997) 119–129.
[52] J. Schulz, H. Hohenberg, F. Pflücker, E. Grtner, T. Will, S. Pfeiffer, R. Wepf, V. Wendel, H. Gers-Barlag, K.P. Wittern, Distribution of sunscreens on skin, Adv. Drug Delivery Rev. 54 (2002) S157–S163.
[53] E. Gontier, C. Habchi, T. Pouthier, P. Aguer, P. Barberet, Y. Barbotteau, S. Incerti, M. Ynsa, J. Surleve-Bazeille, P. Moretto, Nuclear Microscopy and Electron Microscopy Studies of Percutaneous Penetration of Nanoparticles in Mammalian Skin in Vienna, Austria, 2004, pp. 9–11.
[54] A. Mavon, C. Miquel, O. Lejeune, B. Payre, P. Moretto, In vitro percutaneous absorption and in vivo stratum corneum distribution of an organic and a mineral sunscreen, Skin Pharmacol. Physiol. 20 (2006) 10–20.
[55] A. Lansdown, A. Taylor, Zinc and titanium oxides: promising UV-absorbers but what influence do they have on the intact skin? Int. J. Cosmet. Sci. 19 (1997) 167–172.
[56] A. Gamer, E. Leibold, B. Van Ravenzwaay, The in vitro absorption of microfine zinc oxide and titanium dioxide through porcine skin, Toxicol. In Vitro 20 (2006) 301–307.
[57] D.B. Warheit, R.A. Hoke, C. Finlay, E.M. Donner, K.L. Reed, C.M. Sayes, Development of a base set of toxicity tests using ultrafine TiO_2 particles as a component of nanoparticle risk management, Toxicol. Lett. 171 (2007) 99–110.
[58] K. Fujita, M. Horie, H. Kato, S. Endoh, M. Suzuki, A. Nakamura, A. Miyauchi, K. Yamamoto, S. Kinugasa, K. Nishio, Effects of ultrafine TiO_2 particles on gene expression profile in human keratinocytes without illumination: involvement of extracellular matrix and cell adhesion, Toxicol. Lett. 191 (2009) 109–117.
[59] R.K. Shukla, A. Kumar, A.K. Pandey, S.S. Singh, A. Dhawan, Titanium dioxide nanoparticles induce oxidative stress-mediated apoptosis in human keratinocyte cells, J. Biomed. Nanotechnol. 7 (2011) 100–101.
[60] R.K. Shukla, V. Sharma, A.K. Pandey, S. Singh, S. Sultana, A. Dhawan, ROS-mediated genotoxicity induced by titanium dioxide nanoparticles in human epidermal cells, Toxicol. In Vitro (2010).
[61] J. Chen, H. Zhou, A.C. Santulli, S.S. Wong, Evaluating cytotoxicity and cellular uptake from the presence of variously processed TiO_2 nanostructured morphologies, Chem. Res. Toxicol. 23 (2010) 871–879.
[62] N. Lu, Z. Zhu, X. Zhao, R. Tao, X. Yang, Z. Gao, Nano titanium dioxide photocatalytic protein tyrosine nitration: a potential hazard of TiO_2 on skin, Biochem. Biophys. Res. Commun. 370 (2008) 675–680.
[63] D.C. Dorman, B.E. McManus, C.U. Parkinson, C.A. Manuel, A.M. McElveen, J.I. Everitt, Nasal toxicity of manganese sulfate and manganese phosphate in young male rats following subchronic (13-week) inhalation exposure, Inhal. Toxicol. 16 (2004) 481–488.
[64] A. Elder, R. Gelein, V. Silva, T. Feikert, L. Opanashuk, J. Carter, R. Potter, A. Maynard, Y. Ito, J. Finkelstein, Translocation of inhaled ultrafine manganese oxide particles to the central nervous system, Environ. Health Perspect. 114 (2006) 1172.
[65] J.X. Wang, C.Y. Chen, Y. Liu, F. Jiao, W. Li, F. Lao, Y.F. Li, B. Li, C.C. Ge, G.Q. Zhou, Y.X. Gao, Y.L. Zhao, Z.F. Chai, Potential neurological lesion after nasal instillation of TiO(2) nanoparticles in the anatase and rutile crystal phases, Toxicol. Lett. 183 (2008) 72–80.
[66] J. Wang, Y. Liu, F. Jiao, F. Lao, W. Li, Y. Gu, Y. Li, C. Ge, G. Zhou, B. Li, Time-dependent translocation and potential impairment on central nervous system by intranasally instilled TiO_2 nanoparticles, Toxicology 254 (2008) 82–90.
[67] R. Hu, L. Zheng, T. Zhang, G. Gao, Z. Cheng, Y. Cui, J. Cheng, Mechanism of inflammatory responses in brain and impairment of spatial memory of mice caused by titanium dioxide nanoparticules, J. Hazard. Mater. (2011).
[68] L. Ma, J. Liu, N. Li, J. Wang, Y. Duan, J. Yan, H. Liu, H. Wang, F. Hong, Oxidative stress in the brain of mice caused by translocated nanoparticulate TiO_2 delivered to the abdominal cavity, Biomaterials 31 (2010) 99–105.
[69] J. Shin, E. Lee, S. Seo, H. Kim, J. Kang, E. Park, Nanosized titanium dioxide enhanced inflammatory responses in the septic brain of mouse, Neuroscience 165 (2010) 445–454.
[70] K. Takeda, K. Suzuki, A. Ishihara, M. Kubo-Irie, R. Fujimoto, M. Tabata, S. Oshio, Y. Nihei, T. Ihara, M. Sugamata, Nanoparticles transferred from pregnant mice to their offspring can damage the genital and cranial nerve systems, J. Health Sci. 55 (2009) 95–102.
[71] K.S. Hougaard, P. Jackson, K.A. Jensen, J.J. Sloth, K.L. schner, E.H. Larsen, R.K. Birkedal, A. Vibenholt, A.M.Z. Boisen, H. Wallin, Effects of prenatal exposure to surface-coated nanosized titanium dioxide(UV-Titan). A study in mice, Part Fibre Toxicol. 7 (2010) 16.
[72] M. Shimizu, H. Tainaka, T. Oba, K. Mizuo, M. Umezawa, K. Takeda, Maternal exposure to nanoparticulate titanium dioxide during the prenatal period alters gene expression related to brain development in the mouse, Part Fibre Toxicol. 6 (2009) 20.
[73] M. Ema, N. Kobayashi, M. Naya, S. Hanai, J. Nakanishi, Reproductive and developmental toxicity studies of manufactured nanomaterials, Reprod. Toxicol. 30 (2010) 343–352.
[74] V.K. Sharma, Aggregation and toxicity of titanium dioxide nanoparticles in aquatic environment – a review, J. Environ. Sci. Health A 44 (2009) 1485–1495.
[75] R.D. Handy, R. Owen, E. Valsami-Jones, The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs, Ecotoxicology 17 (2008) 315–325.
[76] K.T. Kim, S.J. Klaine, J. Cho, S.H. Kim, S.D. Kim, Oxidative stress responses of *Daphnia magna* exposed to TiO_2 nanoparticles according to size fraction, Sci. Total Environ. 408 (2010) 2268–2272.
[77] M. Heinlaan, A. Ivask, I. Blinova, H.C. Dubourguier, A. Kahru, Toxicity of nanosized and bulk ZnO, CuO and TiO_2 to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*, Chemosphere 71 (2008) 1308–1316.
[78] K. Wiench, W. Wohlleben, V. Hisgen, K. Radke, E. Salinas, S. Zok, R. Landsiedel, Acute and chronic effects of nano-and non-nano-scale TiO_2 and ZnO particles on mobility and reproduction of the freshwater invertebrate *Daphnia magna*, Chemosphere 76 (2009) 1356–1365.
[79] A.L. Neal, What can be inferred from bacterium–nanoparticle interactions about the potential consequences of environmental exposure to nanoparticles? Ecotoxicology 17 (2008) 362–371.
[80] A. Kumar, A.K. Pandey, S.S. Singh, R. Shanker, A. Dhawan, Cellular uptake and mutagenic potential of metal oxide nanoparticles in bacterial cells, Chemosphere (2011).
[81] C. Cherchi, T. Chernenko, M. Diem, A.Z. Gu, Impact of nano titanium dioxide exposure on cellular structure of *Anabaena variabilis* and evidence of internalization, Environ. Toxicol. Chem. (2011).
[82] A. Kumar, A.K. Pandey, S.S. Singh, R. Shanker, A. Dhawan, Engineered ZnO and TiO_2 nanoparticles induce oxidative stress and DNA damage leading to reduced viability of *Escherichia coli*, Free Radic. Biol. Med. (2011).
[83] K. Hund-Rinke, M. Simon, Ecotoxic effect of photocatalytic active nanoparticles (TiO_2) on algae and daphnids, Environ. Sci. Pollut. Res. 13 (2006) 225–232.
[84] A. Kahru, H.C. Dubourguier, From ecotoxicology to nanoecotoxicology, Toxicology 269 (2010) 105–119.
[85] G. Federici, B.J. Shaw, R.D. Handy, Toxicity of titanium dioxide nanoparticles to rainbow trout (*Oncorhynchus mykiss*): gill injury, oxidative stress, and other physiological effects, Aquat. Toxicol. 84 (2007) 415–430.
[86] W. Fan, M. Cui, H. Liu, C. Wang, Z. Shi, C. Tan, X. Yang, Nano-TiO_2 enhances the toxicity of copper in natural water to *Daphnia magna*, Environ. Pollut. 159 (2011) 729–734.
[87] X. Zhang, H. Sun, Z. Zhang, Q. Niu, Y. Chen, J.C. Crittenden, Enhanced bioaccumulation of cadmium in carp in the presence of titanium dioxide nanoparticles, Chemosphere 67 (2007) 160–166.
[88] J. Chen, X. Dong, Y. Xin, M. Zhao, Effects of titanium dioxide nano-particles on growth and some histological parameters of zebrafish (*Danio rerio*) after a long-term exposure, Aquat. Toxicol. 101 (2011) 493–499.

[89] E.J. Park, J. Yi, K.H. Chung, D.Y. Ryu, J. Choi, K. Park, Oxidative stress and apoptosis induced by titanium dioxide nanoparticles in cultured BEAS-2B cells, Toxicol. Lett. 180 (2008) 222–229.
[90] V. Freyre-Fonseca, N.L. Delgado-Buenrostro, E.B. Gutiérrez-Cirlos, C.M. Calderón-Torres, T. Cabellos-Avelar, Y. Sánchez-Pérez, E. Pinzón, I. Torres, E. Molina-Jijón, C. Zazueta, Titanium dioxide nanoparticles impair lung mitochondrial function, Toxicol. Lett. 202 (2011) 111–119.
[91] J.J. Wang, B.J.S. Sanderson, H. Wang, Cyto- and genotoxicity of ultrafine TiO_2 particles in cultured human lymphoblastoid cells, Mutat. Res. Genet. Toxicol. Environ. Mutagen. 628 (2007) 99–106.
[92] C.I. Vamanu, M.R. Cimpan, P.J. Hol, S. Sornes, S.A. Lie, N.R. Gjerdet, Induction of cell death by TiO_2 nanoparticles: studies on a human monoblastoid cell line, Toxicol. In Vitro 22 (2008) 1689–1696.
[93] L.K. Braydich-Stolle, N.M. Schaeublin, R.C. Murdock, J. Jiang, P. Biswas, J.J. Schlager, S.M. Hussain, Crystal structure mediates mode of cell death in TiO_2 nanotoxicity, J. Nanopart. Res. 11 (2009) 1361–1374.
[94] Q. Rahman, M. Lohani, E. Dopp, H. Pemsel, L. Jonas, D.G. Weiss, D. Schiffmann, Evidence that ultrafine titanium dioxide induces micronuclei and apoptosis in Syrian hamster embryo fibroblasts, Environ. Health Perspect. 110 (2002) 797.
[95] E.J. Park, J. Yoon, K. Choi, J. Yi, K. Park, Induction of chronic inflammation in mice treated with titanium dioxide nanoparticles by intratracheal instillation, Toxicology 260 (2009) 37–46.
[96] G. Falck, H. Lindberg, S. Suhonen, M. Vippola, E. Vanhala, J. Catalan, K. Savolainen, H. Norppa, Genotoxic effects of nanosized and fine TiO_2, Hum. Exp. Toxicol. 28 (2009) 339.
[97] A. Di Virgilio, M. Reigosa, P. Arnal, M. Fernández Lorenzo de Mele, Comparative study of the cytotoxic and genotoxic effects of titanium oxide and aluminium oxide nanoparticles in Chinese hamster ovary (CHO-K1) cells, J. Hazard. Mater. 177 (2010) 711–718.
[98] K. Bhattacharya, M. Davoren, J. Boertz, R.P.F. Schins, E. Hoffmann, E. Dopp, Titanium dioxide nanoparticles induce oxidative stress and DNA-adduct formation but not DNA-breakage in human lung cells, Part Fibre Toxicol. 6 (2009) 17.
[99] J. Petković, B. Žegura, M. Stevanović, N. Drnovšek, D. Uskoković, S. Novak, M. Filipič, DNA damage and alterations in expression of DNA damage responsive genes induced by TiO_2 nanoparticles in human hepatoma HepG2 cells, Nanotoxicology 5 (2011) 341–353.
[100] B. Trouiller, R. Reliene, A. Westbrook, P. Solaimani, R.H. Schiestl, Titanium dioxide nanoparticles induce DNA damage and genetic instability in vivo in mice, Cancer Res. 69 (2009) 8784.
[101] N. Li, L. Ma, J. Wang, L. Zheng, J. Liu, Y. Duan, H. Liu, X. Zhao, S. Wang, H. Wang, Interaction between nano-anatase TiO_2 and liver DNA from mice in vivo, Nanoscale Res. Lett. 5 (2010) 108–115.
[102] D.B. Warheit, K.L. Reed, C.M. Sayes, A role for surface reactivity in TiO_2 and quartz-related nanoparticle pulmonary toxicity, Nanotoxicology 3 (2009) 181–187.
[103] R. Landsiedel, L. Ma-Hock, A. Kroll, D. Hahn, J. Schnekenburger, K. Wiench, W. Wohlleben, Testing metal-oxide nanomaterials for human safety, Adv. Mater. 22 (2010) 2601–2627.
[104] E. Bermudez, J.B. Mangum, B.A. Wong, B. Asgharian, P.M. Hext, D.B. Warheit, J.I. Everitt, Pulmonary responses of mice, rats, and hamsters to subchronic inhalation of ultrafine titanium dioxide particles, Toxicol. Sci. 77 (2004) 347.
[105] R. Liu, L. Yin, Y. Pu, G. Liang, J. Zhang, Y. Su, Z. Xiao, B. Ye, Pulmonary toxicity induced by three forms of titanium dioxide nanoparticles via intra-tracheal instillation in rats, Prog. Nat. Sci. 19 (2009) 573–579.
[106] H. Liu, L. Ma, J. Zhao, J. Liu, J. Yan, J. Ruan, F. Hong, Biochemical toxicity of nano-anatase TiO_2 particles in mice, Biol. Trace Elem. Res. 129 (2009) 170–180.
[107] U. Heinrich, R. Fuhst, S. Rittinghausen, O. Creutzenberg, B. Bellmann, W. Koch, K. Levsen, Chronic inhalation exposure of Wistar rats and two different strains of mice to diesel engine exhaust, carbon black, and titanium dioxide, Inhal. Toxicol. 7 (1995) 533–556.
[108] K. Lee, H. Trochimowicz, C. Reinhardt, Pulmonary response of rats exposed to titanium dioxide (TiO_2) by inhalation for two years, Toxicol. Appl. Pharmacol. 79 (1985) 179–192.
[109] L. Renwick, D. Brown, A. Clouter, K. Donaldson, Increased inflammation and altered macrophage chemotactic responses caused by two ultrafine particle types, Occup. Environ. Med. 61 (2004) 442.
[110] N. Kobayashi, M. Naya, S. Endoh, J. Maru, K. Yamamoto, J. Nakanishi, Comparative pulmonary toxicity study of nano-TiO_2 particles of different sizes and agglomerations in rats: different short- and long-term post-instillation results, Toxicology 264 (2009) 110–118.
[111] H.W. Kim, E.K. Ahn, B.K. Jee, H.K. Yoon, K.H. Lee, Y. Lim, Nanoparticulate-induced toxicity and related mechanism in vitro and in vivo, J. Nanopart. Res. 11 (2009) 55–65.
[112] Q. Zhang, Y. Kusaka, X. Zhu, K. Sato, Y. Mo, T. Kluz, K. Donaldson, Comparative toxicity of standard nickel and ultrafine nickel in lung after intratracheal instillation, J. Occup. Health 45 (2003) 23–30.
[113] D.M. Brown, M. Wilson, W. MacNee, V. Stone, K. Donaldson, Size-dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines, Toxicol. Appl. Pharmacol. 175 (2001) 191–199.
[114] A. Elder, R. Gelein, J.N. Finkelstein, K.E. Driscoll, J. Harkema, G. Oberdrster, Effects of subchronically inhaled carbon black in three species. I. Retention kinetics, lung inflammation, and histopathology, Toxicol. Sci. 88 (2005) 614.
[115] R. Kumazawa, F. Watari, N. Takashi, Y. Tanimura, M. Uo, Y. Totsuka, Effects of Ti ions and particles on neutrophil function and morphology, Biomaterials 23 (2002) 3757–3764.
[116] Biological Effects of Titania Nanoparticles and Their Application as Anti-cancer Drug Vehicle in Breast Cancer Therapy, Breast Cancer, Cancer Research. http://wwwcancer-rescom/breast-cancer/biological-effects-of-titania-nanoparticles-and-their-application-as-anti-cancer-drug-vehicle-in-breast-cancer-therapy.html, 2011.
[117] C.M. Liao, Y.H. Chiang, C.P. Chio, Assessing the airborne titanium dioxide nanoparticle-related exposure hazard at workplace, J. Hazard. Mater. 162 (2009) 57–65.
[118] H. Suzuki, T. Toyooka, Y. Ibuki, Simple and easy method to evaluate uptake potential of nanoparticles in mammalian cells using a flow cytometric light scatter analysis, Environ. Sci. Technol. 41 (2007) 3018–3024.
[119] R. Tedja, C. Marquis, M. Lim, R. Amal, Biological impacts of TiO_2 on human lung cell lines A549 and H1299: particle size distribution effects, J. Nanopart. Res. 13 (2011) 3801–3813.
[120] R. Jayakumar, R. Ramachandran, V. Divyarani, K. Chennazhi, H. Tamura, S. Nair, Fabrication of chitin–chitosan/nano TiO_2-composite scaffolds for tissue engineering applications, Int. J. Biol. Macromol. 2 (2011) 336–344.
[121] S. Halappanavar, P. Jackson, A. Williams, K.A. Jensen, K.S. Hougaard, U. Vogel, C.L. Yauk, H. Wallin, Pulmonary response to surface-coated nanotitanium dioxide particles includes induction of acute phase response genes, inflammatory cascades, and changes in microRNAs: a toxicogenomic study, Environ. Mol. Mutagen. 52 (2011) 425–439.
[122] G. Su, B. Yan, Nano-combinatorial chemistry strategy for nanotechnology research, J. Comb. Chem. 12 (2010) 215–221.
[123] H. Zhou, Q. Mu, N. Gao, A. Liu, Y. Xing, S. Gao, Q. Zhang, G. Qu, Y. Chen, G. Liu, A nano-combinatorial library strategy for the discovery of nanotubes with reduced protein-binding, cytotoxicity, and immune response, Nano Lett. 8 (2008) 859–865.
[124] J. Li, Q. Li, J. Xu, X. Cai, R. Liu, Y. Li, J. Ma, W. Li, Comparative study on the acute pulmonary toxicity induced by 3 and 20 nm TiO_2 primary particles in mice, Environ. Toxicol. Pharmacol. 24 (2007) 239–244.
[125] Melbourne Dermatology, Melbourne. http://wwwtreatment-skincarecom/Glossary/Skin-Structure-Diagram.html, 2009.
[126] G. Oberdörster, V. Stone, K. Donaldson, Toxicology of nanoparticles: a historical perspective, Nanotoxicology 1 (2007) 2–25.