Review article

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The potential health challenges of TiO_2 nanomaterials

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ABSTRACT: Titanium dioxide (TiO_2) nanomaterials (NMs) have found widespread applications owing to their attractive physical and chemical properties. As a result, the potential adverse impacts of nano-TiO_2 exposure on humans have become a matter of concern. This review presents the state-of-the-art advances on the investigations of the adverse effects of NMs, including the potential exposure routes of nano-TiO_2 (e.g. respiratory system, skin absorption and digestive system), the physico-chemical characterizations of nano-TiO_2 (e.g. crystal structure, shape,size, zeta potential, treatment media, aggregation and agglomeration tendency, surface characteristics and coatings), risk evaluation of nanotoxicity (e.g. cytotoxicity, ecotoxicity, phototoxicity, and phytotoxicity) and potential mechanisms of adverse effects (e.g. generation of reactive oxygen species, oxidative stress and organelle dysfunction). The review aims to facilitate scientific assessments of health risks to nano-TiO_2, which would guide the safe applications of NMs in our daily life. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: titanium dioxide; nanomaterials; health risk; route; characterization; mechanism

Introduction

With increasing exposure of dispersed nanomaterials (NMs) from products or workplaces, there is an increased opportunity for NMs to enter the body, leading to potential adverse effects. For example, two female patients, who worked in a printing plant, died of respiratory failure (Song *et al*., 2009). Pathological examinations have aroused concern that long-term exposure to NMs without protective measures may lead to serious damage to human lungs (Song *et al*., 2009). Owing to the lack of systematic knowledge of toxicology, epidemiology and workplace exposure of NMs (Kuempel *et al*., 2007), many researchers doubt the adverse effects of NMs (Gilbert, 2009; Brain *et al*., 2010), although the disaster triggered by asbestos dust exposure during the past decades is still fresh in the mind (Frost *et al*., 2008). Recently, Hristozov *et al*. (2014) suggested that the hazard identification of NMs under multiple regulatory frameworks is needed, which should integrate individual studies about physico-chemical and toxicological properties of NMs. Schulte *et al*. (2008) reviewed a conceptual framework using occupational risk protection of engineered NMs, including the potential control of the different routes and adverse factors of NMs exposure. To be doubly sure, there is an urgent need for a better understanding of the risk of emerging NMs [e.g. nanoscale titanium dioxide (nano-TiO_2)] before they are put into practice.

Nano-titanium dioxide (TiO_2), as the most prevalent engineered NMs, is widely used in cosmetics, orthodontic composite, chewing gum, wastewater disinfectant, antibacterial, additive in pharmaceuticals, etc (Chen *et al*., 2013; Heravi *et al*., 2013; Prasad *et al*., 2013; Yang *et al*., 2013). Specifically, sunscreen is a $400 million dollar industry, 70% of which contains nano-TiO_2 (Nel *et al*., 2006). Furthermore, nano-TiO_2 led to ecotoxicity, phytotoxicity, cytotoxicity and genotoxicity in many researches (Diana *et al*., 2010; Gottschalk *et al*., 2013; Zhao *et al*., 2013; Josko and Oleszczuk 2014). Unfortunately, most of the existing literature about nano-TiO_2 focused on the preparation methods of NMs, and many risk assessment studies showed shortcomings in experimental design (Warheit, 2013; Hristozov *et al*., 2014; Wang *et al*., 2014). Up until now, reliable methods to identify relevant health risks of various forms of nano-TiO_2 have not been proposed.

Based on the abundance of risk data generated on nano-TiO_2, four parts are included in this review to facilitate the scientific risk assessments of nano-TiO_2 exposures. (1) Exposure routes of NMs: nano-TiO_2 can interact with the body through various routes, which should be well known for the reasonable design of experiment and risk evaluation. (2) The characterization of nano-TiO_2: different types of nano-TiO_2 have a variable toxicity potential, making it necessary to obtain adequate material characterization for the interpretation of measured results. (3) Risk evaluation in different species: various species have been employed to simulate human exposures, which can help to gauge the relevance of health risks for human beings. (4) The potential mechanisms of nanotoxicity: several major mechanisms have been proposed for inducing the adverse effect of nano-TiO_2, which would guide the usage of NMs in daily life and obtain the benefits of nanotechnology under safe situations.

Exposure route of nano-TiO_2

Various exposure routes of nano-TiO_2 entering the human body through the respiratory system, skin and digestive system have

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Table 1. Risk evaluation of nano-TiO_2 in human cells

Tissues/Cell	Crystal structure	Shape	Size (nm)	Treatment media	Zeta potential (mV)	Aggregation
Human embryonic kidney	Anatase	Spherical	22.9±0.3 (21), 50.7±0.4 (50)	Water	8.71 mV (21 nm), 9.38 mV (50 nm)	No
Human Caco-2_{BBe1} cells	Anatase	Spherical	336±6, 365±5	Cell culture medium	Negative charge above pH=4	Yes
Human erythrocyte and lymphocyte	Anatase/rutile	Spherical	35–56	PBS	-	Yes
Lung fibroblast cells	Rutile/ anatase	Spherical	40–200	-	-	Yes
Dermal fibroblasts	Anatase	-	15	Cell culture medium	-	Yes
Bronchial epithelial cells (BEAS-2B)	Rutile Anatase	Needle-like Spherical	10 × 40 <25	Growth media	-	Yes
Lung fibroblasts (IMR-90)	Anatase	Spherical	<100	Water	+48.8	Yes
Bronchial epithelial cells	Anatase	-	53, 311 461 86, 356	Water PBS Cell culture media	−24 −11 −9	Yes
Macrophage-like human THP-1 cells	Anatase Rutile Spicular	Spherical	<50 <25 10 <5 30–40 10 × 40	-	-	-
Human keratinocytes	-	Spherical	414.9±4.5	Cell culture media	−27.9±0.3	Yes
Keratinocyte HaCaT cells	Anatase	Granular	173.8, 259, 263.9	Cell culture media	-	Yes
Lymphocytes	-	Spherical	90-110	-	-	No

been found. As the production of nanoindustry, nano-TiO_2 may be easily inhaled by workers after respiratory system exposure, leading to the lung damage. The concentration of total airborne TiO_2 particle in the bin filling area of the facility ranges from 15 000 to 156 000 particles/cm^3, and more than 97% of them were less than 100 nm (Berges *et al.*, 2007). An epidemiological study demonstrated that NMs-exposed workers from six European countries showed a higher incidence of lung cancer than general populations (Liao *et al.*, 2008; Wang *et al.*, 2009). As a result, the permissible exposure limit and the immediately dangerous concentration of nano-TiO_2 for worker safety were estimated as 15 mg/m^3 and 7 500 mg/m^3, respectively (Liao *et al.*, 2008; NIOSH 2011; Wang *et al.*, 2009). For the populations heavily exposed to nano-TiO_2, the respiratory system is the major exposure route.

Currently, there is increased usage of nano-TiO_2 in cosmetics industry because of its absorptive properties (Singh *et al.*, 2009), although people argue that the marketing of nano-TiO_2 is ethically undesirable (Jacobs *et al.*, 2010). With the cumulative exposure during our daily life, the interaction between dispersed nano-TiO_2 from cosmetics products and the skin are unavoidable. The amounts of nano-TiO_2 applied on the skin would be 8–37 mg kg^{-1} weight for an adult, assuming that a sunscreen contains 5% nano-TiO_2 (Davis *et al.*, 2010). Wu *et al.* (2009) found that nano-TiO_2 can penetrate through the hairless mice skin after 60-days exposure, inducing diverse pathological lesions, especially in skin and liver. In addition, nano-TiO_2 in a sunscreen penetrated into deeper areas of the stratum corneum in psoriatic skin than healthy skin, because of the looser

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Table 1. (Continued)

Tissues/Cell	Concentration	Exposure duration	Toxicity	Reference
Human embryonic kidney	10–1 000 μg/mL	1 hr, 3 weeks	DNA damage, cell-transformation and cell-anchorage independent growth in soft-agar were observed at the 1000 μg/mL.	(Abrusci *et al.*, 2013)
Human Caco-2_{BBe1} cells	0.35–35 μg/mL	24 hrs	Food grade nano-TiO_2 exposure led to the loss of microvilli from the Caco-2BBe1 cell system.	(Archana *et al.*, 2013)
Human erythrocyte and lymphocyte	25–500 μg/mL	3 hrs	Led to the decreased activity of mitochondrial dehydrogenase, DNA damage, and apoptosis in lymphocyte; interacted with hemoglobin and showed hemolysis in erythrocyte.	(Ghosh *et al.*, 2013)
Lung fibroblast cells	25–400 μg/mL	24, 48 hrs	Intracellular ROS, loss of mitochondrial membrane potential, cell cycle progression alterations, TNF and CYP1A gene expression change were shown after exposure.	(Drevet *et al.*, 2012)
Dermal fibroblasts	1–100 μg/mL	24 hrs	Increased the phosphorylation of H2AX, ATM, and Chk2, and inhibited DNA synthesis and frequency of replicon initiation.	(Vyas *et al.*, 2011)
Bronchial epithelial cells (BEAS-2B)	1–100 μg/cm^2	24, 48, 72 hrs	DNA damage Increased micronuclei	(Falck *et al.*, 2009)
Lung fibroblasts (IMR-90)	2–50 μg/cm^2	2, 4, 6, 24 hrs	DNA adduct formation	(Bhattacharya *et al.*, 2009)
Bronchial epithelial cells	10–40 μg/cm^2	0.5, 1, 2, 4, 24 hrs	Induced lipid peroxidation, lysosomal membrane destabilization, and cell death.	(Hussain *et al.*, 2010)
Macrophage- like human THP-1 cells	20–500 μg/mL	24 hrs	Smaller anatase and larger rutile NMs provoked higher IL-1β production. Spicular NMs induced higher toxicity than similar sized and spherical NMs.	(Morishige *et al.*, 2010)
Human keratinocytes	0.5–10 μg/mL	3 months	Led to changes of cell cycle and the increase of apoptotic cells.	(Kocbek *et al.*, 2010)
Keratinocyte HaCaT cells	47.0–60.2 μg/mL	24 hrs	Affect cell-matrix adhesion	(Fujita *et al.*, 2009)
Lymphocytes	0.25–2 mM	3, 6, 24 hrs	Genotoxicity and cytotoxicity	(Ghosh *et al.*, 2010)

corneocyte organization in the diseased human skin (Pinheiro *et al.*, 2007).

Nano-TiO_2 have been identified in food-grade TiO_2 (Yang *et al.*, 2014) and preferred for drinking water treatment, making them highly possible to be absorbed by digestive system in the process of feeding, and redistributing to other tissues/organs. For instance, Nano-TiO_2 accumulated in mice liver, spleen, kidney, lung and brain after a single oral gavage of 5 000 mg kg^{-1} (Wang *et al.*, 2007). Furthermore, nano-TiO_2 can spread to the environment owing to industrial processes and consumer products, such as the use of wastewater disinfectant (Yang *et al.*, 2013). Nano-TiO_2 present in the environment can affect the water, soil and related organisms, leading to high mortality of medaka (Ma *et al.*, 2012), alteration of bacterial community composition and function in ecosystems (Ge *et al.*, 2011; Binh *et al.*, 2014), and changes in germination and root elongation in plants (Song *et al.*, 2013a, 2013b). These contaminations will finally induce an adverse effect on humans through the digestive system after feeding (e.g. water and vegetables), because humans are at the top of the food chain (D'Agata *et al.*, 2014).

The respiratory system, skin and digestive system are three major routes that nano-TiO_2 can enter the human body to induce potential health risk. Other routes of nanotoxicity assessment, such as intravenous injection, intraperitoneal injection and implantation, have also developed and widely used in animal testing (Patra *et al.*, 2009; Yang *et al.*, 2009). Although the relevance of animal experiments used to simulate human exposures is still under debate, these methods are regarded as part of the above three routes.

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Characterization of nano-TiO_2

NMs can penetrate cells with no need of specific receptors on the outer surface of the cells, which may be attributed to a passive uptake or adhesive interaction through van der Waals forces, electrostatic charges or steric interactions (Rimai *et al*., 2000; Peters *et al*., 2006). To provide an objective evaluation of health risk after nano-TiO_2 exposures, one of the important things is to obtain the adequate material characterization of test samples (Warheit, 2013). Recent studies suggested that the physico-chemical properties of nano-TiO_2 affect the result interpretation *in vitro* and *in vivo* after NMs exposure, such as crystal structure, shape, size, zeta potential, treatment media of NMs, aggregation and agglomeration tendency, surface characteristics and coatings (Scherbart *et al*., 2011; Tyner *et al*., 2011; Silva *et al*., 2013).

Crystal structure

X-ray diffraction (XRD) is the basic tool to analyze the crystal structure of nano-TiO_2 (Chen *et al*., 2013). Anatase, rutile and

Table 2. Risk evaluation of nano-TiO_2 in mice tissues and cells

Tissues/Cell	Crystal structure	Shape	Size (nm)	Treatment media	Zeta potential (mV)	Aggregation	Surface modification
Reproductive system	Anatase	-	208–330	HPMC + K4M	9.28 mV	Yes	-
Airway	Anatase/rutile	-	21	PBS	-	Yes	-
Sperm	Anatase	-	25	PBS with 0.5 % Tween80	-	-	-
Kidney	-	-	21	Water	-	Yes	-
Lung	Rutile	Elongated	12.8–486	Air	-	Yes	Zr, Si, Al
Lung	Anatase/rutile	-	200–300	PBS + DPPC + BSA	-	Yes	-
Lung	Anatase	-	80–110	-	-	Yes	-
Brain	Anatase	-	5	HPMC + K4M	-	-	-
Brain	Anatase	-	2 570	Saline	-	-	-
Brain	Anatase	-	5	HPMC + K4M	-	-	-
JB6 cell line	Anatase/rutile	-	34.9±16.8	PBS	-	-	-
L929 fibroblast cell	Anatase	-	20–50	Culture medium	-	Yes	-
Skin	Anatase	-	4	Caprylic/ LCC + Tween 80 + carbopol + triethanolamine + water	-	-	-
Kidney	Anatase		10				
Liver	Anatase/rutile		21				
	Rutile		25				
	Rutile		60				
	Rutile		90				
Liver	Anatase	-	5	HPMC + K4M	-	-	-
Offspring	Rutile	Elongated	12.8–486	Air	-	Yes	Zr, Si, Al

PBS, phosphate buffered saline; DPPC, dipalmitoyl phosphatidylcholine; BSA, bovine serum albumin; i.p., intraperitoneal administration; i.g., intragastric administration; HPMC, hydroxypropylmethylcellulose; SC, subcutaneous; LCC, capric triglyceride, SOD, superoxide dismutase; MDA, malondialdehyde.

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brookite are the three major crystal structures for nano-TiO_2, and the first two are the most common forms in toxicity assessment of nano-TiO_2 because brookite is rare (Warheit, 2013). One study suggested that anatase nano-TiO_2 showed more toxicity in human lung epithelial cells (A549) than rutile nano-TiO_2 (Sayes *et al.*, 2006). In contrast, the anatase/rutile nano-TiO_2 mixture showed a higher mortality of Zebrafish larvae than anatase nano-TiO_2 under ultraviolet (UV) irradiation, because the crystal structure affected the photocatalytic properties of nano-TiO_2 (Clemente *et al.*, 2014).

Shape, size and specific surface area

With the changes in synthesis condition (e.g. raw material, temperature, acidic and alkaline conditions), nano-TiO_2 with various shapes (e.g. rods, dots and belts), sizes and specific surface areas have been prepared for different applications (Wang *et al.*, 2004; Barnard and Curtiss, 2005). Nano-TiO_2 have structural components smaller than 100 nm in at least one dimension (Cristina *et al.*, 2007), which need transmission electron microscope (TEM) and scanning

Table 2. (Continued)

Tissues/Cell	Concentration	Exposure duration	Toxicity	Reference
Reproductive system	2.5–10 mg/kg	i.g., 90 days	Induced fertility reduction and ovary injury through alteration of inflammation/ follicular atresia-related cytokine expressions.	(Zhao *et al.*, 2013)
Airway	193±8 mg/lung after eight repeated exposures	Inhalation	Increased infiltration of neutrophils in airways of naïve mice, leading to neutrophilic airway inflammation and the loss of weight in asthmatic mice.	(Jing *et al.*, 2014)
Sperm	10–250 mg/kg	Oral, 42 days	Reduced the serum T level and spermatogenesis, inducing the spermatozoa abnormality in epididymides.	(Jia *et al.*, 2014)
Kidney	0.1, 0.25, and 0.5 mg/week	i.g., 4 weeks	Led to renal fibrosis through a ROS/RNS-related HIF-1alpha-upregulated TGF-beta signaling pathway.	(Kim *et al.*, 2013)
Lung	42 mg/m^3	Inhalation, 1 hr/day	Induced long-term lung inflammation.	(Hougaard *et al.*, 2010)
Lung	40 mg/kg	i.p.	Induced acute lung inflammation through oxidant-dependent inflammatory signaling and NF-kB pathway.	(Moon *et al.*, 2010)
Lung	324–2 592 mg/kg	i.p.,7, 14 days	Induced alveolar septal thickening, neutrophil infiltration, thrombosis.	(Chen *et al.*, 2009)
Brain	5-50 mg/kg	i.g., 60 days	Impaired behaviors of spatial recognition memory, disturbing homeostasis of trace elements, enzymes, and neurotransmitter systems.	(Hu *et al.*, 2010)
Brain	1 μg/μL	SC injection on gestational days 6, 9, 12, 15	Changed the gene expression associated with brain development, cell death, oxidative stress, mitochondria, inflammation, and neurotransmitters.	(Shimizu *et al.*, 2009)
Brain	5–150 mg/kg	i.p., 14 days	Translocated into brain, leading to OS, shape changes and inflammation in neurons.	(Ma *et al.*, 2010)
JB6 cell line	1–20 μg/cm^2	24, 48, 72 hrs	Induced the activation of mitochondrial-related pathways and cell apoptosis.	(Zhao *et al.*, 2009)
L929 fibroblast cell	0–300 mg/mL	48 hrs	Suppressed carbohydrate metabolism in L929 cells.	(Jin *et al.*, 2013)
Skin Kidney Liver	400 μg/cm^2	Skin exposure, 60 days	Changed the product of MDA and reduced the content of HYP in skin; saccus lymphaticus was shown in kidney; induced focal necrosis and liquefaction necrosis in the liver.	(Wu *et al.*, 2009)
Liver	5–50 mg/kg	i.g., 60 days	Accumulated in liver, inducing histopathological changes, hepatocytes apoptosis, and dysfunction of mice liver.	(Cui *et al.*, 2011)
Offspring	42 mg/m^3	Inhalation, 1 hr/day	Moderate neurobehavioral alterations	(Hougaard *et al.*, 2010)

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Table 3. Risk evaluation of nano-TiO_2 in rat tissues and cells

Tissues/Cell	Crystal structure	Shape	Size (nm)	Treatment media	Zeta potential (mV)	Aggregation	Concentration	Exposure duration	Toxicity	Reference
Alveolar macrophages	Anatase	-	<100	-	-	-	2.5–100, μg/mL	24 hrs	Cellular response of macrophages to nano-TiO_2 is age dependent. The generation of O_2^- and NO increased in a dose-dependent manner, IL-10 release was shown in the highest exposure of nano-TiO_2.	(Gu *et al.*, 2013)
Liver, lung, kidney and blood cells	Anatase	Spherical, lentil-like	5, 10,150	0.9% Nacl	-	-	16 g/kg	i.p., 3, 12 months	Nano-TiO_2 accumulated inside mononuclear cells and in liver and lung at 3 and 12 months, with higher reactive and biopersistent in lung, and liver cell membrane damage.	(Kunze *et al.*, 2009)
Neuroglia/ brain	-	-	10,20 and 200	MEM	-	-	0.1,1 and 10 mg/kg	Inhalation, 72 hrs	Nano-TiO_2 induced the change of IL-1β, TNF-α and IL-10 levels, blood-brain barrier damage, necrosis, inflammation and cellular oedema in brain.	(Alammar *et al.*, 2013)
PC12	Anatase	-	24–697	Cell culture media	-	Yes	1–100 μg/mL	6, 12, 24, 48 hrs	Induced a time- and dose-dependent cytotoxicity, intracellular accumulation of ROS, and cell apoptosis.	(Chusuei *et al.*, 2013)
Lung	Anatase/rutile	-	100	Aerosol	-	Yes	6 mg/m^3	Inhalation, 4, 12, 24 hrs	Led to increased microvascular OS and nitrosative stress, decreased NO bioavailability, and microvascular dysfunction.	(Nurkiewicz *et al.*, 2009)
Lung	Anatase	Belt	1 536 ± 139	Dispersion media	-30.3 ± 2.8	Yes	20–200 μg/rat	i.t. instilled, 1 day	Induced significant inflammation in BALF, NMs had not cleared from alveolar macrophages by 7 days and recovered from the lung.	(Silva *et al.*, 2013)
Lung	-	-	5–200	-	-	-	0.5–50 mg/kg	i.t., 1 day	Damage cell structure and led to pulmonary alveolar macrophage dysfunction.	(Liu *et al.*, 2010)

i.p., intraperitoneal administration; i.t., intratracheal instillation; BALF, bronchoalveolar lavage fluid; ROS, reactive oxygen species; dispersion medium (ethanol + sterile saline + distearoylphosphatidylcholine + low-endotoxin rat serum albumin)

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Table 4. Risk evaluation of nano-TiO_2 in aquatic animals

Species	Crystal structure	Shape	Size (nm)	Treatment media	Zeta potential (mV)	Aggregation	Surface modification	Concentration	Exposure duration	Toxicity	Reference
Karenia brevis and Skeletonema costatum	Anatase	Granular	<40	F/2 medium	-	Yes	-	125–750 mg/L	24, 48, 72 hrs	Nano-TiO_2 inhibited the growth of *K. brevis* and *S. costatum* through the ROS production and lipid oxidation inside algal cells.	(Davis *et al.*, 2010)
Daphnia magna	Rutile	Spherical	20	Water	-	Yes	-	1 and 10 mg/L	1–24 days	Nano-TiO_2 formed clusters with the algae cells, affecting the transfer of energy throughout depleting the concentration of available food for *Daphnia magna*.	(Wang *et al.*, 2005)
Japanese medaka	Anatase/ rutile	-	22–121	Water	16.5	Yes	-	2-7 mg/L	4 hrs	Showed dose-dependent mortality in *medaka*, and 96 hrs LC50 under laboratory light and solar UV radiation were 294 and 2.46 mg/L, respectively.	(Ma *et al.*, 2012)
Zebrafish (Gills)	-	Spherical	220.8, 687.5	Water	−25.1	Yes	-	1 000 μg/L	2–48 hrs	Changed gene expressions on ribosome structure and activity.	(Griffitt *et al.*, 2009)
Zebrafish (Embryo)	Anatase	Spherical	>50	Water	-	Yes	Cu	10, 20 ppt	2–72 hrs	Nano-TiO_2 penetrated into embryo and led to damaged eyes, abnormal heart and chord formation, increased activities of GSH, CAT, GST, and GSR.	(Yeo and Kang 2009)
Daphnia magna	Anatase/ rutile	-	580.5, 2 349, 3 528.6	Water	-	Yes	-	0.1–100 mg/L 0.1–5 mg/L	24, 48, 72 hrs 21 days	Showed minimal and high toxicity after 48 and 72 hrs exposures to *daphnia*. 21days accumulation induced severe growth retardation, mortality and reproductive defects.	(Zhu *et al.*, 2010)

GSH, glutathione; CAT, catalase; GST, glutathione S-transferase; GSR, glutathione reductase.

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Table 5. Risk evaluation of nano-TiO_2 in bacteria

Species	Crystal structure	Shape	Size (nm)	Treatment media	Zeta potential (mV)	Aggregation	Surface modification	Concentration	Exposure duration	Toxicity	Reference
B. subtilis/ *A. hydrophila*	Anatase Anatase/rutile	-	300–500 22.9	Water	-	Yes -	-	0.5-25 mg/L	1-2 hrs	Led to the reduction of viable *B. subtilis* and *A. hydrophila* under simulated solar illumination.	(Binh *et al.*, 2014)
Soil Bacterial Communities	Anatase/rutile	-	194	Water	-	Yes	-	20 mg/g	288 days	Changed the bacterial community composition and reduced diversity through soil water.	(Ge *et al.*, 2013)
B. subtilis/E. coli	Anatase/rutile	-	320–330	Water	-	Yes	-	10-5 000 ppm	20-26 hrs	Induced a dose-dependent growth reduction in bacteria, such as 2000 ppm NMs led to 99% and 46% growth reduction in *E. coli* and *B. subtilis*, respectively.	(Adams *et al.*, 2006)
B. cereus 905	Anatase/rutile		21	Water	-	-	-	0.002-2 mg/mL	12 hrs	Changed community structure and reduced bacterial population.	(Wang *et al.*, 2009)

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Table 6. Risk evaluation of nano-TiO_2 in plants

Species	Crystal structure	Shape	Size (nm)	Treatment media	Zeta potential (mV)	Aggregation	Concentration	Exposure duration	Toxicity	Reference
Lepidium sativum	-	-	<21	Water	−7.30	-	10–1 000 mg/L	72 hrs	Showed more toxic in water than in soil and inhibited root growth of *L.sativum*.	(Josko and Oleszczuk 2014)
Anabaena sp	Rutile	Spherical	450-650	Culture media	−7.8	Yes	1–50 mg/L	96 hrs	Induced toxicity at concentrations of 10-50 mg/L. Low dose (<1 mg/L) of NMs significantly enhanced the toxicity of Zn^{2+}. Toxicity of Zn^{2+}/ nano-TiO_2 system reduced with increasing NMs doses.	(Tang *et al.*, 2013)
Chlamydomonas reinhardtii	Anatase	Spherical	5	Exposure media	−1.89±0.19μm cmV^{-1} s^{-1}	Yes	1 mg/L	2 hrs	Decreased functions of photosynthesis-related genes, and increased levels of proteasome transcripts encoding subunits.	(Simon *et al.*, 2013)
Marine phytoplankton	Anatase/rutile	Semi-spherical	15–30	Seawater	-	Yes	1–7 mg/L	96 hrs	Induce increased ROS through UV radiation and show toxicity to phytoplankton.	(Miller *et al.*, 2012)
Lactuca sativa	Anatase/rutile	-	27	Water	-	Yes	100–5 000 mg/L	2-15 days	Took up into plant seeds, affecting root elongation in *L. sativa*.	(Song *et al.*, 2013)

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electron microscope (SEM) to image their morphology (Warheit *et al*., 2006; Chen *et al*., 2013). As shown in the published literature, the changes in morphology resulted in the different adverse effects after nano-TiO_2 exposure. For example, compared with TiO_2 nanospheres (diameter 60–200 nm) and short TiO_2 nanobelts (0.8–4 μm long, 60–300 nm wide), long TiO_2 nanobelts (15–30 μm long, 60–300 nm wide) induced significantly higher cytotoxicity in alveolar macrophage after 4-h exposure at dosages of 100 and 200 μg ml^{-1} (Hamilton *et al*., 2009). Another study showed that spicular nano-TiO_2 stimulated a higher production of Interleukin (IL)-1β than spherical nano-TiO_2 at lower concentrations (20 and 100 μg ml^{-1}), although they had a similar size and identical rutile structure (Morishige *et al*., 2010). Park *et al*. (2014) suggested that anatase nano-TiO_2 [180.2 ± 50.8 nm and −11.15 ± 0.46 mV in fetal bovine serum (FBS)] showed a higher accumulation in the heart, lung and liver of mice than brookite nano-TiO_2 (126.8 ± 36.5 nm and −9.66 ± 0.42 mV in FBS), although they had the same rod shape. Besides shape, size is another main determining factor for the distribution of nano-TiO_2 after respiratory tract exposure (Oberdorster *et al*., 2004). For example, amongst the nano-TiO_2 of three different sizes (5, 21 and 50 nm) with a dose more than 5 mg kg^{-1}, 5 nm nano-TiO_2 induced the most severe pulmonary toxicity in rats (Liu *et al*., 2009). The difference in the shape and size also changes the specific surface areas of nano-TiO_2, where a larger specific surface area had been found to induce higher adverse effects *in vitro* and *in vivo* after incubation, especially for the specific surface area-dependent phototoxic effect (Xiong *et al*., 2013; Park *et al*., 2014).

Treatment media

During the whole life-cycle of a product, the pristine characterizations of nano-TiO_2 may be changed during the use and disposal phases (Al-Kattan *et al*., 2014). Compared with the current focus on unaltered NMs, it is necessary to determine the behavior of nano-TiO_2 under a practical situation (e.g. treatment with media) before risk assessment. For instance, the same nano-TiO_2 gave the sizes of 53–311 nm, 461 nm and 86–356 nm in water, PBS and cell culture media, respectively (Hussain *et al*., 2010). The cultured cells have been used widely as the model system for cytotoxicity assessment *in vitro*. However, Prasad *et al*. (2013) found that the treatment media of NMs affected particle uptake, cell–NMs interaction and chromosomal damage through facilitating particle agglomeration. Additives (e.g. bisphenol A) in cell culture media led to synergistic toxicity with NMs through increasing the aggregation level and zeta potential of nano-TiO_2 (Zheng *et al*., 2012). Stomach acid (pH<4) can change the zeta potential of food-grade nano-TiO_2 after intragastric (i.g.) administration, which differ from the test results around pH 7 in deionized water (Yang *et al*., 2014). As a result, the situations concerning nano-TiO_2 should be paid more attention in nanotoxicity assessment (Karunakaran *et al*., 2013).

Modification

Nano-TiO_2 has the most exclusive absorption of UV, which corresponds to 3-5% of solar irradiation. Many modifications are focused on obtaining nano-TiO_2 activity in the range of visible light for the use of solar radiation, such as Fe-doped TiO_2 nanorods, N-doped TiO_2 nanoparticles, phosphate-modified nano-TiO_2 and polymers modified nano-TiO_2 (Liu *et al*., 2011; Chakrabortty and Gupta 2013). The modified nano-TiO_2 can degrade soluble organic pollutants in water through photocatalysis (Orlov *et al*., 2006), in the meantime, nano-TiO_2 with modification (trap centre) can eliminate the generation of free radical under solar energy, differing from the inducing effect of nano-TiO_2 in free radical generation (Wakefield *et al*., 2004).

Risk evaluation of nanotoxicity

Based on the inevitable existence in air, water, soils and organisms, the potential toxicity of nano-TiO_2 to health and the environment has been investigated at cell, tissue and animal levels, including human cells (Table 1), mice (Table 2), rats (Table 3), aquatic animals (Table 4), bacterium (Table 5) and plants (Table 6). Cytotoxicity, ecotoxicity, phototoxicity and phytotoxicity have been found after nano-TiO_2 exposure (Bhattacharya *et al*., 2009; Miller *et al*., 2012; Binh *et al*., 2014; Josko and Oleszczuk 2014).

Cytotoxicity

During the risk evaluation of human health, various human cells have been used to explore adverse effects based on the various exposure routes of nano-TiO_2, such as bronchial epithelial cells (Bhattacharya *et al*., 2009), keratinocytes (Kocbek *et al*., 2010), lymphocytes (Ghosh *et al*., 2010), macrophage (Morishige *et al*., 2010) and hepatocyte (Sha *et al*., 2014). During the risk assessment of NMs *in vitro*, nano-TiO_2 interacted with cultured cells directly. The changes in cell membrane integrity (Vevers and Jha 2008), mitochondrial activity (Di Virgilio *et al*., 2010), apoptosis marker (Veranth *et al*., 2007) and DNA structure (Ghosh *et al*., 2010) were detected after NMs exposure. Consequently, nano-TiO_2 killed cells in dose- and time-dependent manners (Sha *et al*., 2011), through the generation of reactive oxygen species (ROS) (Bhattacharya *et al*., 2009), lysosomal membrane destabilization and lipid peroxidation (Hussain *et al*., 2010), changing the cellular morphology (Goncalves *et al*., 2010), cell–matrix adhesion (Fujita *et al*., 2009) and gene expression (Park *et al*., 2009), etc. Furthermore, genotoxicity, including the DNA damage, the formation of DNA adducts and the changes in the cell cycle, has been observed after NMs incubation (Falck *et al*., 2009; Kocbek *et al*., 2010).

In vitro test plays important roles in assessing the adverse effect of NMs (Corsi *et al*., 2003), which can provide an initial estimation of the toxicity *in vivo* and facilitate a mechanistic understanding of the *in vivo* toxicity (Table 1). Nevertheless, testing may lead to false results of cytotoxicity owing to the different cell microenvironments *in vitro* as compared with its native counterpart (Heng *et al*., 2010), thus an *in vivo* test based on various animals (e.g. mice and rat) has been used to better mimic the response of the human body to NMs (Tables 2, 3).

During animal experiments, NMs can enter mice or rat body through inhalation (Hougaard *et al*., 2010), intraperitoneal (i.p.) administration (Chen *et al*., 2009), i.g. administration (Cui *et al*., 2011) and intratracheal (i.t.) instillation (Silva *et al*., 2013). Nano-TiO_2 interacted with biological components of the body, leading to biodistribution in different tissues during/after exposure (Wu *et al*., 2009), such as the lung (Hougaard *et al*., 2010), brain (Hu *et al*., 2010), liver (Cui *et al*., 2011), heart (Sha *et al*., 2013) and kidney (Chen *et al*., 2009). Regardless of which administration is used to expose animals, the lung is a major injured organ. NMs can migrate to the interstitium of the lung burden, leading to the cell structure damage, alveolar macrophage and microvascular dysfunction, neutrophil infiltration, lung inflammation, thrombosis, alveolar

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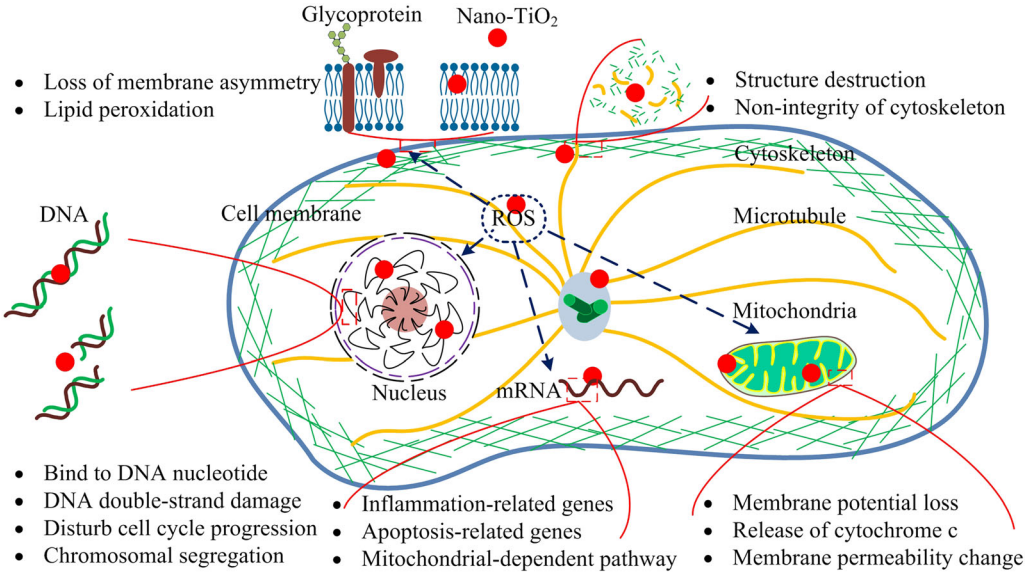

Figure 1. Schematic of titanium dioxide (TiO_2) nanotoxicity at the cellular level. Cell-TiO_2 nanomaterials (NMs) interactions can induce the membrane destabilization, Reactive oxygen species (ROS) production and lipid peroxidation during the penetration process of NMs (Davis *et al.*, 2010). The accumulation of ROS can perturb the antioxidant defense responses and induce OS condition in cells. Intracellular nano-TiO_2 can affect the normal function of macromolecules through protein adsorption, encumbering various signaling pathways, and inserting themselves into DNA base pairs (Li *et al.*, 2010; Drevet *et al.*, 2012; Kim *et al.*, 2013). After distributing to different organelles, the change of mitochondria membrane permeability, membrane potential, DNA conformation, and cell cycle were shown with the destruction of cytoskeleton integrity, DNA double-strand damage, and chromosomal segregation (Tarantola *et al.*, 2009; Trouiller *et al.*, 2009; Zhao *et al.*, 2009; Li *et al.*, 2010).

septal thickening, etc (Hougaard *et al.*, 2010; Moon *et al.*, 2010; Silva *et al.*, 2013). For the brain, Nano-TiO_2 impaired spatial recognition memory behaviors of mice through disturbing the homeostasis of neurotransmitter system and trace elements (Hu *et al.*, 2010), and induced the changes of the gene expression associated with brain development, neuronal apoptosis, oxidative stress (OS), and inflammation after long-term exposure (Shimizu *et al.*, 2009). Nano-TiO_2 accumulated in the liver and led to focal hepatocytes apoptosis and hepatic dysfunction after i.g. administration (Hu *et al.*, 2010), after histopathological changes, such as loss of the sinusoid space, hydropic degeneration with minor fatty change, and inflammation (Chen *et al.*, 2009; Cui *et al.*, 2011). Saccus lymphaticus, proteinic liquids and dilatation in the renal tubular, and swelling in the renal glomerulus were shown after the accumulation of nano-TiO_2 in the kidney (Chen *et al.*, 2009; Wu *et al.*, 2009). More notably, nano-TiO_2, which came from the i.g. administration, can distribute into the reproductive system, leading to ovary injury and fertility reduction through the regulation of inflammation/follicular atresia-related cytokine expressions (Zhao *et al.*, 2013). Further, inhaled nano-TiO_2 enhanced prepulse inhibition of exposed female offspring and altered their neurobehavior (Hougaard *et al.*, 2010).

Ecotoxicity

Nano-TiO_2 can induce ecotoxicity owing to industrial processes and consumer goods, such as the use of food-grade nano-TiO_2 (Yang *et al.*, 2014) and wastewater disinfectant (Yang *et al.*, 2013). After NMs spread to water, nano-TiO_2 showed adverse effects on aquatic animals (Table 4), including high mortality of medaka (Ma *et al.*, 2012), changes in gene expressions about the ribosome structure in zebrafish gills (Griffitt *et al.*, 2009), severe growth retardation, mortality and reproductive defects in daphnia magna (Zhu *et al.*, 2010) and developmental neurotoxicity in zebrafish through phototoxicity after UV radiation (Wang *et al.*, 2014). For hydrophyte, nano-TiO_2 affected the functions of photosynthesis-related genes and increased ROS levels through UV radiation (Miller *et al.*, 2012; Simon *et al.*, 2013).

In addition, nano-TiO_2 can interact with other metals (e.g. Pb) and modify their bioavailability and toxicity in aquatic environments. Nano-TiO_2 exhibited synergistic effects with heavy metal ions (e.g. Zn^{2+}) to increase the phytotoxicity on the photosynthetic capacity and algae survival rate (Tang *et al.*, 2013). Compared with Pb treatment alone, the bioconcentration and toxicity of Pb were significantly increased when combined with nano-TiO_2 (0.1 mg l^{-1}) in zebrafish larvae, which led to the disruption of thyroid endocrine and the neuronal system through affecting gene transcription (Sun *et al.*, 2014).

Nano-TiO_2 present in the soil also affects the bacteria (Table 5) and terrestrial plants (Table 6). For soil bacterial communities, microbial biomass and diversity were reduced after nano-TiO_2 exposure, which may alter the composition and function of the bacterial community in ecosystems (Ge *et al.*, 2011; Ge *et al.*, 2013; Binh *et al.*, 2014). Nano-TiO_2 can be absorbed from soil by terrestrial plants as they grow, accumulating in stems, leaves and fruits through plant metabolic systems, and affecting the germination, root elongation of seed and seedlings (Song *et al.*, 2013a, 2013b). Certainly, to avoid the effects of the soil's properties and various components in it, hydrophonic culture was used in nanotoxicity assessment of plants, which suggested the more toxic be in water than in soil (Josko and Oleszczuk, 2014).

Considering the top position of people in the food chain, the ecological environment is necessary for humans (D'Agata *et al.*, 2014). As a result, ecotoxicity, induced by nano-TiO_2 exposure in the water and soil, will translate to cytotoxicity after the interaction between people and the surroundings.

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Potential mechanisms

In vitro, direct cell-TiO_2 NMs interactions can stimulate adverse effects, inducing ROS production, mitochondrial dysfunction, DNA damage, cell apoptosis, etc (Di Virgilio *et al.*, 2010; Ghosh *et al.*, 2010). *In vivo*, nano-TiO_2 can penetrate into different tissues (e.g. lung, liver, spleen and kidney), and affect the normal function of cells in tissues after complicated biodistribution, metabolism and clearance (Chen *et al.*, 2009; Wu *et al.*, 2009). The mechanisms of nanotoxicity at the cellular level are shown schematically in Fig. 1.

ROS generation and OS

As one of the widely accepted toxic mechanisms, the enhanced generation of ROS is shown after NMs exposure (Gonzalez *et al.*, 2008). The adverse effects of ROS production on cells have been reported for various NMs, including nano-TiO_2 (Long *et al.*, 2007). The accumulation of ROS can perturb the biological antioxidant defense responses (oxidant/antioxidant balance) (Xia *et al.*, 2004; Foster *et al.*, 2006). Owing to the increased ROS levels, NMs can deplete GSH (Sha *et al.*, 2013), dopamine (DA), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in a dose-dependent manner (Hussain *et al.*, 2006). The ROS generated by NMs exposure can rapidly damage biological targets (e.g. bronchial epithelial cells) (Bhattacharya *et al.*, 2009) and trigger the apoptotic process through activating cytosolic caspase-3 and chromatin condensation (Park *et al.*, 2008).

The imbalance between the production and detoxification of reactive oxygen will induce a state of OS in cells (Finkel and Holbrook, 2000). In the OS model, antioxidant defense, pro-inflammatory effects and cytotoxicity are three classes of OS aggravated by the incremental cellular responses under the production of ROS (Nel *et al.*, 2006; Meng *et al.*, 2009). Based on the published literature, the transcriptional activation of the antioxidant response element in phase II enzyme promoters can mediate the homeostatic redox balance pathway (Xia *et al.*, 2006). Then, mitogen-activated protein kinase (MAPK), AP-1 and nuclear factor kappa-B (NF-kB) signaling cascades can activate cytokine and chemokine expressions (Xiao *et al.*, 2003). The increased levels of transcription factor NF-kB and oxidant-dependent inflammatory signaling can induce the cellular inflammatory response when lungs are exposed to nano-TiO_2 (Moon *et al.*, 2010). Nano-TiO_2 can induce an increased level of IL-8 mRNA through both transcriptional and post-transcriptional pathways (Park *et al.*, 2008). Finally, mitochondrial perturbation mediates the toxic OS and activates cytotoxic cell death. Exposure of the cultured cells to NMs can induce the activity of OS-related genes, such as catalase and thioredoxin reductase (Park *et al.*, 2008), and cell apoptosis was triggered by the activation of caspase-8/Bid and the mitochondrial signaling pathways (Zhao *et al.*, 2009).

Organelle dysfunction

NMs are similar in size to many biological molecules and structures (~0.1 to 100 nm), enabling NMs to easily enter cells, organelles and functional biomolecular structures and interact with vital biological systems (Fischer and Chan, 2007). Most importantly, intracellular NMs may have observable toxicity because they are not bound to the cell membrane and interact with the macromolecule directly (Geiser *et al.*, 2005). For example, the adsorption of proteins onto the NMs surface can also change protein conformation (Lynch *et al.*, 2006). Consequently, the abnormal behavior of protein can encumber various signaling pathways, leading further to cell death (Berntsen *et al.*, 2010).

Mitochondria supply energy to cells and keep their survival (Calabrese *et al.*, 2001). After nano-TiO_2 exposure, mitochondrial membrane permeability experience significant change, which may lead to the release of cytochrome c from mitochondria to the cytosol (Zhao *et al.*, 2009). Mitochondrial perturbation, such as the loss of mitochondrial membrane potential and mitochondrial damage, leads to cell death (Hussain *et al.*, 2005).

Cytoskeleton (CSK) is a compact network of structural proteins and crucially important to intracellular transport and cellular division (Bursac *et al.*, 2005). An *in vitro* study suggested that NMs can affect cell viability through the destruction of CSK integrity (Tarantola *et al.*, 2009). During the process of cell penetration, NMs can change the normal function of the cytoskeletal network via caveolin, endocytosis and adhesive interactions (Rothen-Rutishauser 2009). After neonatal rat ventricular myocytes have been exposed to nano-TiO_2, the myofibrils showed a less organized structure than the non-exposed cells. TEM examinations revealed that nano-TiO_2- exposed cells exhibit thick electron dense Z-lines, compared with the control (Helfenstein *et al.*, 2008).

For cell nucleus, DNA damage caused by exposure to NMs is one of the key reasons behind the development of cell death and tumor. After the exposure of the liver cell to anatase nano-TiO_2, which inserted themselves into DNA base pairs or binds to DNA nucleotide, resulting in the change of DNA conformation (Li *et al.*, 2010). Nano-TiO_2 can cause the formation of gamma-H2AX foci and 8-hydroxy-2′-deoxyguanosine, inducing oxidative DNA double-strand damage in a mice model (Trouiller *et al.*, 2009). NMs also disturb cell-cycle progression and lead to further chromosomal segregation and cell transformation. After long-term exposure to nano-TiO_2, cultured fibroblast cells demonstrated increasing numbers of multinucleated cells and micronucleus, G2/M delay and slower cell division (Huang *et al.*, 2009).

Conclusions

In this paper, we detail the current knowledge of the adverse effects induced by nano-TiO_2. Based on the abundance of risk studies on nano-TiO_2, the detailed information, such as, evaluative object, physico-chemical properties of nano-TiO_2, exposure concentration, exposure duration and toxicity, were listed in Tables 1–6. However, there are limited informational values for nanotoxicity assessment in many published reports, and the lack of toxicity standard limits the deeper understanding of potential toxicity.

First, the physico-chemical properties of nano-TiO_2 were not described adequately. The crystal structure, shape, size, treatment media, zeta potential, aggregation and agglomeration tendency, and modification of nano-TiO_2 as the essential information were collected in our tables. Various forms of nano-TiO_2 led to different toxicity, just like the different responses of bronchial epithelial cells (BEAS-2B) as a model (Bhattacharya *et al.*, 2009; Falck *et al.*, 2009). Unfortunately, it is hard to compare the different adverse effects between various nano-TiO_2 mainly because of missing the essential description of nano-TiO_2 used.

Second, extremely high doses of nano-TiO_2 have been used in acute toxicity research, which may be impractical (Chen *et al.*, 2009). Animal experiments have enabled researchers to perform, replicate and quantify the adverse factors detected from *in vitro* studies, such as ROS, signal pathway activity, OS, cell cycle and inflammation (Poland *et al.*, 2008; Ryman-Rasmussen *et al.*, 2009). However, the adverse effects, as shown *in vitro* and *in vivo* after a

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high dose of nano-TiO_2 exposure, are not adequate to provide an accurate prediction of human body responses.

Third, to take into account the difference between exposure routes using *in vivo* studies is also important to identify and quantify the nanotoxicity. For example, the increased usage of nano-TiO_2 in cosmetics suggests that the skin is a route for human to get exposed to NMs. Wu *et al.* (2009) found that nano-TiO_2 penetrated through the skin into different tissues and induced diverse pathological lesions after 60 days exposure in hairless mice. In contrast, Adachi *et al.* (2013) found no evidence of nano-TiO_2 skin penetration after 56 days exposure of NMs emulsion to the hairless rat. To avoid the difference of NMs and species, the meaningful risk assessments should be the epidemiological study of heavily-exposed populations, which can reflect the real effects through the respiratory system after nano-TiO_2 exposure (Warheit, 2013). For example, based on the available data and hygiene standards, Swidwinska-Gajewska *et al.* (2014) concluded that 0.3 mg m^{-3} nano-TiO_2 is the maximum admissible concentration in the workplace in Poland. In addition, more experiments on inhalation exposure are needed to measure the relevance of risk effects for human.

Based on the nanotoxicity assessment studies, nano-TiO_2 induced health risks, such as cytotoxicity, ecotoxicity, phototoxicity and phytotoxicity, as caused by the generation ROS and OS, organelle dysfunction, etc. Actually, the life cycles of nano-TiO_2 can be determined with the development of the nano-risk framework, the adverse effect of nano-TiO_2 will effectively decrease with the improvement of strict preventive procedures if consistent definition and standard of low toxicity is established. Better understanding of the toxicity of nano-TiO_2 holds promise for strict prevention of exposure to NMs, and will facilitate the use of the superior power of these double-edged weapons.

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Conflict of interest

None of the authors has any conflicts of interest related to this manuscript.

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