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Rethinking Nano-TiO_2 Safety: Overview of Toxic Effects in Humans and Aquatic Animals

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Titanium dioxide nanoparticles (nano-TiO_2) are widely used in consumer products, raising environmental and health concerns. An overview of the toxic effects of nano-TiO_2 on human and environmental health is provided. A meta-analysis is conducted to analyze the toxicity of nano-TiO_2 to the liver, circulatory system, and DNA in humans. To assess the environmental impacts of nano-TiO_2, aquatic environments that receive high nano-TiO_2 inputs are focused on, and the toxicity of nano-TiO_2 to aquatic organisms is discussed with regard to the present and predicted environmental concentrations. Genotoxicity, damage to membranes, inflammation and oxidative stress emerge as the main mechanisms of nano-TiO_2 toxicity. Furthermore, nano-TiO_2 can bind with free radicals and signal molecules, and interfere with the biochemical reactions on plasmalemma. At the higher organizational level, nano-TiO_2 toxicity is manifested as the negative effects on fitness-related organismal traits including feeding, reproduction and immunity in aquatic organisms. Bibliometric analysis reveals two major research hot spots including the molecular mechanisms of toxicity of nano-TiO_2 and the combined effects of nano-TiO_2 and other environmental factors such as light and pH. The possible measures to reduce the harmful effects of nano-TiO_2 on humans and non-target organisms has emerged as an underexplored topic requiring further investigation.

1. Introduction

Titanium dioxide nanoparticles (nano-TiO_2, < 100 nm) are widely used, in industry, technology, and consumer products[1,2] due to their desirable physicochemical characteristics including large specific surface area, high reactivity and photocatalytic activity, ultraviolet (UV) shielding function as well as unique quantum and electron-tunneling effects.[3] Cosmetics and personal care products (such as the sunscreens and toothpastes) account for >50% of the nano-TiO_2 use.[4] Humans are thus increasingly exposed to nano-TiO_2 through skin penetration, ingestion and inhalation.[5] Furthermore, nano-TiO_2 is released into the environment either directly from loss during production and use, or indirectly via sewage sludge and the effluent of waste water treatment plants.[4,6] Aquatic environments including rivers, lakes, estuaries and coastal zones receive a large fraction (≈20–35%) of the environmental load of nano-TiO_2.[4] Recent estimates predict high concentrations of nano-TiO_2 in the coastal waters, up to 16.8 μg L^{-1} in European waters and up to 103 μg L^{-1} in San Francisco Bay[7] and even higher levels in the sediment.[8] In summer, the concentrations of nano-TiO_2 may exceed 900 μg L^{-1} in the surface water near popular beaches.[9] As a result, nano-TiO_2 can affect the health of humans directly through the occupational exposures and use of nano-TiO_2-containing

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products, and indirectly through environmental exposure to unintentionally released nano-TiO_2.[1,10] Nano-TiO_2 can also have impact on aquatic organisms exposed to increasing levels of nanoparticles in water and sediments.

Given the high volume of production and release of nano-TiO_2, its safety is of major concern for human and ecosystem health. Although nano-TiO_2 was originally classified as biologically inert material, there is a growing body of evidence concerning the toxicity of nano-TiO_2 to humans and non-target organisms.[5,11] Several recent reviews provide an excellent overview of the mechanisms of nano-TiO_2 toxicity and highlight the potential for adverse health effects of nano-TiO_2 requiring further research and improved regulatory practices.[10,12–15] However, a comprehensive assessment of the adverse impacts of nano-TiO_2 requires quantitative approaches that can objectively summarize the results of multiple studies and assess the relative importance of different biological responses in the context of the environmentally relevant nano-TiO_2 exposures. Meta-analysis and bibliometric analysis can provide such quantitative approaches. Meta-analysis is a statistical tool that systematically assesses the results of multiple independent studies, determines effect sizes for the studied response variables and allows generalizations about the available findings in a certain research area, such as nano-TiO_2 toxicity. Bibliometric analysis uses statistical methods to visually analyze a body of published research and determine the research hot spots.[16–18]

Here, we provide a systematic review on the toxicity of nano-TiO_2 toxicity including acute studies focusing on elucidation of the toxic mechanisms, as well as the studies conducted at environmentally relevant concentrations to assess the potential risk of nano-TiO_2 to aquatic organisms.

We also present the results of a meta-analysis to systematically review the possible health hazards from nano-TiO_2 to humans using studies on humans and animal models. Bibliometric analysis encompassing studies in humans, mammalian models and non-target aquatic organisms is used to evaluate the structure of the current body of research on nano-TiO_2 toxicity, to identify the best studied areas, and reveal the knowledge gaps urgently requiring further investigation. The summary of the data used in this review is available in Supporting Information.

2. Methods

2.1. Meta-Analysis

For studies on humans and model organisms, the information on nano-TiO_2 was extracted from articles published from 2006 to 2019 either manually (if presented in the tables) or using Plot Digitizer 2.6.8 (available in http://plotdigitizer.sourceforge.net/) to extract data from figures.

Stata 15.1 (available in www.stata.com) was used to conduct meta-analysis, and the random-effect model[19] was chosen. Unstandardized mean difference between the experimental and control group was used to calculate the effect size as follows:

$$D = \overline{x_1} - \overline{x_2} \tag{1}$$

$$Var(d) = \frac{s_1^2(n_1-1)+s_2^2(n_2-1)}{(n_1+n_2-2)} \times \frac{n_1+n_2}{n_1 n_2} \tag{2}$$

where D is the effect size, $Var(d)$ is the variance of D, $\overline{x_1}$ and $\overline{x_2}$ are the means of outcomes of treatment and control group, respectively; s_1 and s_2 are the standard deviation of outcomes of treatment and control group, s_2 respectively, and n_1 and n_2 are the sample sizes of treatment and control groups, respectively.

The effect size of each study was used to calculate the effect size of the population, θ_{pop},Using the random-effect model assuming that the study effect sizes are different and the collected studies represent random samples from a larger population of studies. θ_{pop} was calculated as follows:

$$V_{D_i}^* = Var(d)_i + T^2 \tag{3}$$

$$W_i^* = \frac{1}{V_{D_i}^*} \tag{4}$$

$$\theta_{pop} = \frac{\sum_{i=1}^{k} W_i^* V_{D_i}^*}{\sum_{i=1}^{k} W_i^*} \tag{5}$$

where $Var(d)_i$ is the variance of D of each experiment, W_i^* is named as weight, k is the total number of experiments, T^2 represents the between-study variability. The 95% of confidence interval (95%CI) of effect size was calculated as follows:

$$V_{\theta_{pop}} = \frac{1}{\sum_{i=1}^{k} W_i^*} \tag{6}$$

$$S_{\theta_{pop}} = \sqrt{V_{\theta_{pop}}} \tag{7}$$

$$95\%CI = \left[\theta_{pop} - 1.96 \times S_{\theta_{pop}}, \theta_{pop} + 1.96 \times S_{\theta_{pop}}\right] \tag{8}$$

where $V_{\theta_{pop}}$ is the variance of θ_{pop}, $S_{\theta_{pop}}$ is the standard deviation of θ_{pop}.

The selection criteria for inclusion of the articles in meta-analysis were:

1) The sample size, mean and standard deviation of the experimental group and the control group can be obtained from the article.
2) The more effect sizes of an observed value are obtained, the closer the calculated population effect size is to the real value. If we cannot get a sufficient number of observation values to calculate an effect size, such observation value is not suitable for meta-analysis.
3) The units of the observed variables in each study must be consistent and interconvertible into the same units between different articles.

Because of the relatively small number of published studies on nano-TiO_2 toxicity that met these stringent criteria, the scope of the present meta-analysis was limited (Supporting Information). And the animals and exposure methods of the studies used in **Figures 1–3** are shown in Supporting Information. Therefore, to complement the meta-analysis,

bibliometric analysis was used to determine the main foci of the current research on nano-TiO_2, to identify the gaps in the authors' knowledge and to propose future research directions to strengthen the field.

2.2. Bibliometric Analysis

A search was conducted in the Web of Science using the key words "nano-TiO_2" and "titanium dioxide nanoparticle" in the titles, key words and abstracts across all publication years. It is worth noting that this search is unlikely to be exhaustive due to the lack of the unified nomenclature for nano-TiO_2. Nevertheless, a total of 49 948 articles were found and exported from Web of Science (Supporting Information) into VOSviewer 1.6.11 (available in www.vosviewer.com) to build bibliometric maps of hot keywords. The number of occurrences of a keyword was shown as the dot size and the co-occurrence between hot keywords as line links in the maps. The minimum number of occurrences of a keyword was set to five and all co-occurrence links received the same weight. The range of the publication year of the data was color coded by VOSviewer. Further information and the data to explore the bibliometric maps in VOSviewer in more details can be found in Supporting Information.

3. Brief Description of Nano-TiO_2 Properties

Nano-TiO_2 has a high photocatalytic potential because of its high surface area and unique physicochemical properties.[20] Brookite, anatase and rutile are the main polymorphs of nano-TiO_2.[20,21] Anatase and rutile are often used for nanotoxicology research, so that most studies in this review focus on these polymorphs. Nano-TiO_2 anatase has more oxygen vacancy defects and therefore higher photocatalytic activity than rutile.[22] For the detailed discussion of the photocatalytic properties of different nano-TiO_2 polymorphs, we refer the reader to excellent reviews by Friehs et al.[20] and Schneider et al.[23] The particle size can also affect the nano-TiO_2 properties, and the decrease of nano-TiO_2 sizes can enhance photoredox reactions.[24] Furthermore, nano-TiO_2 is commonly surface-modified, causing a change in its properties. Binary silica (SiO_2) and alumina (Al_2O_3) compounds have been applied to promote the dispersion of nano-TiO_2 and increase UV protection of the clear polyacrylic composite.[25] TiO_2 nanoparticles used in sunscreens are usually coated with silica and alumina.[26]

4. Results and Discussion

4.1. Toxic Effects of Nano-TiO_2 in Humans and Model Vertebrates

Nano-TiO_2 can enter the human body via ingestion (as a common food additive), inhalation, or through dermal penetration of nano-TiO_2-containing cosmetic products. Dietary nano-TiO_2 intake is a significant contributor to human exposures, with children consuming from ≈2- to 11-fold more nano-TiO_2 per kg body mass than adults.[27,28] While the absorption efficiency of nano-TiO_2 via the oral route is low in humans (≈0.02%), Ti is poorly eliminated and can accumulate over the lifetime causing effects on multiple organs.[27] The human exposure to nano-TiO_2 through inhalation has not yet been sufficiently quantified, but studies with animal models indicate that this intake route poses high health risks due to the direct intake of the nano-TiO_2 by the respiratory epithelia and transport to lungs, brain, and other vital organs.[29] Transdermal absorption is not considered a major exposure route for humans because nano-TiO_2 does not penetrate the deeper layers of skin.[30]

4.1.1. Neural System

In Vivo Effects: Nano-TiO_2 can cause neurotoxicity by crossing the blood–brain barrier or entering the brain via axonal translocation through the nose-to-brain pathway.[31] Once the nano-TiO_2 is translocated into the central nervous system (CNS), it is slowly eliminated and therefore accumulates. It subsequently causes pathological changes, such as inflammation, immunological response, edema, cell necrosis or cell injury, and can ultimately lead to neurodegenerative diseases and psychiatric disorders.[31]

The efficiency of nano-TiO_2 uptake in the brain through inhalation depends on the size, shape and the surface properties of the nanoparticles.[32] For example, intranasal instillation of the hydrophilic silica-coated nano-TiO_2 led to high accumulation of Ti in the olfactory bulb and most brain regions of mice, and this accumulation was higher than during instillation of other forms of nano-TiO_2.[33] Accumulation of Ti was associated with neuronal loss and damage, increased content of glutamic acid, decreased monoamine neurotransmitters and enhanced oxidative stress.[32,33]

Maternal exposures to nano-TiO_2 can strongly influence the fetal brain development and function since nano-TiO_2 can cross the placental barrier.[31] Nano-TiO_2 reduced cell proliferation in the hippocampus and impaired their memory and learning ability in the offspring in the exposed pregnant Wistar rats.[34] This impairment can be attributed to the excessive autophagy and apoptosis leading to suppressed dendritic outgrowth of the hippocampal neurons.[35] Similarly, oral exposure of female mice to nano-TiO_2 during pregnancy and lactation resulted in thinning of cerebral and cerebellar cortex, loss of neurons, edema, and nuclear condensation, dysplasia of neurites in hippocampal pyramidal cells, thinning in pyramidal cell layer in hippocampus, and decrease in learning and memory of offspring mice.[36]

In Vitro Cytotoxicity to CNS Cells: Cytotoxicity of nano-TiO_2 to the brain is commonly studied using the PC12 cell line as an in vitro model of dopaminergic neurons.[37] The nano-TiO_2 anatase particles (20-40 nm) showed concentration- and time-dependent toxic effects on PC12 cells. At high concentrations of up to 100 mg L^{-1} the nano-TiO_2 particles induced oxidative stress, dysfunction of the protein quality control systems and increased apoptosis in PC12 cells. Free radical scavengers such as *N*-(mercaptopropionyl)-glycine or *N*-acetylcysteine mitigated these harmful effects.[38,39] High concentrations of nano-TiO_2 decreased viability and intracellular dopamine levels in ref. [40], but concentrations < 1 mg L^{-1} had no effect on viability or levels

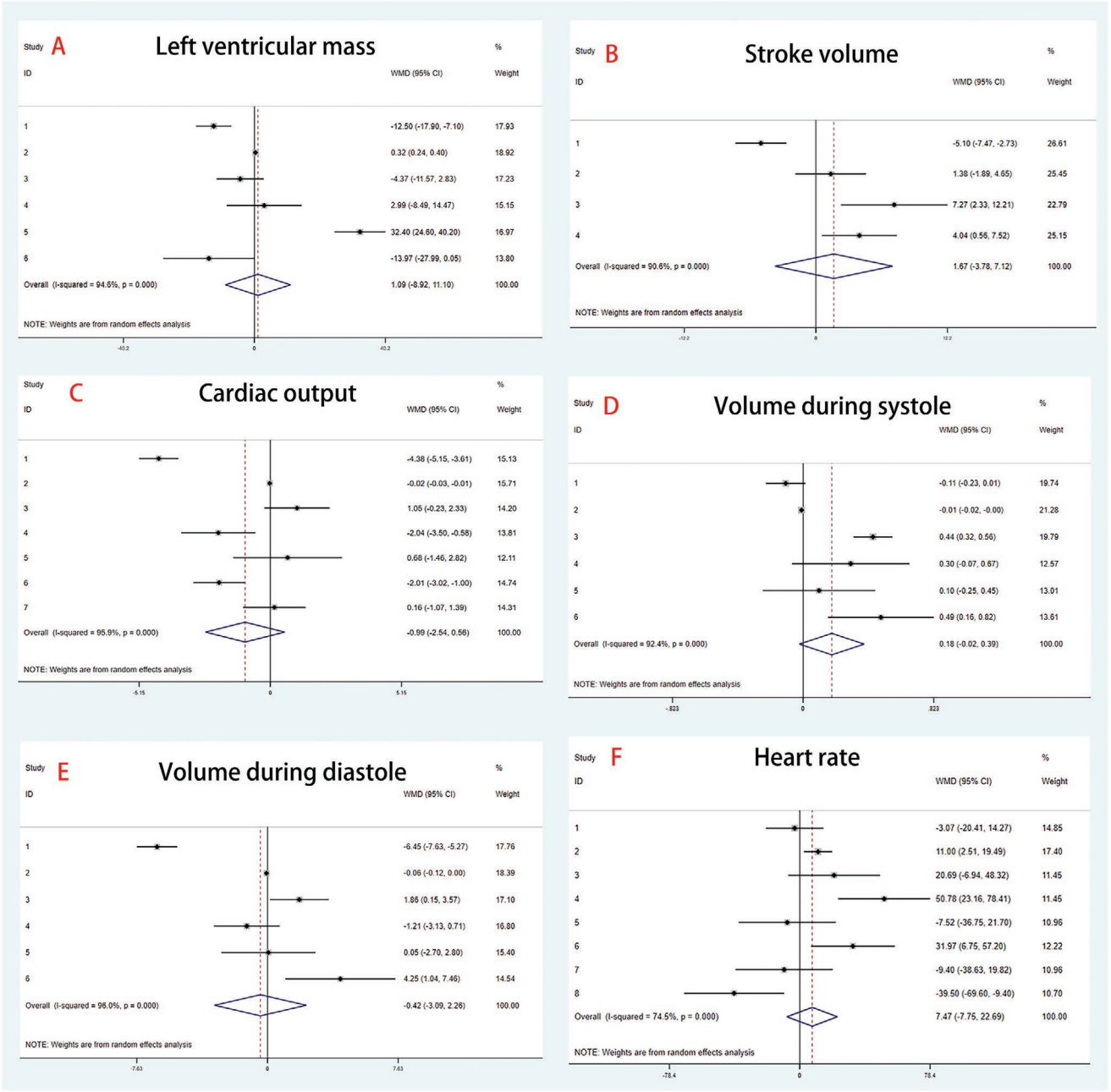

Figure 1. Meta-analysis of parameters of circulatory system. Nano-TiO_2 effects on heart functions: A) left ventricular mass, B) stroke volume, C) cardiac output, D) volume during systole; E) volume during diastole, F) heart rate. The dot represents the effect size of each study. The line through the dot represents 95%CI of the effect size. The square represents the weight, W_i^*. The bigger the square, the bigger the weight. The broken line represents the effect size and the horizontal length of the diamond the 95%CI of the effect size for all studies combined.

of cell injury in PC12 cells.[41] Furthermore, a high concentration of nano-TiO_2 particles (up to 200 mg L^{-1}) elevated the levels of α-Synuclein (α-Syn), and caused a concentration-dependent aggregation of α-Syn, a phenotype commonly found in Parkinson's disease.[39] Although the high concentrations of nano-TiO_2 (≥100 mg L^{-1}) used in these studies are likely not relevant physiologically, the findings of these acute in vitro exposures shed light on the cytotoxic mechanisms of nano-TiO_2 in the brain. Interestingly, long-term exposure to low concentration of nano-TiO_2 (1 mg L^{-1}) led to a decline in the cell numbers and total cell length of PC12 cells, indicating that non-cytotoxic concentrations of nano-TiO_2 might impair cell proliferation and suppress neurite outgrowth.[41]

In vitro studies indicate that high levels of nano-TiO_2 can inhibit the growth of neuroblastoma in the brain. Exposure to > 10 mg L^{-1} of nano-TiO_2 for 72 h suppressed viability and induced autophagy and apoptosis of human neuroblastoma cell line due to the elevated oxidative stress.[42,43] Cytotoxicity and induction of apoptosis have also been found in the human microglia N9 cells exposed to nano-TiO_2.[44] The uptake and

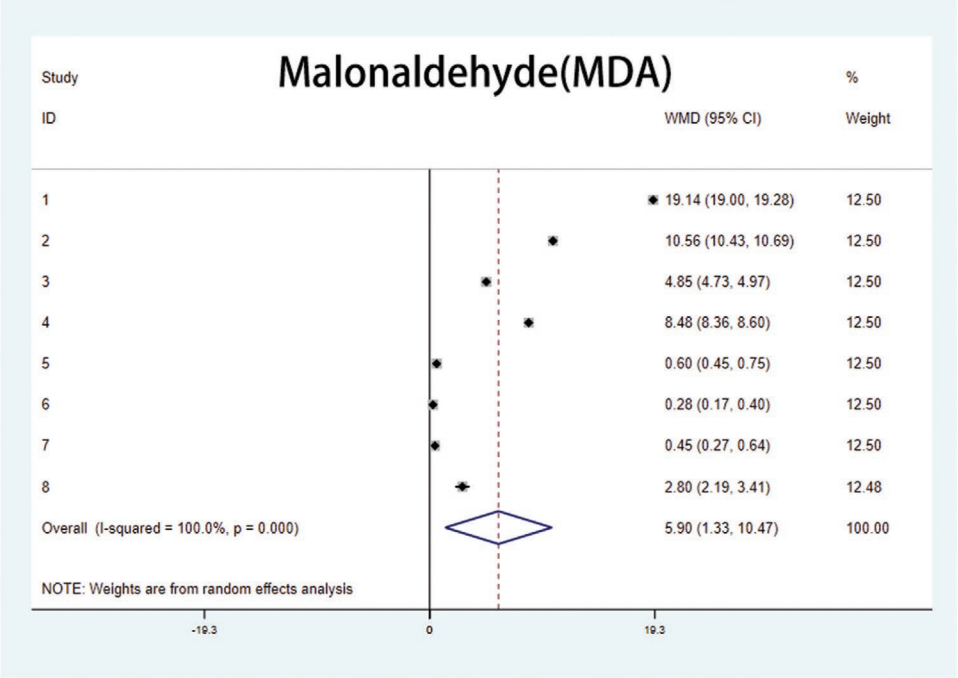

Figure 2. Meta-analysis of Malondialdehyde (MDA) in liver. The dot represents the effect size of each study. The line through the dot represents 95%CI of the effect size. The size of the square represents the weight, W_i^*. The broken line represents the effect size and the horizontal length of the diamond the 95%CI of the effect size for all studies combined.

internalization of nano-TiO_2 by glial cells inhibited proliferation, disturbed the mitochondrial production of ATP and stimulated brain microglia to produce reactive oxygen species (ROS).

It is worth noting that nano-TiO_2 has positive effects on some types of brain cells even though it is toxic to the mature neurons. Thus, exposure of the mouse neural stem cells to 200 mg L^{-1} silica-coated nano-TiO_2 for 7 days led to a prominent increase in the β-tubulin positive cells.[45] This indicated that the nano-TiO_2 could induce the C17.2 differentiate into neurons. Future studies in this area could provide important tools for local manipulation of the brain cell differentiation and viability and facilitate research of the nervous system.

Taken together, these studies indicate that nano-TiO_2 exposure has the potential to cause neurotoxicity in the exposed organisms and their offspring, especially when administered orally or through inhalation. Thus, the neurotoxicity mechanisms of nano-TiO_2 are of particular concern for the occupational exposures that commonly involve exposures to high levels of nano-TiO_2 through the (most dangerous) inhalation route.[29] However, certain doses of nano-TiO_2 particles might be beneficial for the treatment of neuroblastoma and have some positive effects on the brain. The assessment of the associated health risks due to the routine exposures is difficult due to the lack of the clear understanding about the levels of exposure and accumulation of nano-TiO_2 throughout the life time, which require further investigations.

4.1.2. Circulatory and Cardiovascular System

Exposures of model vertebrates to nano-TiO_2 through injection or inhalation show that the nanoparticles can enter the vital organs such as heart, liver and brain through the circulatory system.[46–48] Furthermore, during the long-term exposures nano-TiO_2 can translocate among organs and pass through the blood-brain and blood-heart barrier, as was shown in zebra fish.[37] Our meta-analysis indicates that nano-TiO_2 particles have negative but life-stage specific effects on the vascular and cardiac functions, and that at least some of these effects might be mediated by a mitochondrial regulator microRNA-378a.

In mammals, inhalation of nano-TiO_2 (≈21 nm) results in cardiopulmonary impairment and negative effects on microcirculation as a result of oxidative damage and inflammation.[49–54] Nano-TiO_2 impairs endothelium-dependent vasodilation in subepicardial arterioles blunting response to acetylcholine and impairing flow-induced dilation.[51] The vasodilatory response of the aorta was less sensitive to nano-TiO_2.[55] Mitochondrial dysfunction caused by nano-TiO_2 contributes to cardiac dysfunction, partially mediated by overexpression of microRNA-378a.[56] Residing within the first intron of the PGC-1b,[56] microRNA-378a acts as a negative regulator of mitochondrial oxidative metabolism and mitochondrial biogenesis pathways.[57] The expression of MiRNA-378a increased after inhalation of nano-TiO_2, and the MiRNA-378a knock-out showed a cardioprotective effect.[55] According to the meta-analysis, the left ventricular mass of mice increased slightly under TiO_2 exposure based on the effect size calculated across all studies (Figure 1A). MiRNA-378a knock-out mice showed a more significantly increased stroke volume than heterozygous knockout mice (Figure 1B). Similar to inhalation, oral exposure to nano-TiO_2 resulted in cardiac injury, decreased the heart rate and systolic blood pressure, and increased the diastolic blood pressure in rats.[58]

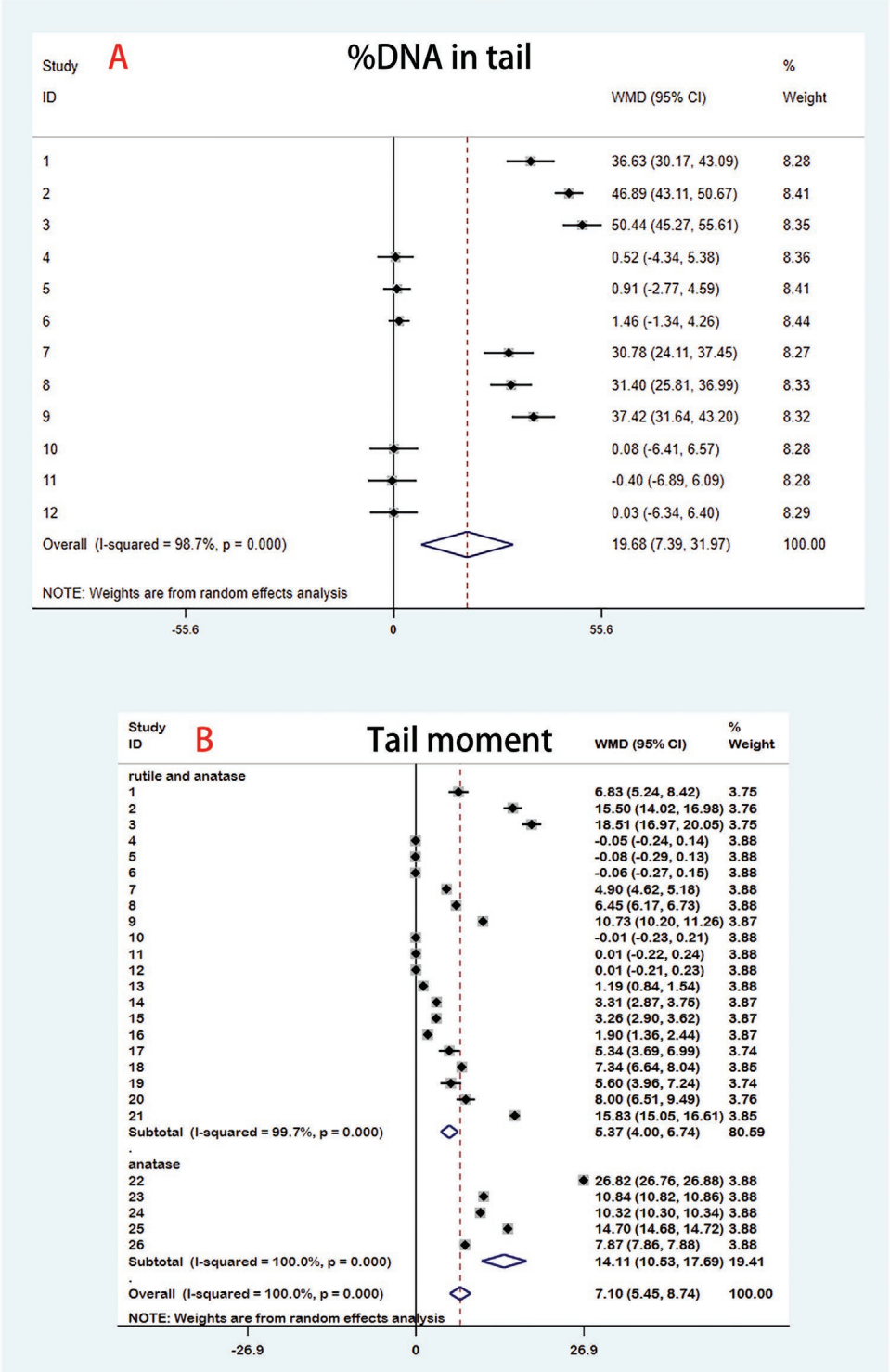

Figure 3. Meta-analysis of parameters of DNA. A) the percentage of DNA in tail. B) tail moment. The dot represents the effect size of each study. The line through the dot represents 95%CI of the effect size. The size of the square represents the weight, W_i^*. The broken line represents the effect size and the horizontal length of the diamond the 95%CI of the effect size for all studies combined.

The meta-analysis showed that nano-TiO_2 exposure via different routes tend to reduce the cardiac output albeit the effect depends on the life stage and the genetic background (Figure 1C). Thus, fetal mice (prenatally exposed to ≈10 mg m^{-3} of nano-TiO_2[54,59]) showed negligible change in cardiac output. In adult isogenic[59] and heterozygous mice,[56] nano-TiO_2 caused more significant increased cardiac output, whereas in adult wild type and MiRNA-378a knockout mice the cardiac

output notably decreased in response to nano-TiO_2 inhalation (Figure 1).

The meta-analysis indicates that exposure to nano-TiO_2 might increase the heart volume during systole and decrease the volume during diastole but has no significant effect on the heart rate (Figure 1D–F). Some variability of response among the life stages was also evident. For example, the heart volume during systole decreased in response to nano-TiO_2 in pregnant females but not in fetal mice (Figure 1D). In mice, exposure to 10 mg cm^{-3} TiO_2 aerosols causes a significant but transient increase in the stroke volume at 3 h that declines again at 4 h, indicating the adjustment of heart to nano-TiO_2 exposure (Figure 1B). Intraperitoneal (IP) injections of nano-TiO_2 in rodents led to pathological changes in myocardium, and blocked microcirculation.[13] The negative impacts of nano-TiO_2 IP injections on the blood (such as increased oxidative stress, depletion of antioxidants, and lysis and agglutination of erythrocytes) might play a role in the cardiovascular dysfunction induced by nano-TiO_2.[60–62] Furthermore, nano-TiO_2 is cytotoxic to endothelial cells and, to a lesser degree, smooth muscle cells causing oxidative stress, endoplasmic reticulum stress, inflammation and cell loss. This might contribute to the vasculatory disorders during nano-TiO_2 exposures.[13,63] Impairment of the endothelium-dependent vasodilation in the fetal aorta, the coronary arterioles and mitochondrial dysfunction have also been found in the offspring of the nano-TiO_2-exposed female rats.[64,65] The cardiac contractile dysfunction in the offspring of mice gestationally exposed to nano-TiO_2 was a result of the oxidative stress and inflammation in the heart as well as the direct effects on the fetal vasculature.[59,65]

4.1.3. Hepatotoxicity

General Hepatotoxicity and Effect on Metabolism: Translocation of nano-TiO_2 into the liver during inhalatory or oral exposure, as well as during experimental injections, results in hepatotoxicity. When exposed to nano-TiO_2 orally, through IP, or intravenous (i.v.) injections, indicators of the liver (assessed by an increase of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in blood plasma[48]) increased in a concentration-dependent manner in the serum of mice.[37,66,67] Mice IP-treated with a high dose of nano-TiO_2 (2592 mg kg^{-1} body weight) showed anorexia, diarrhea, lethargy, tremor, body weight loss and lusterless skin, indicating acute hepato- and gastrointestinal toxicity.[37] Similar signs were initially found mice exposed to the medium-dose (324 mg kg^{-1} body weight) but these signs gradually disappeared indicating adjustment to the mild toxic stress.[37] Chronic oral exposures to less than 50 mg kg^{-1} nano-TiO_2 for 90 days caused slight hepatotoxicity in rats indicated by elevated serum levels of albumins and globulins (but no increase ALT or AST) in the plasma.[68]

Nano-TiO_2 exposure can alter liver metabolism. Following oral exposure (50 mg kg^{-1} dose), nano-TiO_2 particles were reported to cluster together in the hepatocytes and alter the expression of the metabolic genes of liver in mice.[69] Levels of mRNA encoding the organic anion transporting polypeptide Oapt1 increased by > 7-fold and elevated mitochondrial numbers and swelling of the endoplasmic reticulum were found in most liver cell types.[69] Metabolomics studies in the rats orally exposed to 50 mg kg^{-1} nano-TiO_2 showed significant changes in the pathways involved in the metabolism of amino acids in the liver including alanine, aspartate, D- and L- glutamate and D-glutamine.[68]

Oxidative Stress: Oxidative stress (i.e., misbalance between ROS production and antioxidant defense and the resulting damage to proteins, lipids and DNA) might contribute to nano-TiO_2-induced liver injury. In mammals, nano-TiO_2 exposure results suppressed antioxidant levels in the liver in a concentration-dependent manner. Thus, activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), as well as the levels of glutathione (GSH) significantly decreased in response to a higher dose (>25 mg kg^{-1} body weight) of nano-TiO_2 via IP injection, while the low dose (5 mg kg^{-1}) had no effect.[37,66] After oral administration of 10–50 mg kg^{-1} of nano-TiO_2, SOD and GPx decreased in first 30 days but increased after 90 days.[67,68] However, the elevated levels of SOD and GPx after the long-term exposure failed to restore the normal redox status as indicated by a depletion of GSH and elevated levels of oxidized to reduced glutathione.[68]

Malondialdehyde (MDA) is a common product of lipid peroxidation and indicator of oxidative membrane damage. In mammals, nano-TiO_2 exposure induces a significant increase of MDA in liver according to the meta-analysis (Figure 2). Thus, chronic nano-TiO_2- oral-exposure caused accumulation of MDA in the liver of rats.[68] The exposure of 150 mg kg^{-1} body weight TiO_2 for 2 weeks induced a significant increase of MDA in liver while the exposure to 64 mg kg^{-1} body weight of nano-TiO_2 per day for 28 weeks induced slight increase of MDA in liver (Figure 2). Intratracheal instillation to 3.5–17.5 mg kg^{-1} body weight of nano-TiO_2 on alternate days for 5 weeks had little impact on the MDA level in the liver (Figure 2). Vitamin E, carnosine, and idebenone can alleviate the nano-TiO_2-induced increase of MDA (Figure 2).

Elevated oxidative damage in the livers of nano-TiO_2-exposed animals can lead to increased cell death through apoptosis and necrosis, which in turn can induce systemic inflammation. Thus, in mice intratracheally instilled with a single dose of 0.162 mg nano-TiO_2, eosinophilic necrosis of single hepatocytes was observed near central venules.[70] During chronic oral exposures of mice to nano-TiO_2 (50 mg kg^{-1} daily for 90 days) an increase in the levels of inflammatory cytokines IL-1α, IL-4, and TNF was found[68] whereas a shorter (14 days) exposure to high nano-TiO_2 doses (250 and 500 mg kg^{-1} daily) did not upregulate transcription of the inflammatory cytokines in mice.[69] In the latter study, no increase in the mRNA levels of apoptotic genes BAC, Bcl-xl, Bcl-2, and BIM was found in the liver, consistent with the lack of the inflammatory response.[69] However, longer oral exposures (30 days at 10–50 mg kg^{-1} daily) or i.v. injection (25–50 mg kg^{-1}) led to a strong upregulation of apoptotic gene expression and in increase in the percentage of apoptotic cells in the liver of exposed rats.[66,67]

4.1.4. Respiratory System

Current studies show that inhalation of nano-TiO_2 increases the potential pulmonary health risks. The effects of nano-TiO_2

on the respiratory system varied with the concentration, particle size, exposure time, and particle surface area. Histopathological analyses show that lower dose of nano-TiO_2 caused the changes of lung tissues while higher dose led to the accumulation of the particles in the lung. In mice, a low dose of nano-TiO_2 (0.5 mg kg^{-1}) led to aggregation and accumulation of lymphocytes and macrophages, induced pulmonary emphysema and disruption of alveolar septa whereas exposure to a higher dose (4 mg kg^{-1}) led to thickening of the alveolar wall, collapse of terminal bronchioles and interstitial thickening.[71] Similarly, female mice receiving a single intratracheal instillation of 18 μg rutile nano-TiO_2 showed no signs of pulmonary neutrophilic inflammation[72] . As the exposure dose increased to 32 mg kg^{-1} of nano-TiO_2, infiltration of inflammatory cells into the lung was observed in mice.[71] Furthermore, lactic dehydrogenase (a general marker of cell injury), alkaline phosphatase (a marker of type II epithelial cell toxicity) and gamma-glutamyl transpeptidase (a marker for damage to Clara and type II epithelial cells) all increased after nano-TiO_2 exposure (≥50 mg m^{-3}, ≥20.80 mg m^{-2}, ≥500 mg kg^{-1} body weight,)[73–76] indicating lung injury.[73]

Respiratory function, breathing rate, and specific airway resistance were not significantly altered at lower doses (314 and 826 mg m^{-3}) of nano-TiO_2 treatments administered for 4 h/day for 2 days while breathing rate was significantly increased under the same exposure regime at 3638 mg m^{-3} dose of nano-TiO_2.[77] These results indicate that the toxicity of nano-TiO_2 on the respiratory system is concentration-dependent, but the currently available data are insufficient for establishing a safe exposure threshold. Interestingly, the exposure time was found to be not an important factor affecting the toxicity of nano-TiO_2, with short-term inhalation showing similar respiratory toxicity with 90-days exposures.[73]

Nano-TiO_2 can enter the blood circulation and lymphatic circulation in the lung interstitium.[75] However, no associations between the pulmonary TiO_2 exposure and vasodilatory dysfunction has so far been found. Nano-TiO_2 exposure caused a modest increase in plaque progression in the aorta, but no change in the vasodilatory functions in mice lung tissue.[55] The mice exposed to fine and photocatalytic TiO_2 did not show altered vasodilatory function or lung tissue inflammatory gene expressions.[55]

The effect of nano-TiO_2 on lung functioning is affected by the particle surface coating and particle size. Pure nano-TiO_2 caused greater inflammation than nano-TiO_2 embedded in a paint matrix.[78] The size (and thus the specific surface area) of nano-TiO_2 is a key factor in determining the toxicity of nanoparticles to the respiratory system. Thus, the inflammatory response of mice measured as neutrophil influx was larger during exposures to 10.5 nm nano-TiO_2 compared to 38 nm nano-TiO_2.[70] In a study by Sager et al.,[74] differences in pulmonary inflammation were also observed between groups of rats intratracheally instilled with two different sizes of TiO_2 particles until 42 days post-instillation. In rats exposed by intratracheal instillation to various doses of TiO_2, smaller particles induced greater inflammation in the lungs than the larger sized nano-TiO_2 immediately after exposures.[74,79] However, after >1 week post-instillation, pulmonary inflammation was remarkably decreased in all the TiO_2 particle-exposed groups regardless of particle size,[79] showing progressive adjustment.

4.1.5. Genotoxic Effects

Extensive dose-dependent DNA damage was observed in the liver of Wistar rats exposed to nano-TiO_2 through caudal vein injection, including double strand breaks and DNA misrepair.[66] IP injection of 50 mg kg^{-1} of nano-TiO_2 and intratracheal instillation at any tested doses showed a high rate of DNA damage.[70,80] DNA comet assay is a common way to assess the DNA damage by measuring the percentage of DNA in the comet tail and the tail momentum. Meta-analysis shows that nano-TiO_2 can cause a significant increase of percentages of DNA in the tail (Figure 3A) and the tail momentum (Figure 3B). Antioxidants could partially alleviate the nano-TiO_2 induced DNA damage assessed by the comet assay. Thus, chlorophyllin, a potent antioxidant, prevented the DNA damage caused by nano-TiO_2 whereas vitamin E, carnosine, and idebenone mitigated but could not fully prevent the genotoxic effects of nano-TiO_2 (Figure 3A,B). Interestingly, the composition of nano-TiO_2 appeared to affect its genotoxicity as the nanoparticles containing only anatase promoted a greater increase of DNA tail moment than those containing anatase and rutile (Figure 3B).

The expression levels of genes for DNA damage sensing and DNA repair are commonly up-regulated during TiO_2 exposure.[81] Thus, nano-TiO_2 anatase/rutile mixture activated the p53-mediated DNA damage checkpoint signals in lymphocytes.[82] In NIH 3T3cells and human fibroblast HFW cells exposed to nano-TiO_2 concentrations from 0.0005 to 100 mg L^{-1}, polo-like kinase 1 and the DNA damage checkpoint was activated thereby affecting mitotic progression.[83] This effect was likely related to intercalation of nano-TiO_2 anatase into DNA base pairs and/or binding to nucleotide to alter the conformation of DNA.[84]

4.2. Toxicity of Nano-TiO_2 in Aquatic Animals

4.2.1. The sources and Fate of Nano-TiO_2 in the Aquatic Environment

Engineered TiO_2 nanoparticles are released into the aquatic environment from multiple point- and non-point sources.[4,85] The fate of nano-TiO_2 in the aquatic environment depends on their aggregation and sedimentation rates, transport with water and sediments and interactions with the living and non-living components of the ecosystem[85–87] (Figure 4). Salinity and pH (and more generally the presence of cations) as well as organic matter (including humic acids, dissolved organic matter (DOM) and particulate (POM) organic matter) may strongly affect the aggregation and sedimentation rates of nano-TiO_2.[85,88,89] Humic acid can increase the suspension stability of nano-TiO_2 and present a steric hindrance barrier between a cell and the nanoparticle thereby diminishing bioavailability.[85,90] Furthermore, nano-TiO_2 is readily incorporated into organic matter aggregates such as marine snow which affects its sedimentation rates.[88] Prolonged suspension of DOM-bound TiO_2 nanoparticles and incorporation of the nanoparticles into marine snow makes them more accessible to filter feeders such as bivalves, sedentary annelids and crustaceans.

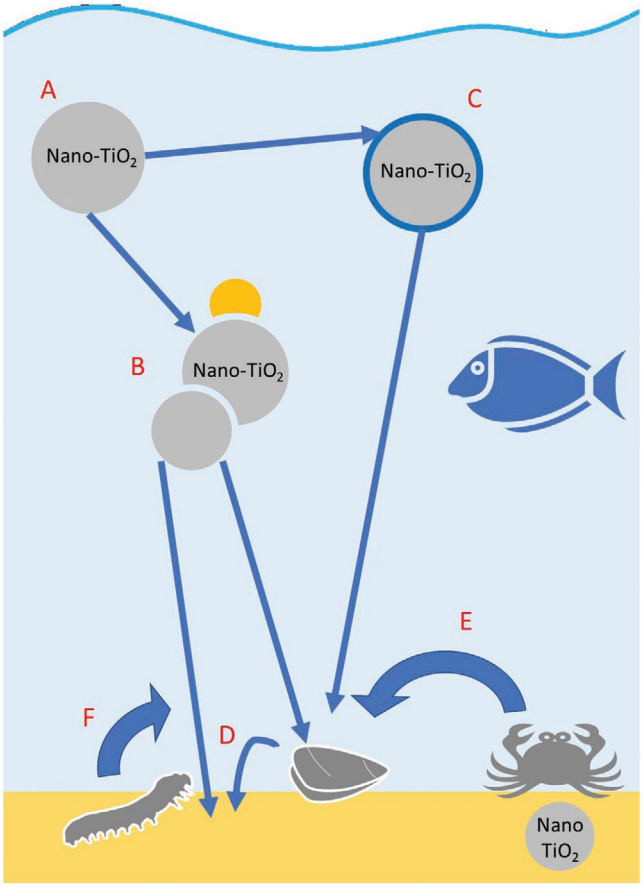

Figure 4. The fate of nano-TiO_2 in the aquatic environment. A) Free titanium dioxide nanoparticles. B) Agglomerations between TiO_2 NPs or TiO_2 NPs and organics have the potential to settle or become more bioavailable. C) Uptake of free TiO_2 NPs or after surface modifications due to binding of simple organics. D) Transport of TiO_2 NPs to the sediment via feces and pseudofeces. E) Surface disturbance and F) bioturbation leads to TiO_2 NP resuspension and reexposure.

Large agglomerates of nano-TiO_2 with other particles (heteroaggregates) or POM will eventually be deposited on the bottom and impact benthic organisms such as sediment-dwelling bioturbators and benthic deposit feeders. Sediment burrowing animals such as mollusks and annelids can bury the nanoparticles into the deeper sediment layers making them less accessible to the organisms on the sediment surface and in the water column, whereas surface feeders (such as crabs or fish) could disturb the sediment surface causing resuspension and re-exposure of TiO_2 to filter feeders and pelagic organisms (**Figure 4**).

As discussed in the introduction, estimated environmental concentrations of nano-TiO_2 can reach up ≈100 µg L^{-1} in the coastal waters,[7] albeit higher levels have been reported locally in the sediments[8] and the water column near popular beaches.[9] For the purpose of this review, we assume 100 µg L^{-1} as the hazard threshold with regard to the present-day concentration of nano-TiO_2 in the aquatic environment, so that results of the studies carried out at the exposures at or below 100 µg L^{-1} are considered environmentally relevant. We also discuss the results of the studies carried out at higher nano-TiO_2 (>100 µg L^{-1}) concentrations. Even though such studies cannot be directly used for the environmental risk assessment, they provide important insights into the mechanisms of toxicity of nano-TiO_2 in aquatic organisms.

Abiotic Factors as Potential Modulators of Nano-TiO_2 Toxicity: The toxicity of nano-TiO_2 in aquatic environments may be modulated by other abiotic factors such as temperature, pH, salinity, and UV radiation (**Figure 5**). In particular, visible and UV light can strongly potentiate the nano-TiO_2 toxicity through photocatalytic reactions that generate hydroxyl and superoxide radicals causing oxidative stress and damage to cellular components.[86] DOM (such as humic acids) can attenuate the sunlight-induced generation of ROS via a ROS quenching mechanism and thus diminish the oxidative stress in the presence of photocatalytic nano-TiO_2.[85,91]

Hypoxia and low pH can enhance the toxic effects of nano-TiO_2 in aquatic organisms as was shown in bivalves,[92–94] whereas the interactions of nano-TiO_2 with dissolved metals are more variable.[94–97] Nano-TiO_2 can also serve as a carrier for other environmental pollutants including divalent metals such as Cu^{2+}, Zn^{2+}, Pb^{2+}, and Cd^{2+} and metalloids such as arsenic (As).[98,99] The implications of increased pollutant binding to nano-TiO_2 depend on the exposure mode and the type of toxicant. Thus, pre-exposure to nano-TiO_2 followed by exposure to dissolved Cd and Zn increased the uptake and toxicity of the dissolved metals in the freshwater crustacean *Daphnia magna*,[100] whereas the concomitant exposure to nano-TiO_2 and dissolved Cd had no effect on Cd uptake or toxicity in freshwater invertebrates *D. magna*, *Lumbriculus variegatus*, and *Corbicula fluminea*.[101,102] Co-exposure of *D. magna* to nano-TiO_2 and dissolved Cu, Ag and As increased body burdens and toxicity of Ag but decreased accumulation and toxicity of As and Cu compared with single metal exposures.[95,97,103–107] Overall, the published studies to date show that potentiation of nano-TiO_2 toxicity is possible under certain multiple stressor scenarios, but the data are presently insufficient to permit broad generalizations and require further investigations.

4.2.2. Mollusks

Mollusks (including bivalves and gastropods) include many species of critical ecological importance and high economic value around the world. Due to their filter-feeding habits, bivalves are particularly vulnerable to nanopollutants.[108–110] Bivalves can take up nano-TiO_2 from the water, phytoplankton and suspended sediment and internalize them through digestion, cellular, and trans-epithelial transport.[111] Accumulation of Ti was found during exposure to waterborne nano-TiO_2 in all major organs of the bivalves indicating bio-distribution within the organism.[111,112]

Oxidative Damage: Oxidative stress is a hallmark of nano-TiO_2 toxicity in mollusks as shown by elevated production of ROS, upregulation of antioxidant enzymes and accumulation of oxidative lesions. Upregulation of antioxidant enzymes (including SOD, CAT, and glutathione-S-transferase) was found in the Mediterranean clam *Ruditapes decussatus* and the mussels *Mytilus galloprovincialis* and *Mytilus coruscus* exposed to nano-TiO_2,[112–115] albeit in some species this response was

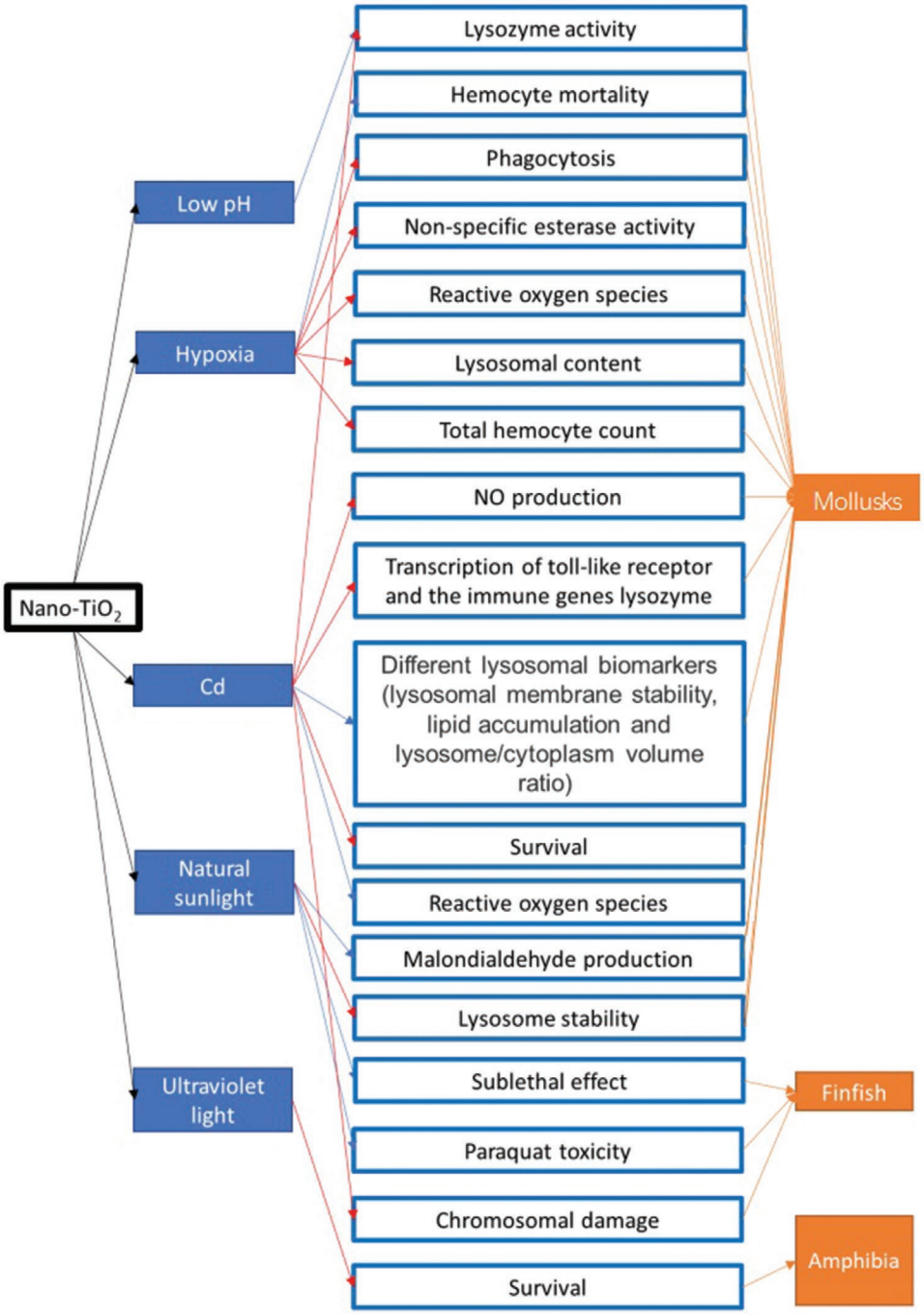

Figure 5. The combined effects of nano-TiO_2 and other environmental factors on aquatic organisms. The black arrows point to the environmental factors that influence the toxic effect of nano-TiO_2. The red and blue arrows indicate the adverse outcome pathways for toxicity, with a decrease (red) and increase (blue) in the respective traits. The orange arrows indicate in which organisms these effects have been reported.

tissue-specific.[114,115] In the freshwater mussel *Unio tumidus*, exposure to nano-TiO_2 resulted in elevated levels of ROS, depletion of GSH and activation of SOD.[116] Increased lipid peroxidation (indicated by MDA accumulation) was found in the nano-TiO_2 exposed clams *R. decussatus*, *C. fluminea*, oysters *Crassostrea virginica*, and abalone *Haliotis diversicolor*.[86,102,112,117]

The intensity of oxidative stress responses of mollusks exposed to nano-TiO_2 appears to depend on the size of nano-TiO_2. Thus, in the mussel *Unio tumidus* exposure to 75 nm nano-TiO_2 (100 µg L^{-1} for 14 days) suppressed the SOD activity in the digestive glands.[116] In *R. decussatus* exposure to 12.5 nm nano-TiO_2 (100 µg L^{-1} for 14 days) elevated the SOD level in the digestive glands.[112] Similarly, following exposure to 55 nm nano-TiO_2 (1 mg L^{-1}), the level of SOD in several bivalves was decreased, while exposure to 20–21nm nano-TiO_2 stimulated the SOD activity.[102,118,119] Thus, it appears that smaller

nano-TiO_2particles are stronger inducers of oxidative stress in bivalves as evidenced by a compensatory increase in the SOD activity.

Immunotoxicity: Exposure to waterborne nano-TiO_2 causes immunotoxicity in marine bivalves. The blood cells (hemocytes) are the main cell type involved in the innate immune response of bivalves (that lack the adaptive immunity). Hemocytes also play an important role in nanoparticle uptake and are thus an important target of nano-TiO_2 toxicity. Suppression of hemocyte viability and phagocytosis are common responses to nano-TiO_2 exposures in marine bivalves as shown in the clam *Tegillarca granosa*,[120,121] and the mussels *M. coruscus*,[122] *M. galloprovincialis* and *Perna viridis*.[94,123] It is worth noting that most studies to date have been conducted at the high nano-TiO_2 concentrations (10 mg L^{-1}) well above the present-day environmental hazard threshold. Elevated ROS production, accumulation of oxidative lesions to proteins and lipids and DNA damage are commonly found in hemocytes of nano-TiO_2 exposed bivalves, consistent with the pro-oxidant mechanisms of nano-TiO_2 toxicity.[92,122–125]

Neurotoxicity: Neurotoxicity of nano-TiO_2 has not been extensively studied in bivalves, but a recent study in the blood clam *T. granulosa* indicates potential involvement of this toxic mechanism. In *T. granulosa,* exposure to waterborne nano-TiO_2 (0.1, 1, and 10 mg L^{-1}) increased the concentrations of the neurotransmitters dopamine and acetylcholine and γ-aminobutyric acid, decreased the activity of acetylcholine esterase, and suppressed the transcript levels of the genes encoding to neurotransmitter modulatory enzymes and neurotransmitter receptors.[126]

Energy Metabolism: Nano-TiO_2 exposure negatively impacts metabolism and energy balance of bivalves. Thus, exposures to high levels of waterborne nano-TiO_2 (2.5 and 10 mg L^{-1}) suppressed the filtration activity, food absorption efficiency and aerobic scope for growth in the mussel *M. coruscus*.[127] The specific dynamic action (reflecting the energy demand for food digestion and absorption) and activity of the digestive enzymes decreased in nano-TiO_2 exposed mussels, which reflected suppressed feeding.[93,128] Similarly, exposure to 50 and 100 µg L^{-1} nano-TiO_2 decreased filtration activity of the Mediterranean clam *R. decussatus*.[112]

4.2.3. Crustaceans

Similar to the bivalves, nano-TiO_2 in crustaceans appear to target the digestive processes, energy metabolism, and redox balance, albeit most studies to date in crustaceans used high concentrations of nano-TiO_2 above the assumed hazard threshold of 100 µg L^{-1}. Thus, prolonged exposure to a nano-TiO_2 anatase/rutile mixture exposure (>500 µg L^{-1}) caused severe growth retardation, reproductive defects and mortality of *Daphnia magna*.[129] Nano-TiO_2 accumulated in guts could also induce oxidative stress directly or through interference with the digestive processes as shown in *Artemia salina*.[130] Nano-TiO_2 anatase accumulated in guts could also adsorb Cd,[100] Zn,[100] and As[131] thereby inducing oxidative stress and thus increasing toxicity to *D. magna*.

Nano-TiO_2 can also induce oxidative stress as a result of UV-induced phototoxicity as shown in the freshwater amphipod *Hyalella Azteca*.[132] Under simulated solar radiation, the toxicity of a mixture of nano-TiO_2 anatase/rutile to *D. magna* and *Daphnia similis* was also enhanced due to the light-induced ROS generation around nano-TiO_2 nanoparticles.[133,134] However, the nano-TiO_2 anatase/rutile mixture might also have a protective effect on *D. magna* against UV-B radiation by nano-TiO_2 adsorption of UV-B light.[135] Therefore, the phototoxicity of nano-TiO_2 in natural conditions might be more complex.

Surface modifications and crystalline polymorph of nano-TiO_2 can modulate its uptake and toxicity in crustaceans. Thus, surfactants were reported to decrease the toxicity of TiO_2 nanoparticles to *D. magna* by inhibiting the accumulation and/or facilitating the depuration of nano-TiO_2 anatase.[136] Nano-TiO_2 accumulation in crustaceans also depends on the nano-TiO_2 crystalline polymorph. For example, nano-TiO_2 mixtures with higher anatase/rutile ratios (4:1 and 1:1 at a concentration of 1 mg L^{-1}) were more bioavailable to *D. magna* than nano-TiO_2 with another 1:4 anatase/rutile mixtures or titanium tetrachloride.[137]

4.2.4. Aquatic Vertebrates

Oxidative Stress: Nano-TiO_2 at high concentrations (>100 µg L^{-1}) can induce oxidative stress in fish, as was shown during exposure of the juvenile olive flounder *Paralichthys olivaceus* to a mixture of nano-TiO_2 anatase/rutile[138] and after exposure of zebrafish to a nano-TiO_2 reduced graphene oxide (RGO) composite.[139] In the latter study, it was difficult to distinguish between the toxic effects of nano-TiO_2 and RGO. However, the toxic effects of the TiO_2 and RGO composite (including oxidative stress, cardiotoxicity, and teratogenicity) was found only at the extremely high (>30 mg L^{-1}) but not at the lower exposure concentrations (0.25–3 mg L^{-1}).[139] Exposure to 1 mg L^{-1} of nano-TiO_2 anatase/rutile mixture negatively affected the genome template stability of European sea bass *Dicentrarchus labrax,* likely reflecting oxidative DNA damage.[140] However, exposure to 0.1 mg L^{-1} nano-TiO_2 did not affect the SOD activity in catfish indicating lack of oxidative stress response. Taken together, these data indicate that at environmentally relevant concentrations (≤100 µg L^{-1}), nano-TiO_2 is unlikely to induce oxidative stress in fish.

Immunotoxicity: Nano-TiO_2 anatase (2 ng g^{-1} and 10 mg g^{-1} body weight) showed immunotoxic impacts on fathead minnow *Pimephales promelas* by reducing the bactericidal function of its neutrophils.[141] In the European sea bass *D. labrax*, a mixture of nano-TiO_2 anatase/rutile (1 mg L^{-1}) negatively affected the transcript abundance of immune-related genes in the spleen.[142] Negative shift in immune gene expression profile and function of neutrophils in the fathead minnow *P. promelas* exposed to nano-TiO_2 anatase (0.1 mg L^{-1}) also indicated potential interference with the innate immune responses.[143]

Reproduction and Development: Exposure to a high concentration of TiO_2 (>31 mg L^{-1}) suppressed body growth and delayed development of the tadpoles of a model amphibian, *X. laevis*, while the low concentration (0.31 mg L^{-1}) induced no effects.[144] Zebrafish *Danio rerio* exposed for 14 days to 1 mg L^{-1} nano-TiO_2, remained capable of reproductive behavior and produced viable embryos.[145] However, at the higher concentration of 4 mg L^{-1} of nano-TiO_2 spermatogenic cells and testicle

morphology of zebrafish were negatively affected and necrotic areas were detected.[146] Nano-TiO_2 had no conspicuous impact on the gonadal morphology or histology of exposed zebrafish as shown by no occurrence of ova-testis, no evidence of reactive hyperplasia, or gonadal atrophy in exposed groups.[145] However, the survival rate of embryos decreased and frequency of malformation increased as the parental fish were exposed to increasing nano-TiO_2 concentrations.[180] Embryonic malformations including diffuse edemas coupled with microcephaly, gut abnormalities and/or axial defects were also found in the offspring of African frog *X. laevis* whose parents were in a concentration-dependent manner.[147] Thus, activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), as well as the levels of glutathione (GSH) significantly decreased in response to a higher dose (>25 mg kg^{-1} body weight) of nano-TiO_2 via IP injection, while the low dose (5 mg kg^{-1}) had no effect in a concentration-dependent manner.[147] Nano-TiO_2 might also affect the CNS development as was shown by inhibition in carboxylesterase activity and increases in acetylcholinesterase activity in *X. laevis* embryos after exposure to high concentrations of nano-TiO_2 (160 and 320 mg L^{-1}).[148]

Factors Modulating the Nano-TiO_2 Toxicity: Similar to bivalves, the polymorph, and size of nano-TiO_2 modulate nanoparticle toxicity to aquatic vertebrates including fish and amphibians. Thus, *Piaractus mesopotamicus* exposed to a nano-TiO_2 anatase/rutile mixture under UV light had a higher glutathione S-transferase activity than their counterparts exposed to nano-TiO_2 anatase, indicating the higher toxicity of nano-TiO_2 anatase/rutile mixture.[149] Under ultraviolet light, small diameter nano-TiO_2 (5 and 10 nm) significantly reduced the survival of *X. laevis* tadpoles while 32 nm nano-TiO_2 had no effect on the survival,[144] indicating that the phototoxicity of nano-TiO_2 on *X. laevis* tadpoles is enhanced by the small size (and thus high surface area) of the nanoparticles.

Co-occurrence of other pollutants with nano-TiO_2 can also modify the toxicity of the mixture. The bioaccumulation of perfluorooctane sulfonate (a persistent organic pollutant) in zebrafish was facilitated by nano-TiO_2 anatase/rutile mixture exposure.[150] However, during co-exposures to strong prooxidants such as paraquat, dioxins, or toxic metals, nano-TiO_2 might exert protective effects in fish. Thus, in a common carp, exposure to nano-TiO_2 anatase/rutile mixture mitigated the toxic effects of paraquat under the UV light.[151]

Similar to fish, the toxic effects of nano-TiO_2 in amphibians are typically observed at concentrations 1–3 orders of magnitude higher than the estimated present-day levels in the surface waters. This indicates that under the current conditions, nano-TiO_2 may not present a significant acute health risk to aquatic vertebrates. However, due to the scarcity of the studies in this animal group and a heavy bias of existing research towards the model species, the field is not yet ready for the broad generalization concerning the potential hazard to fish and amphibians in environmentally relevant settings.

4.3. Bibliometric Analysis

The bibliometric analysis of published research on nano-TiO_2 revealed several key focal points that changed over time (**Figure 6**). During 2012, the main hot keywords were related to

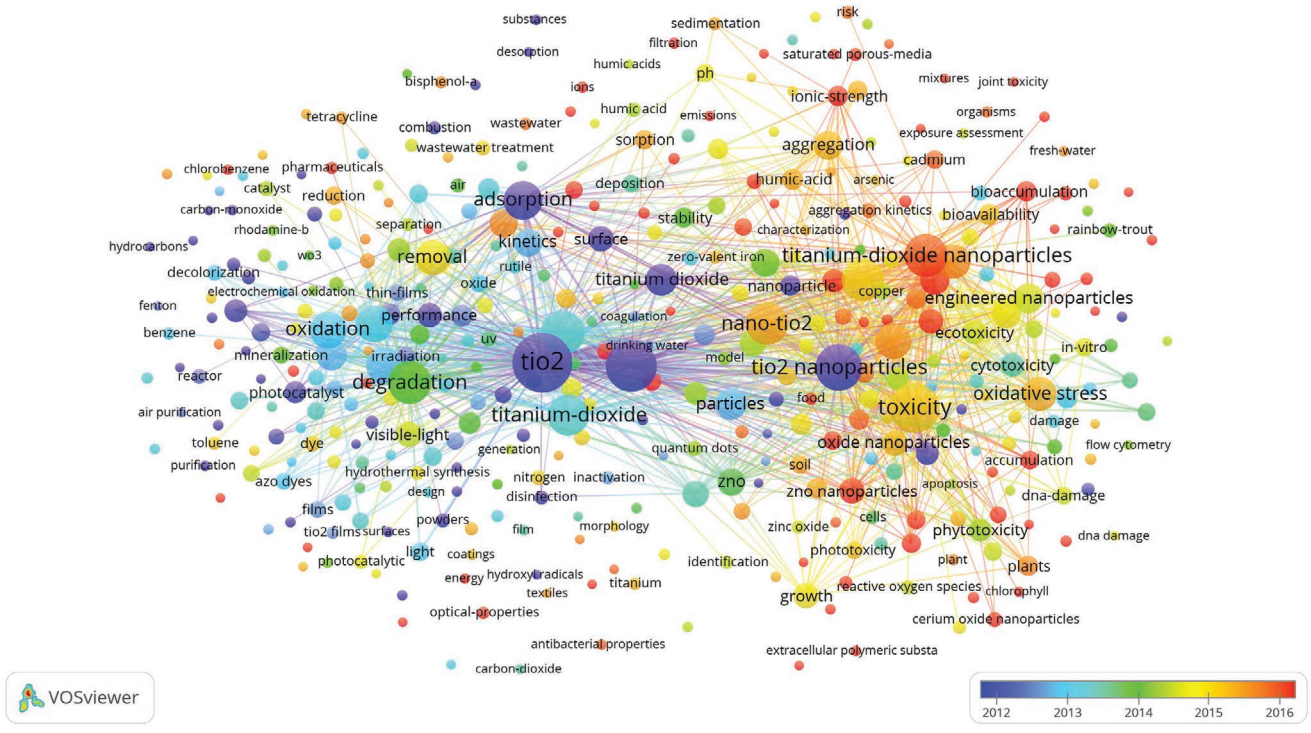

Figure 6. Bibliometric map of environmental science about nano-TiO_2. The size of the dot is proportional to the frequency that a certain keyword appeared in the analyzed articles. The line between two dots means that these two keywords appeared in the same article. The thicker the line, the more frequently the two keywords appeared in the same articles. The color represents the year when a certain keyword most frequently appeared in articles.

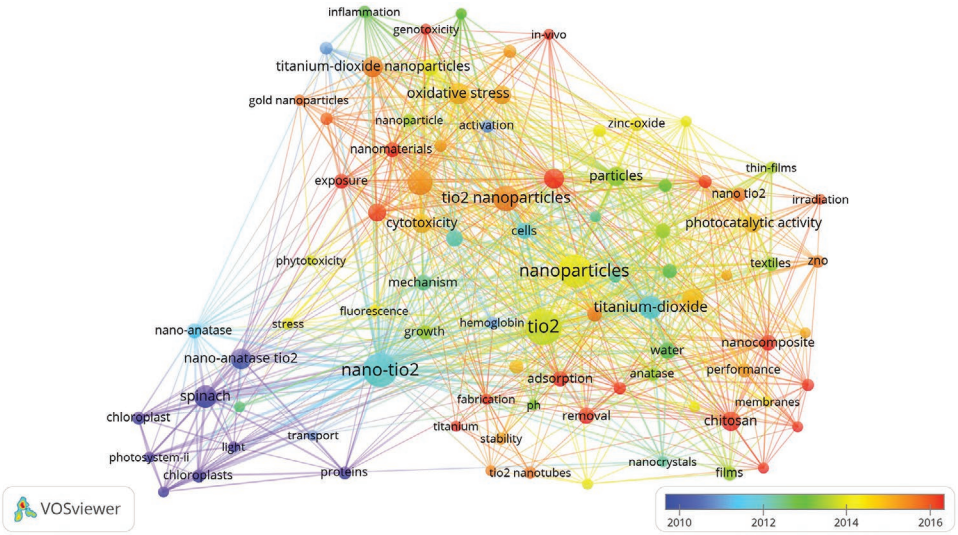

Figure 7. Bibliometric map of the molecular biology studies on the effects of nano-TiO_2. The size of the dot is proportional to the frequency that a certain keyword appears in the analyzed articles. The line between two dots means that these two keywords appeared in the same article. The thicker the line, the more frequently the two keywords appeared in the same articles. The color represents the year when a certain keyword most frequently appeared in articles.

the basic physico–chemical properties of nano-TiO_2 including "adsorption", "performance", "photocatalyst", "combustion", and "desorption", indicating focus on the investigation of the properties and development of novel applications for this engineered nanomaterial. In 2012–2014, the research on nano-TiO_2 physico-chemical properties (e.g., "degradation", "stability", "deposition") continued, but the focus shifted towards investigations of its biological effects in cell lines, plants and model vertebrates, as shown by the high frequency of the keywords "cytotoxicity", "phytotoxicity" and "rainbow trout". A high frequency of the keyword "ZnO" indicates that the combined effects of these nanoparticles and nano-TiO_2 have been explored, possibly due to the joint applications of nano-ZnO/TiO_2 in some technologies such as the determination of chemical oxygen demand[152] and in sunscreen formulations.[153] Between 2014 and 2015, the topics "toxicity", "oxidative stress", "ecotoxicity", "DNA damage" were highly represented in the published research on nano-TiO_2. "Visible light" and "pH" effects were also often discussed in the nano-TiO_2 in this period. Since 2015, "bioaccumulation" and "ionic-strength" became additional foci of research on nano-TiO_2, and frequent occurrence of the keyword "cadmium" indicates increasing concern about nano-TiO_2 as a potential carrier of toxic metals.

In the molecular biology subfield of nano-TiO_2 research (**Figure 7**), "nano-anatase" impacts on the "photosystem" have become a hot topic since 2010. The keywords "chloroplasts" and "spinach" indicate a common model system in which the effects of nano-TiO_2 on photosynthesis were explored.[154] Around 2012, investigations of "mechanisms" how nano-TiO_2 affects "cells" were highly represented among the published studies. "Inflammation", "oxidative stress" and "genotoxicity" emerge as important research topics in nano-TiO_2-related molecular biology research since 2013. "Gold nanoparticles", "ZnO" and "irradiation" are also considered. Similar to ZnO, gold nanoparticles are used in technological applications to increase the efficiency of photocatalysis.[155] In the natural environment, gold nanoparticles and TiO_2 NPs may aggregate. This increases the photocatalytic properties of the nanoparticles and promotes the production of cytotoxic ROS.

The toxic effects (**Figure 8**) of nano-TiO_2 have been extensively studied since about 2011. Investigation at the organismal (as opposed to the cellular and molecular) level on effects of nano-TiO_2 has become an important topic of the scientific exploration in 2011–2013 (cf. Figures 2 and 8). Nevertheless, the studies of the molecular and cellular effects of nano-TiO_2 continue to dominate the field as shown by the frequent occurrence of the keywords "oxidative stress", "genotoxicity", and "inflammation". The scope of the model organisms increases during this time, as shown by the appearance of the keywords "rats", "algae", "rainbow-trout", "danio-rerio", and "daphnia-magna". This might reflect an increasing awareness of the potential off-target effects of nano-TiO_2 in aquatic environments and increasing investigation of the ecotoxicological effects of nano-TiO_2, in addition to the continuing research in biomedical models. The toxicity of diverse nanoparticles such as "ZnO", "CeO_2", and dissolved metals such as "silver" and "cadmium" in combination with nano-TiO_2 is intensively tested during this period, reflecting the new developments of photocatalytic applications that use novel combinations of nanoparticles.[155,156]

5. Conclusion and Perspective

The existing and still growing body of evidence demonstrates the potential for toxic effects of nano-TiO_2 particles in humans, model vertebrates, and other organisms via different exposure routes including (depending on the organism) inhalation, ingestion, injection, and exposure in the water and in sediments. The cellular mechanisms of nano-TiO_2 are shared in different studied organisms and among different modes of exposures (**Figure 9**). Nano-TiO_2 particles and their aggregates

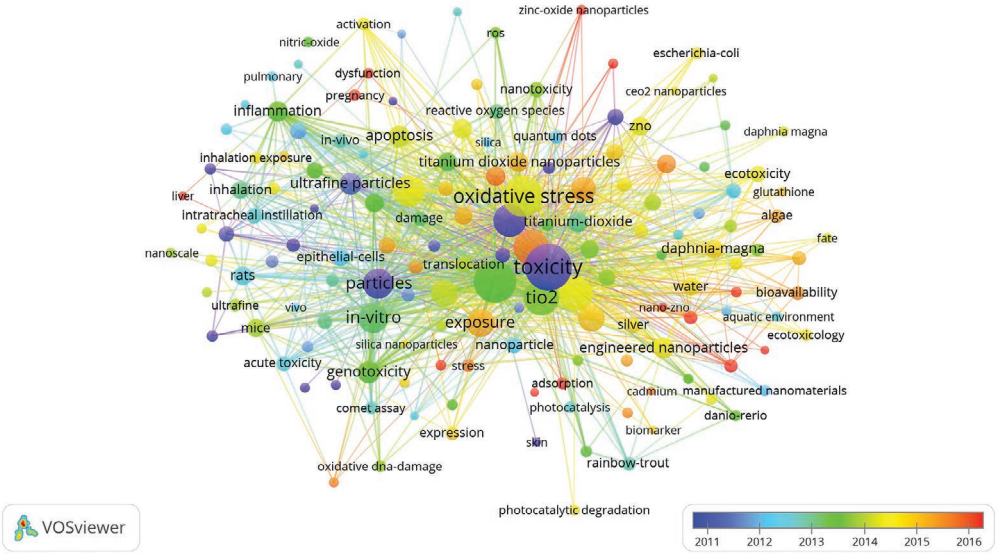

Figure 8. Bibliometric map of the studies on the toxic effects of nano-TiO_2. The size of the dot is proportional to the frequency that a certain keyword appears in the analyzed articles. The line between two dots means that these two keywords appeared in the same article. The thicker the line, the more frequently the two keywords appeared in the same articles. The color represents the year when a certain keyword most frequently appeared in articles.

can attach to the cell membrane and interact with the surface receptors including those involved in phagocytosis and micropinocytosis, leading to internalization of nano-TiO_2.[157] Mechanical stress due to interactions of cells with nano-TiO_2 can impair the integrity of the cell membrane and affect ion homeostasis and activity of the membrane-associated receptors and enzymes.[157] External (free of membrane-associated) nano-TiO_2 can also photocatalytically generate reactive oxygen species (most notable hydrogen peroxide),[158,159] which contribute to the observed membrane damage. Internalized nano-TiO_2 is transported by phagosomes to lysosomes causing lysosomal stress and damage, accumulation of nano-TiO_2 in the lysosomally-derived multilamellar vesicles, and eventually release into the cytosol where it can interact with different cellular components[160,161] (Figure 9). Intracellular accumulation of nano-TiO_2 leads to DNA damage, changed DNA conformation due to nano-TiO_2 binding, whereas altered gene expression affects the induced oxidative stress and inflammation.[69,84,162] If left unchecked, the accumulating damage to the cellular organelles and macromolecules can lead to the induction of autophagic and apoptotic pathways leads to cell loss and organ injury.[163]

Further studies are needed to deeply explore the cytotoxic mechanisms of nano-TiO_2 and develop strategies for mitigation of the cellular damage. This might be achieved, for example, by preventing the binding to DNA, mitigating oxidative stress with antioxidants (e.g., alloxan, vitamin E, idebenone or chlorophyllin), stimulating cellular repair mechanisms such as protein quality control or DNA repair, or targeting the inflammatory and autophagic pathways. It is worth noting that while the cytotoxic mechanisms of nano-TiO_2 are relatively well understood, our understanding of the consequences of nano-TiO_2 exposures on the organismal performance and health (including behavior, growth, reproduction, and different systemic physiological

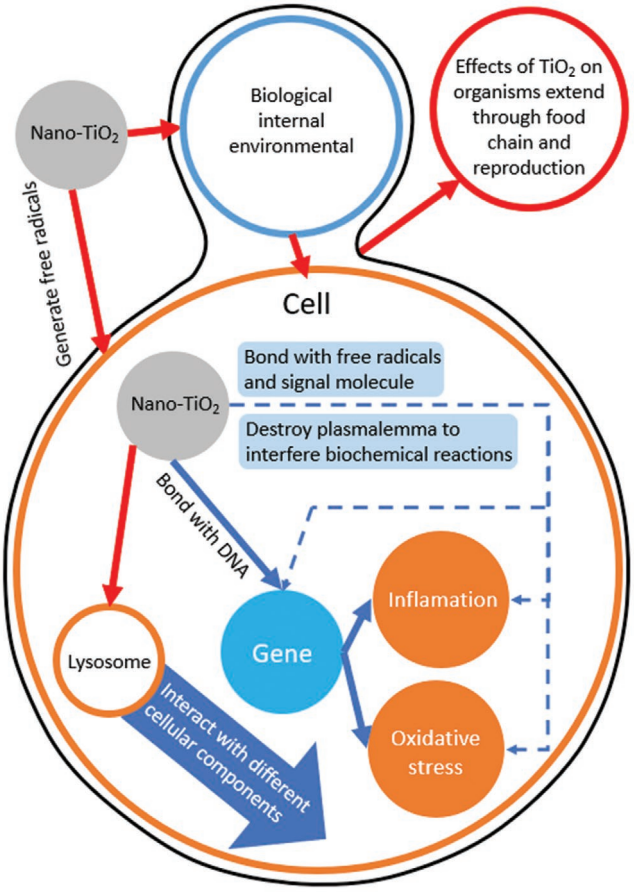

Figure 9. The likely adverse outcome pathways underlying the effects of nano-TiO_2. The dotted lines represent suspected pathway. The solid lines represent demonstrated pathways.

activities such as energy metabolism, excretion, osmoregulation, endocrine homeostasis) lags significantly behind. Further studies are urgently needed to assess the systemic and holistic impacts of nano-TiO_2 on the individual health and performance of humans and wildlife.

While mitigation of the cellular toxicity of nano-TiO_2 might be possible in some cases, such as during acute occupational exposure, the ever increasing use of nano-TiO_2 in multiple applications and consumer products and its release into the environment require strategies to minimize exposure of humans and wildlife to nano-TiO_2. The existing weight-of-evidence for toxic effects of nano-TiO_2 at environmentally relevant exposure concentrations requires critical reappraisal of the current criteria for environmental policies and the regulatory framework for minimizing the cradle-to-grave release and impacts of nano-TiO_2 during production and use. Further strategies to minimize the environmental and health impacts of nano-TiO_2 should include development of environmentally-friendly alternatives to nano-TiO_2 and its efficient recycling. Further environmental testing and remediation measures are also urgently needed to eliminate nano-TiO_2 from polluted environments, particularly sediments and soil that act as sinks for nano-TiO_2.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

bibliometric analysis, ecotoxicity, health, meta-analysis, titanium dioxide nanoparticles, toxicity

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