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REVIEW

Neurotoxicity of Titanium Dioxide Nanoparticles: A Comprehensive Review

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Abstract: The increasing use of titanium dioxide nanoparticles (TiO_2 NPs) across various fields has led to a growing concern regarding their environmental contamination and inevitable human exposure. Consequently, significant research efforts have been directed toward understanding the effects of TiO_2 NPs on both humans and the environment. Notably, TiO_2 NPs exposure has been associated with multiple impairments of the nervous system. This review aims to provide an overview of the documented neurotoxic effects of TiO_2 NPs in different species and in vitro models. Following exposure, TiO_2 NPs can reach the brain, although the specific mechanism and quantity of particles that cross the blood-brain barrier (BBB) remain unclear. Exposure to TiO_2 NPs has been shown to induce oxidative stress, promote neuroinflammation, disrupt brain biochemistry, and ultimately impair neuronal function and structure. Subsequent neuronal damage may contribute to various behavioral disorders and play a significant role in the onset and progression of neurodevelopmental or neurodegenerative diseases. Moreover, the neurotoxic potential of TiO_2 NPs can be influenced by various factors, including exposure characteristics and the physicochemical properties of the TiO_2 NPs. However, a systematic comparison of the neurotoxic effects of TiO_2 NPs with different characteristics under various exposure conditions is still lacking. Additionally, our understanding of the underlying neurotoxic mechanisms exerted by TiO_2 NPs remains incomplete and fragmented. Given these knowledge gaps, it is imperative to further investigate the neurotoxic hazards and risks associated with exposure to TiO_2 NPs.

Keywords: TiO_2 NPs, neurotoxic effects, oxidative stress, neuronal damage, neurotoxic mechanisms

Introduction

Nanomaterials (NMs) are materials defined as having at least one dimension ranging from 1 to 100 nanometers (nm). Titanium dioxide nanoparticles (TiO_2 NPs) rank among the top five NMs used in consumer products, such as food additives, toys, cosmetics, electronic products, and pharmaceuticals (Figure 1).[1,2] Consequently, the presence of TiO_2 NPs in air, water, soil, and other environmental media has gradually increased due to their widespread use.[3] This growing application and contamination have made human and animal exposure to TiO_2 NPs unavoidable. Apart from skin exposure, inhalation, and oral exposure, other routes of exposure to TiO_2 NPs include intraperitoneal injection, subcutaneous injection, and intramuscular injection.[4] Importantly, regardless of the route of exposure, TiO_2 NPs can ultimately enter the systemic circulation and translocate to various tissues and organs (Figure 2).[4] As the accumulation of TiO_2 NPs in the body increases, the associated health hazards become more severe.[5]

Before the rise of nanotechnology, TiO_2 was widely used in the form of fine particles (FPs), which were considered as poor soluble and low toxicity particles.[6] However, some studies have contradicted this view, such as lung tumors in rats exposed to high levels of TiO_2 FPs for two years.[7] Furthermore, TiO_2 has been classified as a Group 2B carcinogen (possibly carcinogenic to humans) by the International Agency for Research on Cancer.[8] Although the specific carcinogenicity of TiO_2 FPs is still debated, there is no doubt that TiO_2 FPs pose a health risk.[9] Compared to TiO_2 FPs,

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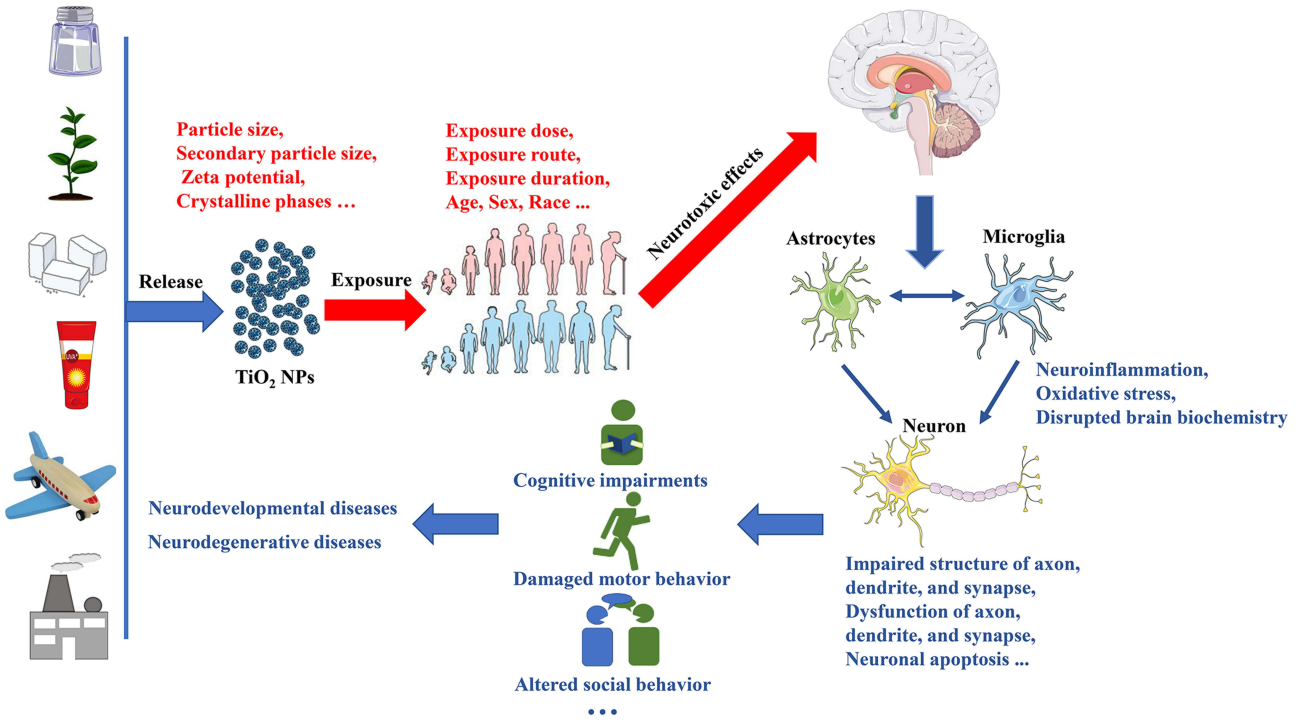
Graphical Abstract



TiO_2 NPs present stronger catalytic activity and bioactivity due to their nanoscale dimensions.[9] Consequently, the toxicity of TiO_2 NPs cannot be solely inferred from the known toxicology of TiO_2 FPs, nor can it be determined using conventional methods.[10] In recent years, extensive exploration of the effects of TiO_2 NPs exposure on human

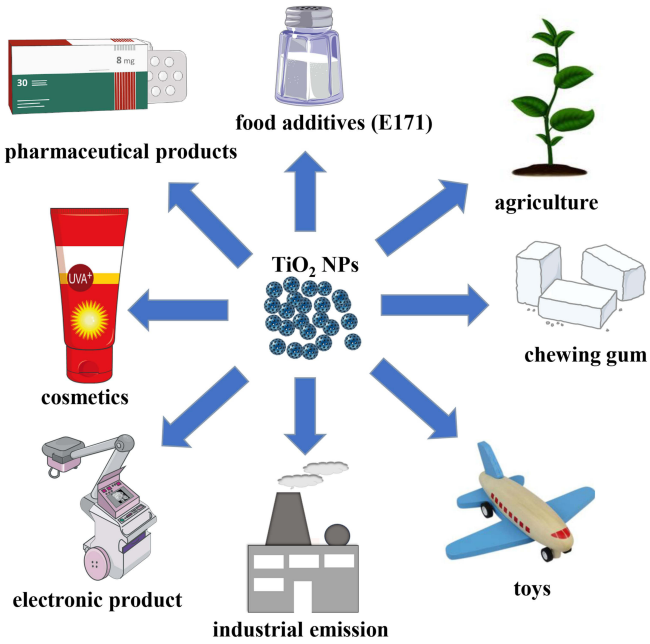


Figure 1 Application of TiO_2 NPs.

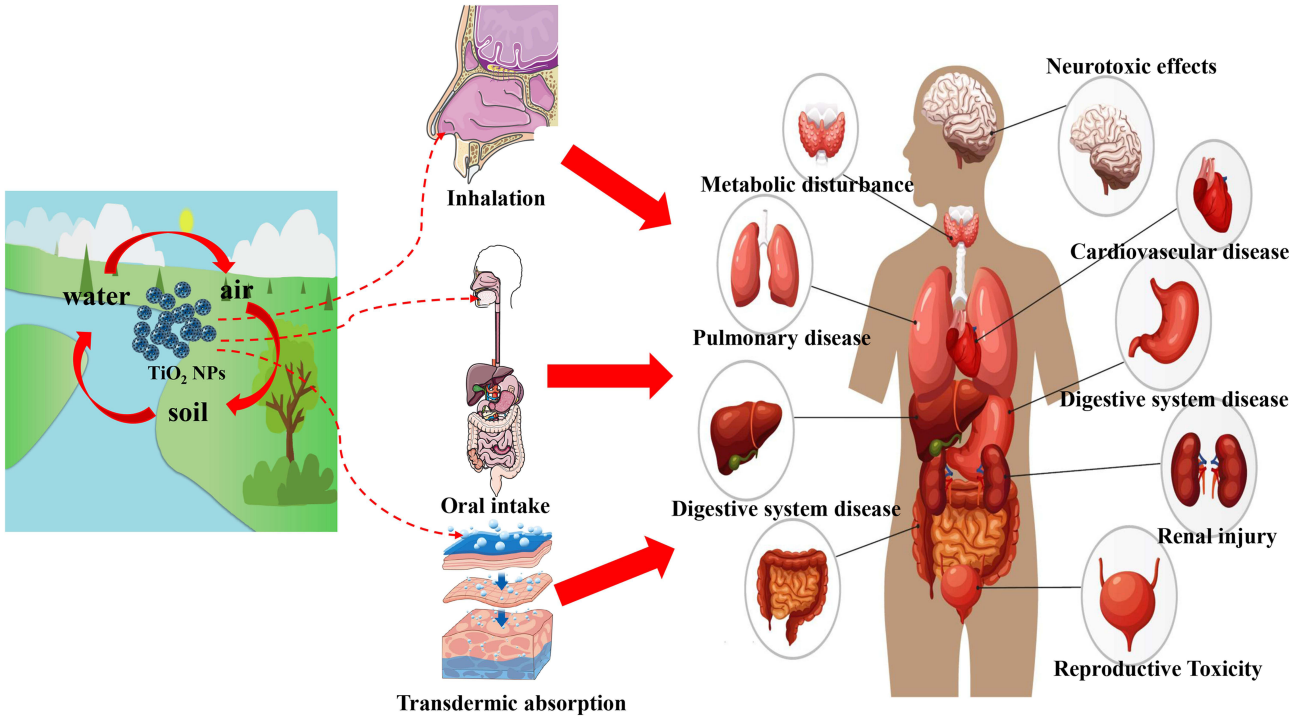


Figure 2 TiO_2 NPs can enter the human body through different ways and accumulate in the body, posing a threat to human health.

health also reflects the high concern about the safety of TiO_2 NPs. In addition to considering all relevant exposure scenarios and biological intermediate steps, understanding the final toxic outcome is critical for human health risk assessment.[11] Results from another child cohort study in China indicated that Ti can cross the placental barrier (PB) to harm fetuses that are extremely sensitive to environmental threats.[12] Several epidemiological studies have confirmed that Ti exposure increases the risk of adverse birth outcomes, including neural tube defects, preterm birth, fetal distress, and low birth weight.[13–15] Moreover, TiO_2 NP exposure can have detrimental effects on the health of the population beyond the fetus. Emerging epidemiological evidence suggests that higher levels of urinary or blood Ti are associated with an increased risk of various adverse health effects, including diabetes and cardiopulmonary disorders (Figure 2).[16–19] In recent years, laboratory studies on the toxicity of TiO_2 NPs have surpassed epidemiological studies. Common animal models such as mice, rats, zebrafish, and *Drosophila* have been used to study TiO_2 NPs. In vivo, studies have shown that TiO_2 NPs exposure may be linked to lung inflammation, pneumoconiosis, cardiovascular disease, reproductive toxicity, retinal impairments, etc.[20–24] In vitro, studies have also supported these toxic effects of TiO_2 NPs.[25–28] Given that nanoparticles can enter the brain, concerns regarding their neurotoxic effects, including those of TiO_2 NPs, have gained significant attention.[29]

The entry of TiO_2 NPs into the brain mainly occurs through the blood-brain barrier (BBB), via absorption-mediated transversion or intranasal pathways.[30,31] However, the mechanisms by which nano-titanium dioxide penetrates and targets different brain regions remain unknown. The degree of TiO_2 NPs accumulation in each brain region closely correlates with the extent of neurotoxic effects. Common neurotoxic effects include behavior deficits, nervous system dysfunction, and structural changes induced by oxidative stress, autophagy, inflammation, or the activation of specific signaling pathways.[32] Although emerging studies support the role of TiO_2 NPs exposure as an environmental risk factor for human health, conscientious and systematic investigations are scarce into the extent of TiO_2 NPs translocation to different brain regions and the resulting damage to the neuronal system in relation to particle dose and particle size. The lack of information on the neurotoxicity of TiO_2 NPs also complicates risk assessment following exposure. Therefore, this paper will mainly focus on current studies concerning the neurotoxicology of TiO_2 NPs, while also

reviewing the molecular mechanisms underlying their neurotoxic effects to mitigate potential damage resulting from exposure.

Evidence from Epidemiological and Human Exposure Studies

In earlier years, population exposure to TiO_2 NPs was primarily investigated among occupational populations. Welding fumes, industrial waste combustion, and mineral mining can all result in environmental contamination by TiO_2 NPs, thereby increasing the exposure risk for workers.[33] Exposure to fumes from metal-inert gas soldering has been found to increase the risk of Parkinson's disease (PD).[34] Although these fumes mainly consist of zinc, copper, and iron, Andujar et al discovered an excessive accumulation of Ti in the lung tissue sections of welders in 2014.[35] Industrial waste, pesticides, and automobile exhaust are common sources of environmental pollutants associated with neurotoxic effects.[36] Among various environmental pollutants, NPs can easily penetrate the BBB and induce neurotoxicity by activating innate immune responses in astrocytes, microglia, and neurons.[36] TiO_2 NPs are a major component among environmental pollutants, with up to 760 tons of TiO_2 NPs being released into the soil through sewage and sludge each year.[37,38] Currently, there is no direct evidence of neurotoxic effects caused by TiO_2 NPs exposure in mineral miners, but a previous study suggested a significantly increased inflammatory response in mineral miners exposed to TiO_2 NPs.[39] It is well known that the occurrence of inflammatory reactions in other organs is closely related to nervous system damage.[40] With the increasing application of TiO_2 NPs, concerns have also arisen regarding the neurotoxic effects of non-occupational populations exposed to TiO_2 NPs. A recent cohort study demonstrated that high levels of urinary Ti during pregnancy were significantly associated with impaired language development, suggesting that TiO_2 NPs might act as developmental neurotoxicants.[41] Furthermore, elevated levels of Ti in maternal hair were also significantly associated with an increased risk of neural tube defects.[42] However, epidemiological studies on the neurotoxic effects caused by TiO_2 NPs are still limited. Currently, laboratory studies are the main basis for evaluating the neurotoxicity of TiO_2 NPs.

Literature Search

To review the neurotoxic potential of TiO_2 NPs, a comprehensive literature search was conducted using the "Pubmed" database, covering articles from 1991 to September 29, 2023. The search utilized combinations of the following keywords: Titanium dioxide nanoparticles exposure; E171 exposure; Titanium dioxide nanoparticles neuron; Titanium dioxide nanoparticles brain; Titanium dioxide nanoparticles behavior; and Titanium dioxide nanoparticles neurotoxicology. Our search strategy involved an initial screening of all titles and abstracts, followed by a full-text review of the pertinent review articles. One hundred forty-seven papers were selected, including one hundred twenty-one research papers. Citations within twenty-six reviews were also screened for additional studies not identified in the electronic search; however, no additional research papers were found through these references. In the end, a total of one hundred and twenty-one research papers were selected, all of which included neurotoxicity endpoints in their experimental designs.

For experimental research, we identified in vitro or in vivo studies involving the administration of TiO_2 NPs. The researched organism groups mainly consisted of rodents, zebrafish, and cells. Fifty-five studies were conducted on rodents, twenty on zebrafish, and two on *Caenorhabditis elegans* (*C. elegans*), while one each on *Pheretima hawayana, Tegillarca granosa*, and *Drosophila melanogaster*. Additionally, forty-one studies used in vitro cells, including various animals and human-derived neuronal cells (Figure 3).

Neurotoxic Effects of TiO_2 NPs in Rodents

The results of the literature search demonstrate that rodents were the most commonly used species in neurotoxicity studies of TiO_2 NPs, forming the basis of 55 articles. Among these, 23 articles focused on rat models, with the Wistar rat being the most commonly employed. The exposed TiO_2 NPs generally had a particle size of less than 20 nm, and the exposure doses ranged below 200 mg/kg (Table 1). Intratracheal instillation, intragastric administration, intravenous injection, and intraperitoneal injection are common methods of rat exposure to TiO_2 NPs. Pregnancy and lactation have been identified as critical periods of neurodevelopment and have been selected as exposure windows to toxicants in many neurotoxicological studies, including the neurotoxicity of TiO_2 NPs.[43–45] Exposure to TiO_2 NPs (10 nm, 100 mg/kg)

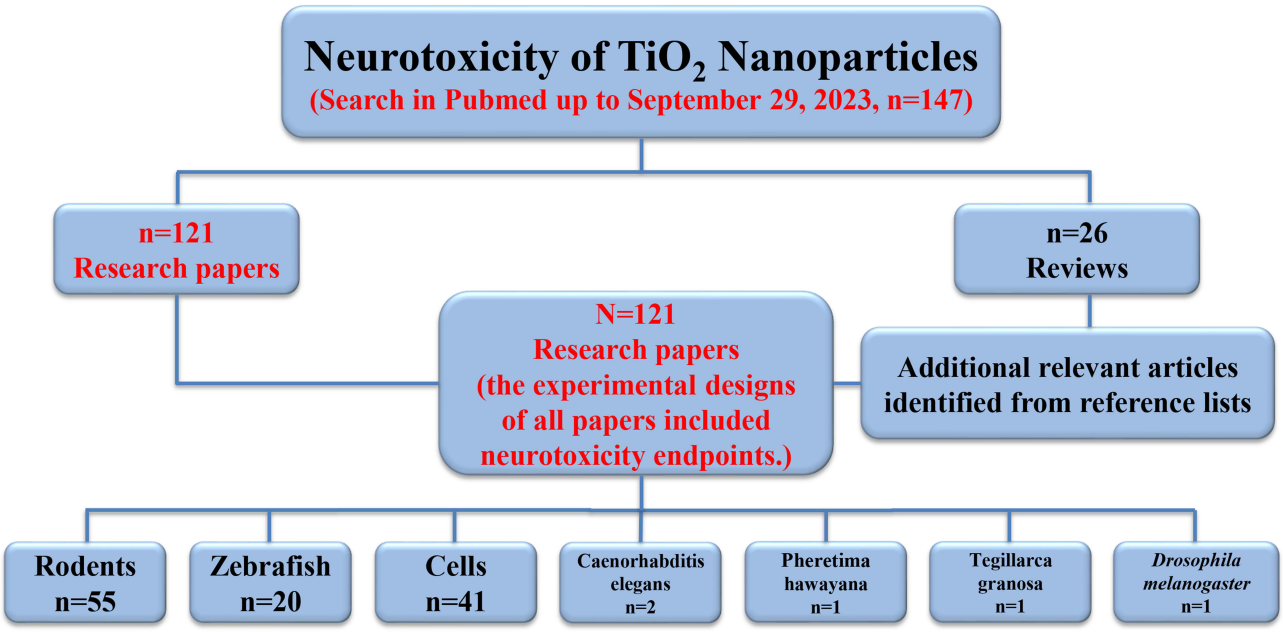


Figure 3 Study selection flow diagram. The flow chart illustrates the number of citations and resources that underwent screening, exclusion, and/or inclusion in the review.

during pregnancy or lactation has been found to impair memory and learning in Wistar offspring rats by reducing hippocampal cell proliferation.[44,46] Prenatal exposure to TiO_2 NPs (5 nm, 1 μg/μL) via subcutaneous injection has been shown to enhance depressive-like behavior in adult Sprague-Dawley (SD) rats.[47] Additionally, Engler-Chiurazzi et al conducted locomotion, learning, and anxiety tests on male adult SD rats and observed significant cognitive impairments in offspring resulting from inhalation exposure to TiO_2 NPs (170.9 ± 6.4 nm, 10.4 ± 0.4 mg/m^3) during pregnancy.[48] In earlier studies, the roles of age and sex in early-life exposure at different developmental stages were often overlooked. However, a recent study demonstrated that female pups were more susceptible to adverse outcomes after early exposure to oral TiO_2 NPs (postnatal day, PND 2–5 or PND 7–10) compared to male pups, while male pups exhibited more severe motor deficits following exposure to nano-titanium dioxide during late lactation (PND 17–20).[45] The behavioral impairments resulting from TiO_2 NPs exposure during pregnancy and lactation involve multiple mechanisms, including oxidative damage, changes in neurotransmitter concentrations, and metabolic disturbances.[45,47] Furthermore, exposure to TiO_2 NPs during pregnancy has been associated with neuronal apoptosis, decreased neurogenesis, altered expression of brain-derived neurotrophic factor (BDNF), and impaired synaptic plasticity.[49–51]

Adolescence is a transitional period of physical and behavioral development between childhood and adulthood, and it represents a particularly vulnerable neurodevelopmental phase.[67,68] In a study by Cui et al exposure to TiO_2 NPs (5 nm, 20 mg/kg) during adolescence via intravenous injection induced anxiety-like behavior, cognitive impairment, neuroinflammation, and oxidative damage in the hippocampus.[63] Similar results were observed in another study using adult Wistar rats, where subacute exposure to 20 mg/kg TiO_2 NPs increased the anxious index.[61] Furthermore, motor functional damage and spatial cognitive impairments were observed in adult Wistar rats exposed to TiO_2 NPs.[54,57,60] Certain regions of the mammalian brain continue to exhibit neurogenesis throughout adulthood, such as the subventricular zone of the lateral ventricles and the subgranular zone of the hippocampal dentate gyrus.[69] Adult neurogenesis is a multiple-step process, and abnormalities at any stage can impair neurogenesis and brain function, leading to cognitive impairment and neurodegenerative diseases.[70] Exposure to TiO_2 NPs in adulthood also induces various neurotoxic effects, including mitochondrial dysfunction, oxidative stress, cell apoptosis, alterations in neuronal architecture, neuroinflammation, decreased neurogenesis, and electrophysiological alterations.[52–54,56,58,59,64]

To date, slightly more neurotoxicology studies on TiO_2 NPs have been conducted using mice as animal models compared to rats, totaling 32 studies. Among them, 21 studies used ICR mice, 7 studies used C57BL/6, 2 studies used Albino mice, while BALB/c mice and Swiss Webster mice were used in 1 study each (Table 2). The exposure methods

Table 1 Overview of Literature Investigating Neurotoxic Effects of TiO_2 NPs in Rats

Model System	Particle Size	Exposure Method	Exposure Time	Exposure Dose	Neurotoxic Effects	Ref.
Wistar rats	<30 nm	Intragastric administration, Intraperitoneal injection	GD 2–21	200 mg/kg	Oxidative stress, Altered expression of BDNF and IL-6	[49]
	10 nm	Intragastric administration	GD 2–21	100 mg/kg BW	Impaired memory, Decreased hippocampal cell proliferation	[46]
	<100 nm	Intragastric administration	GD 2–21 and PND 2–21	100 mg/kg BW	Apoptosis, Decreases neurogenesis	[50]
	10 nm	Intragastric administration	PND 0–21	0, 100 mg/kg	Impaired learning and memory	[44]
	5–10 nm	Intragastric administration	60 days	0, 50, 100, 200 mg/kg BW	Neuroinflammation response	[52]
	5–12 nm	Intragastric administration	Per week for 8 weeks	0, 50, 100, 200 mg/kg BW	Neuronal degeneration, Apoptosis	[53]
	32.34±2.37 nm	Intravenous injection	5 days	5 mg/kg BW	Motor functional damage, Mitochondrial dysfunction, Neuronal architecture alterations	[54]
	21 nm	Intravenous injection	/	2, 10 mg/kg BW	Oxidative stress, Neuroinflammation	[55]
	34 ± 9 nm	Intravenous injection	/	1, 5, 4, 16 g/kg BW	Cell apoptosis, Decreases neurogenesis	[56]
	/	Intravenous injection	Acute	20 mg/kg BW	Spatial cognitive impairments, Biochemical and structural changes	[57]
	28 nm	Intratracheal instillations	28 days	0, 1, 3, 10 mg/kg	Electrophysiological alterations	[58]
	15 x 65 nm	Intratracheal instillations	28 days	0, 5, 8, 10 mg/kg BW	Mitochondrial dysfunction, Oxidative stress, Cell apoptosis	[59]
	10,100 nm	Intratracheal instillations	30 days	5, 18 mg/kg BW	Motor functional damage	[60]
	20, 30 nm	Intraperitoneal injection	Every 2 days for 20 days	20 mg/kg BW	Increased anxiety index	[61]
	32.34 ± 2.37 nm	/	1 h	5, 10, 50 μg/mL	Mitochondrial dysfunction	[62]
SD rats	5 nm	Subcutaneous injection	GD 6, 9, 12, 15, 18.	1 μg/ μL	Depressive-like behaviors, Oxidative damage	[47]
	5 nm	Intravenous injection	30 days	0, 20 mg/kg	Oxidative stress, Neuroinflammation, Anxiety-like behavior, Cognitive dysfunction	[63]
	/	Intraperitoneal injection	28 days	0, 80, 120, 160 mg/kg	Oxidative stress, Histological alterations, Reduced cell viability	[64]
	170.9 ± 6.4 nm	Inhalation	GD 7–20	10.4 ± 0.4 mg/m^3	Working impairments	[48]
	21 ± 5 nm	Intragastric administration	PND 2–5, PND 7–10, PND 17–20	0, 10 mg/kg BW	Damaged locomotor behavior, Perturbation of brain biochemistry	[45]
	<25 nm	Intragastric administration	GD 2–21	0, 2 mg/kg BW	Impaired synaptic plasticity, Oxidative damage	[51]
Fisher F344 rats	21.5 ± 7.2 nm	Inhalation	Per 5 days for 4 weeks	0, 10 mg/m^3	Neuroinflammation, BBB dysfunction, Decreased neuronal synaptophysin	[65]
Albino rats	60 nm	Oral route	14 days	0, 150 mg/kg BW	Altered expression of neurobiomarkers	[66]

Note: The reported particle size reflects the diameter of primary particles.

Table 2 Overview of the Literature Investigating Neurotoxic Effects of TiO_2 NPs in Mice

Model System	Particle Size	Exposure Method	Exposure Time	Exposure Dose	Neurotoxic Effects	Ref.
ICR mice	25–70 nm	Subcutaneous injection	GD 6–15	100 μg/μL	Altered gene expression	[88]
	25–70 nm	Subcutaneous injection	GD 6, 9, 12, 15	0, 1 μg/μL	Altered gene expression	[89]
	25–70 nm	Subcutaneous injection	GD 6, 9, 12, 15, 18	0, 1 mg/mL	Increased dopamine levels	[90]
	12 nm	Subcutaneous injection	3, 7, 10, 14 days	0, 50, 100 ml of 1 mg/mL	Increased depression-like behavior in neonatal mice	[91]
	5 nm	Intraperitoneal injection	14 days	0, 5, 10, 50, 100, 150 mg/kg BW	Oxidative stress, Neuroinflammation	[92]
	7.97 ± 2.36 nm	Intraperitoneal injection	14 days	0, 50, 100, 150 mg/kg	Oxidative stress, Hippocampal cell apoptosis	[93]
	80, 155 nm	Intranasal administration	2, 10, 20, 30 days	0, 500 μg	Oxidative damage, Neuroinflammation	[73]
	25, 80, 155 nm	Intranasal administration	2, 10, 20, 30 days	0, 50 mg/kg	Affected the releases and metabolisms of monoaminergic neurotransmitters	[94]
	<100 nm 50 nm	Intranasal administration	30 days	50 μg/μL	Changed the morphology of neurons in the cerebral cortex, Altered the monoamine neurotransmitter levels	[74]
	80, 155 nm	Intranasal administration	30 days	0, 500 μg	Oxidative stress, Increased GFAP-positive astrocyte, Changed the morphology of neurons in the CA4 region	[77]
	5.5 nm	Intranasal administration	90 days	0, 2.5, 5, 10 mg/kg	Neuroinflammation, Impaired spatial memory	[76]
	5–6 nm	Intranasal administration	90 days	0, 2.5, 5, 10 mg/kg BW	Oxidative stress, Overproliferation of all glial cells, Tissue necrosis, Hippocampal cell apoptosis, Altered gene expression	[72]
	5–6 nm	Intranasal administration	90 days	0, 2.5, 5, 10 mg/kg BW	Overactivation of the p38-Nrf-2 signaling pathway, Oxidative stress	[78]
	208–330 nm	Intranasal administration	90 days	0, 2.5, 5, 10 mg/kg	Hippocampus injury, Decreased spatial recognition, Decreased long-term potentiation, Altered gene expression	[80]
	5–6 nm	Intranasal administration	9 months	0, 1.25, 2.5, 5 mg/kg BW	Imbalance of glutamate metabolism, Inhibitions of glutamate receptor expression	[75]
	5 nm	Intragastric administration	60 days	0, 5, 10, 50 mg/kg BW	Impaired spatial recognition memory ability, Altered the homeostasis of trace elements, enzymes, and neurotransmitter systems	[95]
	6.5 nm	Intragastric administration	60 days	0, 5, 10, 50 mg/kg	Hippocampal apoptosis, Impaired spatial recognition memory	[96]
	5,10, 60, 90 nm	Intragastric administration	60 days	0, 5, 10, 50, 100, 150, 200 mg/kg	Ruptured and cracked nerve cells, Neuroinflammation	[97]
	6.5 nm	Intragastric administration	Prenatal day 0 to PND 21	0, 1, 2, 3 mg/kg BW	Brain retardation, Impaired cognitive ability	[85]

(*Continued*)

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Table 2 (Continued).

Model System	Particle Size	Exposure Method	Exposure Time	Exposure Dose	Neurotoxic Effects	Ref.
	6.5 nm	Intragastric administration	Prenatal day 7 to PND 21	0, 1.25, 2.5, 5 mg/kg	Excessive activation of ERK1/2/MAPK signaling pathway Retarded axonal and dendritic outgrowth	[84]
	6.5 nm	Intragastric administration	Prenatal day 7 to PND 21	0, 1, 2, 3 mg/kg BW	Oxidative stress, Hippocampal cell apoptosis, Excessive autophagy, Changed the morphology of neurons	[86]
C57BL/6J mice	21 nm	Intragastric administration	GD 8–21	0, 150 mg/kg	Reduced cortical thickness, Dilatation of lateral ventricles, Neural tube defects	[83]
	21 nm	Intragastric administration	GD 8–21	0, 150 mg/kg	Oxidative stress	[82]
	21 nm	Intragastric administration	30 days	0, 150 mg/kg	Neurobehavioral impairments, Disrupted the gut-brain axis	[98]
	21 nm	Intraperitoneal injection	/	0, 1 mg/mL	Intestinal dysbiosis Neurobehavioral impairments	[99]
	101 nm	Intravenous injection	GD 9	100, 1000 μg	Disturbed the gut microecology, Impaired locomotor activity	[100]
	21 nm	Intranasal administration,	GD 10.5	0, 0.25, 1, 4 μg/μL	Neuroinflammation	[79]
	26.2 ± 10.7 nm	Oral exposure	28 days	0, 150 mg/kg	Induced behavioral deficits relevant to ASD and related neurodevelopmental disorders	[101]
Albino mice	10–25 nm	Intragastric administration	GD 0–16	0, 30, 150, 300, 500 mg/kg BW	Altered the DNA methylation, Altered gene expression	[102]
	<75 nm	Intragastric administration	21 days	0, 500 mg/kg BW	No substantial neuropathological changes	[103]
BALB/c mice	10 nm	Intragastric administration	45 days	0, 10, 25, 50 mg/kg	Destruction of dopaminergic neurons, Increased risk of PD	[87]
Swiss Webster mice	<100 nm	Intragastric administration	24 days	0, 500 mg/kg	Genotoxic and mutagenic to brain tissue	[104]

Note: The reported particle size reflects the diameter of primary particles.

used in most of the neurotoxicological studies of TiO_2 NPs using mice as animal models mimic the actual pathways of human exposure to TiO_2 NPs. TiO_2 NPs can enter the human body via intentional or unintentional inhalation; therefore, intranasal administration was chosen as the exposure method in 10 studies. Intranasal drug administration is a promising method to bypass BBB, resulting in higher bioavailability and greater brain exposure compared to oral administration at the same dose.[71] Several studies have shown that intranasal administration of TiO_2 NPs in mice resulted in various neurological damages, including hippocampal cell apoptosis, tissue necrosis, oxidative damage, neuroinflammation, imbalance of glutamate metabolism, and altered gene expression.[72–79] These structural and functional impairments in the nervous system are closely related to behavioral deficits. Ze et al found that exposure to TiO_2 NPs (208–330 nm, 2.5 mg/kg body weight, BW) for 90 consecutive days via intranasal administration damaged the hippocampus structure, decreased long-term potentiation, altered gene expression, and caused spatial recognition deficits.[80] Intragastric administration, a method commonly used to assess the effects of oral exposure in the hazard assessment of environmental toxicants, was employed in 13 neurotoxicological studies of TiO_2 NPs using mice as animal models.[81] Pregnancy exposure to TiO_2 NPs (21 nm, 150 mg/kg) via intragastric administration caused the delayed appearance of neurobehavioral impairments in both dams and offspring, which may be related to disruption of the gut-brain axis.[82,83] Furthermore, exposure to low levels of TiO_2 NPs (6.5 nm, < 5 mg/kg) via intragastric administration during pregnancy and lactation retarded axonal and dendritic outgrowth, impaired cognitive ability, and increased hippocampal neurons apoptosis.[84–86] In addition to common impairments in the nervous system associated with TiO_2 NPs exposure, such as neuroinflammation, oxidative stress, and altered gene expression, Wang et al found that exposure to TiO_2 NPs (10 nm) increased the risk of PD.[87]

Neurotoxic Effects of TiO_2 NPs in Zebrafish

Zebrafish (Danio rerio) is commonly used as in vivo model system for studying the toxicity of nanomaterials due to its low cost, rapid growth, and significant homology to humans.[105] A total of 20 studies have investigated the neurotoxic effects of TiO_2 NPs in zebrafish, with 11 of them examining co-exposure to other compounds (Table 3). Among the 11 studies, 8 studies selected the embryonic stage of zebrafish for TiO_2 NPs exposure, 2 studies selected adult zebrafish, and one study selected zebrafish larvae. The most commonly used dose of TiO_2 NPs in studies involving co-exposure to other compounds was 100 μg/L. So far, TiO_2 NPs have been shown to enhance Pb,[106,107] decabromodiphenyl oxide (BDE-209),[108] cypermethrin,[109] triphenyl phosphate,[110] bisphenol A,[111,112] difenoconazole,[113] tetracycline,[114] and microcystin-LR[115] -induced neurotoxicity. TiO_2 NPs mainly enhance the neurotoxicity of these compounds by increasing their bioconcentration and bioavailability in zebrafish. Interestingly, co-exposure with TiO_2 NPs did not alter pentachlorophenol-induced neurotoxicity.[116]

Exposure to TiO_2 NPs alone is also able to induce a variety of neurotoxic effects in zebrafish. The embryonic stage of zebrafish is the most commonly used exposure stage for TiO_2 NPs exposure models, which may be attributed to the incomplete development of the BBB during this period.[126] TiO_2 NPs exposure during the embryonic stage of zebrafish significantly alters motor behavior, social behavior, and spatial recognition memory.[117–120] In addition to behavioral impairments, TiO_2 NPs exposure causes oxidative stress, promotes neuronal proliferation, decreases motor neuron axon length, alters gene expression, and increases cell apoptosis.[120–123] Two study chose the adult stage of zebrafish for TiO_2 NPs exposure, and their results suggested that TiO_2 NPs exposure caused cognitive deficit, promoted neuroinflammation, and altered biochemical constituents of the brain.[124,125]

Neurotoxic Effects of TiO_2 NPs in Other Animal Models

In vivo, studies investigating the potential neurotoxic effects of TiO_2 NPs exposure on animals other than rodents and zebrafish are relatively scarce. To date, only five studies investigated the neurotoxicity of TiO_2 NPs exposure in other animal models, namely *C. elegans, Tegillarca granosa, Pheretima hawayana*, and *Drosophila* melanogaster (Table 4).

The nervous system of the *C. elegans* model is structurally and functionally similar to that of mammals. Its small size, short life cycle, and high reproductive rate make *C. elegans* an advantageous model in neuroscience.[132] Long-term exposure to TiO_2 NPs (10 nm) resulted in severe defects in the development of AVL and DVB neurons that control

Table 3 Overview of Literature Investigating Neurotoxic Effects of TiO_2 NPs in Zebrafish

Model System	Particle Size	Exposure Dose	Neurotoxic Effects	Ref.
Zebrafish embryos	5 nm	0, 100 μg/L	Enhanced Pb-induced neurotoxicity	[106]
	5 nm	0, 100 μg/L	Enhanced BPA-induced neurotoxicity	[111]
	5–10 nm	0, 100 μg/L	Enhanced DIF-induced neurotoxicity	[113]
	7.04 nm	0, 100 μg/L	Enhanced Pb-induced neurotoxicity	[107]
	7.04 nm	0, 100 μg/L	Enhanced BDE-209-induced neurotoxicity	[108]
	7.04 nm	0, 1 mg/L	Enhanced CYP-induced neurotoxicity	[109]
	100, 300 nm	0, 100 μg/L	Enhanced TPhP-induced neurotoxicity	[110]
	25 nm	0, 100 μg/L	No changed PCP-induced neurotoxicity	[116]
	6.5 nm	0, 5, 10, 20, 40 μg/L	Decreased spatial recognition memory, Altered biochemical constituents of the brain, Over proliferation of glial cells, Cell apoptosis	[117]
	7.04 nm	0, 0.1 mg/L	Altered motor and social behaviors, Cell apoptosis, Oxidative stress, Promoted neuronal proliferation	[118]
	14.1 ± 0.6 nm	0, 0.1, 1 mg/L	Altered motor and social behaviors, Cell apoptosis, Oxidative stress	[119]
	21 nm	0, 0.01, 0.1, 1.0 mg/L	Altered motor behavior, Decreased CNS neurogenesis, Decreased motor neuron axon length, Altered gene expression	[120]
	30 nm	0, 100 μg/L	Cell apoptosis	[121]
	33.4 ± 1.9 nm	0, 0.1, 1, 10 μg/mL	Oxidative stress, Loss of DA secretion, Altered gene expression	[122]
	50 nm	0.1 mg/mL	Oxidative stress	[123]
Zebrafish larvae	/	0.5 mg/L	Enhanced TC-induced neurotoxicity	[114]
Adult zebrafish	5 nm	0, 100 μg/L	Enhanced BPA-induced neurotoxicity	[112]
	26.98 ± 0.85 nm	0, 100 μg/L	Enhanced MCLR-induced neurotoxicity	[115]
	20 nm	0, 10, 100 ppm	Altered biochemical constituents of the brain	[124]
	/	10 μg/mL	Caused cognitive deficit, Caused neuroinflammatory	[125]

Note: The reported particle size reflects the diameter of primary particles.

Table 4 Overview of the Literature Investigating Neurotoxic Effects of TiO_2 NPs in Other Animal Models

Model System	Particle Size	Exposure Dose	Neurotoxic Effects	Ref.
C. elegans	10 nm	0, 100, 100 mg/L	Impaired AVL and DVB neurons	[127]
	32 ± 2.9 nm	0, 100, 500 μg/mL	Decreased the length of axon, Impaired motor behavior	[128]
Drosophila melanogaster	20.68 ± 4.21 nm	0, 5, 10, 15, 20 mg/kg	Impaired motor behavior, Damaged the morphology of the NMJ, Altered gene expression	[129]
Tegillarca granosa	35 ± 5 nm	0, 1, 10 mg/L	Altered biochemical constituents of nervous system, Altered gene expression	[130]
Pheretima hawayana	200 nm	0, 1, 10, 100 μg/kg	Altered biochemical indices	[131]

Note: The reported particle size reflects the diameter of primary particles.

defecation in nematodes.[127] Neurons exposed to both anatase and rutile TiO_2 NPs exhibited shorter axon growth, which may be the reason for defective locomotion behavior in nematodes.[128]

Drosophila melanogaster is an excellent animal model for evaluating the neurotoxicity of various NPs due to its low-cost, physiological similarities to humans, and well-known behavioral and developmental characteristics.[133,134] The results of a recent study indicated that chronic exposure to TiO_2 NPs (approximately 20 nm, 20 mg/kg) induced deficits in motor behavior by disrupting the development of the neuromuscular junction (NMJ) in Drosophila.[129]

Tegillarca granosa and *Pheretima hawayana* are rarely studied in neurotoxicological research involving NPs. *Tegillarca granosa* inhabits intertidal mudflat, where the concentration of NPs is predicted to be higher than in other parts of the ocean.[135] Selecting *Tegillarca granosa* as an animal model provides better insights into the neurotoxicity of NPs in marine bivalve mollusks.[130] Exposure to TiO_2 NPs (200 nm) increased neurotransmitters concentrations, suppressed the activity of acetylcholinesterase (AChE), and decreased the expression of neurotransmitter-related genes, which may disrupt various physiological processes in *Tegillarca granosa*.[130] While TiO_2 NPs are increasingly being released into the soil, their effects on soil biota remain largely unknown.[136] The traditional sentinel species for soil toxicity testing is the earthworm, and *Pheretima hawayana* is a species of Egyptian earthworm. Exposure to TiO_2 NPs altered biochemical indices related to the function of the nervous system, such as inhibiting AChE, increasing antioxidant enzymes, and accumulating malondialdehyde (MDA).[131]

Neurotoxic Effects of TiO_2 NPs in vitro Models

In vitro models are widely used to assess neurotoxic effects on cellular functions.[137] Several studies have evaluated the neurotoxic effects of TiO_2 NPs using in vitro models. Primary hippocampal and cortical neurons are widely used in vitro models for neurotoxicology testing as they are easily polarized and form unique axons and dendrites. In addition, these models are used to study neuronal polarization, axon/dendrite morphology, synaptic formation, and central nervous system (CNS) functions.[138] Exposure to TiO_2 NPs impairs neuronal function, inhibits neuroblast proliferation, reduces cell viability, and increases cell apoptosis by promoting oxidative stress in both primary hippocampal and cortical neurons.[139–144] Furthermore, TiO_2 NPs inhibit neurite outgrowth of hippocampal neurons by interfering with glutamate metabolism and impairing N-methyl-D-aspartic acid (NMDA) receptor function.[145] According to some previous studies, the suppression of axonal development, dendritic development, and synapse development by TiO_2 NPs was associated with decreased expression of axon growth-related factors and inhibition of the Wnt/β-catenin and BDNF-TrkB pathways.[146–148] See Table 5 for details.

Rat pheochromocytoma (PC12) cell line and human SH-SY5Y neuroblastoma cell line have been used as models for neurotoxicity testing of TiO_2 NPs (Table 5). PC12 cell line shows morphological and functional differentiation similar to sympathetic neurons. PC12 cell line is a suitable model for studying the chemical disruption of neuronal differentiation, synthesis, storage, and release of neurotransmitters, function and regulation of ion channels, and the interaction of compounds with membrane-bound receptors.[160] A previous study revealed that treatment of PC12 cells with TiO_2 NPs (< 36 nm, < 200 μg/mL) decreased cell viability, increased cell apoptosis via oxidative stress, inhibited the neurite outgrowth, disturbed cell cycle, and disrupted the ubiquitin-proteasome system.[149–151] The human-derived SH-SY5Y cell line is preferred over the PC12 cell line as it avoids interspecific differences in chemical action.[161] The SH-SY5Y cell line is an excellent model for studying toxicity on proliferating or differentiated cells because it can be maintained as neuroblasts or induced to differentiate into more neuron-like morphologies.[161] TiO_2 NPs were shown to cause endoplasmic reticulum (ER) stress, autophagy, inhibition of cell proliferation, disturbance of the microtubule dynamics, and membrane damage in SH-SY5Y cells.[153–158] Several in vivo studies investigated the neurotoxic effects of TiO_2 NPs on mouse hippocampus. However, one in vitro study explored the neurotoxic effects of TiO_2 NPs on mouse hippocampal neuronal HT22 cells. The study revealed that TiO_2 NPs increased apoptosis of HT22 cells via oxidative stress- and calcium imbalance-mediated ER stress.[159]

Acute or prolonged exposure to TiO_2 NPs is associated with toxic effects on neuronal and glial cells.[162] Glial cells are critical cells of the nervous system, which serve as tissue-resident macrophages. Microglia are crucial regulators that influence nervous system development, maintenance of the neural environment, and response to injury and repair.[163] The immortalized mouse microglia cell line BV2 is often used as an alternative for primary microglia in cell experiments. Some previous studies showed that exposure of BV2 cells to TiO_2 NPs was associated with mitochondrial dysfunction and increased oxidative stress.[28,164] Astrocytes play a key role in innate and adaptive immune responses in CNS injury.[165] Due to advancements in cell culture technology, primary astrocytes have become a common primary cell model. Previous studies revealed that TiO_2 NPs induced mitochondria damage, oxidative stress, autophagy, neuroinflammation, and cell apoptosis in primary rat cortical astrocytes.[166–168] Other studies employed human glial cell lines as in vitro models for neurotoxicity studies to eliminate species differences. Some previous studies revealed that TiO_2 NPs

Table 5 Overview of the Literature on Neurotoxic Effects of TiO_2 NPs on Primary Neuron and Nerve Cell Lines

Model System	Particle Size	Exposure Dose	Neurotoxic Effects	Ref.
Primary hippocampal rat neurons	5.5 nm	0, 5, 15, 30 μg/mL	Decreased cell viability, Increased levels of LDH, Cell apoptosis	[139]
	5.5 nm	0, 5, 15, 30 μg/mL	Inhibited neurite outgrowth by interfering with glutamate metabolism, Impaired NMDA receptor function	[145]
	5.5 nm	0, 1.25, 2.5, 5 μg/mL	Inhibited dendritic development, Inhibition of the Wnt/β-catenin pathway	[147]
	36.83 nm	0, 5, 15, 30 μg/mL	Inhibited axonal development	[146]
	/	0, 5, 15, 30 g/mL	Inhibited synapse development, Inhibition of the BDNF-TrkB pathway	[148]
Primary rat cortical neurons	26.2 ± 10.7 nm	0, 30, 100 μg/mL	Limited hazard for neuronal function	[140]
	6–142 nm	0, 3.1, 6.3, 12.5, 50 μg/mL	Decreased cell viability	[141]
	200–700 nm	0, 5, 10, 15, 20 μg/mL	Decreased proliferation of neuroblasts	[142]
Primary mouse cortical neurons	20–80 nm	20, 50 mg/cm^2	Oxidative stress	[144]
	< 100 nm	0.01–300 μg/cm^2	Oxidative stress	[143]
PC12 cells	20–50 nm	0, 10, 50, 100 μg/mL	Oxidative stress Cell apoptosis	[149]
	< 25 nm	0, 50, 100, 200 μg/mL	Oxidative stress, Dysfunction of the ubiquitin-proteasome system, α-Syn aggregation	[150]
	< 36 nm	0, 0.01, 0.1, 1, 10, 100 μg/mL	Inhibited the neurite outgrowth	[151]
	Anatase-20 nm Rutile-20 nm Micro-1000 nm	0, 25, 50, 100, 200 μg/mL	Decreased cell viability, Increased levels of LDH, Oxidative stress, Cell apoptosis, Disturbed cell cycle, Altered gene expression	[152]
SH-SY5Y cells	5 nm	0, 5, 10, 50, 100 μg/mL	Cell apoptosis, Oxidative stress, ER stress	[153]
	20 nm	0, 2, 10, 50, 100 μg/mL	Disturbed cell cycle, Oxidative stress, Membrane damage, Autophagy	[154]
	25 nm	0, 80, 120, 150 μg/mL	Disturbed cell cycle	[155]
	100–150 nm	0, 100 μg/mL	Altered cellular morphology, Disturbed the microtubule dynamics	[156]
	115.73 ± 0.67 nm	0.75–75 μg/mL	Inhibited cell proliferation	[157]
	/	0, 5, 10, 20, 40, 80, 160 μg/mL	Decreased cell viability, Increased levels of LDH, Promoted inflammation	[158]
HT22 cells	50 nm	0, 50, 100, 200 μg/mL	Cell apoptosis, Oxidative stress, ER stress	[159]

Note: The reported particle size reflects the diameter of primary particles.

inhibited cell proliferation, induced morphological changes, decreased immuno-location of F-actin fibers, and increased cell apoptosis in U374 cells.[169,170] Furthermore, several studies have investigated the neurotoxic effects of TiO_2 NPs in a co-culture of glial cells and other cells. For example, Yang et al showed that TiO_2 NPs stimulate the inflammatory reaction in brain microglia and damage neuron using a co-culture model of primary microglia and PC12 cell line.[171] Similarly, TiO_2 NPs was shown to stimulate the inflammatory reaction in brain microglia and damage neurons in co-culture models of BV2 and N27 mesencephalic neurons, and BV2 and N2a neuroblastoma cells.[172,173] See Table 6 for details.

Most in vivo and in vitro studies have evaluated the neurotoxic effects of TiO_2 NPs in the cortex, hippocampus, and cerebellum. However, to the best of our knowledge, no studies have evaluated the neurotoxic effects of TiO_2 NPs on other brain regions. The BBB is effective in protecting the brain from chemical damage. Therefore, there is a need to understand the effects of TiO_2 NP_S on the BBB. A previous study exploring the effects of TiO_2 NPs on an in vitro model of BBB established by co-culturing primary human brain microvascular endothelial cells (HBMECs) and primary human astrocytes, revealed that TiO_2 NPs increased the permeability of the BBB.[31] Another study showed that acute or long-term exposure of an in vitro model of the BBB established by co-culturing primary rat endothelial cells and glial cells to TiO_2 NPs was associated with BBB dysfunction related to increased inflammatory response and altered expression of the ABC transporter.[174] Moreover, treatment of T98G human glioblastoma cells with TiO_2 NPs was associated with changes in the transcriptome, suggesting that exposure to TiO_2 NPs could compromise BBB integrity and cause neuroinflammation.[175] Furthermore, TiO_2 NPs can be internalized by dorsal root ganglion cells (DRG) and cause damage via apoptosis.[176,177] Yu et al showed an association between the toxic effects of TiO_2 NPs on olfactory bulb neuron cells and its pathogenicity to neurodegenerative diseases.[178] Furthermore, exposure to TiO_2 NPs was associated with varying degrees of cytotoxicity to the human cerebral endothelial cell line (HCECs), human neural stem cell line (hNSCs), and neuroectodermal stem cell line (1C11) models.[179–181] See Table 7 for details.

Table 6 Overview of the Literature on Neurotoxic Effects of TiO_2 NPs in Primary Glial Cells and Glial Cell Lines

Model System	Particle Size	Exposure Dose	Neurotoxic Effects	Ref.
BV2 microglia	20–30 nm	0.1–200 μg/mL	Mitochondrial dysfunction, Oxidative stress	[28]
	30 nm	2.5–120 ppm	Oxidative stress, Mitochondrial dysfunction	[164]
Primary rat cortical astrocytes	10, 20 nm	0, 6.25, 12.5, 25, 50, 100 mg/L	Cell apoptosis, Morphological changes	[168]
	50 nm	116 μg/mL	Mitochondria damage, Oxidative stress, Autophagy, Neuroinflammation	[167]
	Anatase-360 nm P25-540 nm Rutile-360 nm	0, 25, 50, 100 mg/kg	Mitochondria damage, Oxidative stress	[166]
C6 and U373 cells	< 50 nm	0, 20 μg/cm^2	Oxidative stress, Mitochondrial damage, Cerebral damage, Neurodegenerative diseases	[170]
	40–200 nm	0, 2.5, 5, 10, 20, 40 μg/cm^2	Inhibited cell proliferation, Morphological changes, Decreased immuno-location of F-actin fibers, Cell apoptosis	[169]
Primary microglia and PC12 cells	20 nm	0, 0.25, 0.5 mg/mL	Neuroinflammation	[171]
BV2 microglia and N27 mesencephalic neurons	< 330 nm	2.5–120 ppm	Promoted inflammation, Cell apoptosis, Altered cell cycle, Decreased energy metabolism	[172]
Human astrocytoma cells-D384 and SH-SY5Y cells	69.3 ± 0.4 nm	0, 15, 31, 125 μg/mL	Disturbed cell cycle, Membrane damage, Mitochondrial dysfunction	[162]
BV2-N2a, ALT-N2a, ALT-BV2 co-culture	44.4 ± 0.2 nm	0, 5, 30, 100 μg/mL	Decreased cell viability, Oxidative stress, Promoted inflammation	[173]

Note: The reported particle size reflects the diameter of primary particles.

Table 7 Overview of the Literature on Neurotoxic Effects of TiO_2 NPs in Other Cells

Model System	Particle Size	Exposure Dose	Neurotoxic Effects	Ref.
T98G human glioblastoma cells	786.9 ± 176.7 nm	0, 20 μg/mL	Disrupted BBB integrity, Neuroinflammation	[175]
Primary HBMECs Primary human astrocytes	29.56 ± 10.72 nm	0, 12.5, 25, 50, 100 μg/mL	Stimulated F-actin stress fiber formation, Induced formation of paracellular gaps, Up-regulated ROCK II	[31]
Primary rat glial cells	25.2 nm	0–500 mg/mL	Inflammatory response	[174]
Endothelial cells		0–100 mg/mL	Altered expression of ABC transporter	
DRG cells of chick embryos	25 nm	0, 0.5, 5 μg/mL	Oxidative stress, Promoted inflammation, Cell apoptosis, Altered axonal retrograde transport	[176]
	200–400 nm	0, 250 μg/mL	Cell apoptosis	[177]
Olfactory bulb neurons	≤20 nm	0, 1, 5, 10 mg/mL	Cell apoptosis, Decreased expression of OMP and tyrosine hydroxylase (TH)	[178]
HCECs	21 nm	0.12–75 μg/mL	Oxidative stress, Autophagy	[179]
hNSCs	80 nm, < 44 μm	0, 0.01, 0.1, 1.0 mg/mL	Acute membrane permeability	[180]
1C11	22 nm	5, 10, 25, 50 μg/mL	Oxidative stress, Neuroinflammation, Altered neuronal signaling, Altered neuronal homeostasis	[181]

Note: The reported particle size reflects the diameter of primary particles.

Factors Influencing the Neurotoxic Potential of TiO_2 NPs

The neurotoxic effects of TiO_2 NPs are influenced by various factors. The exposure characteristics, such as exposure dose, method, duration, and species, can influence the toxic effects of TiO_2 NPs in vivo. A review of the literature showed that the exposure dose in vivo and in vitro experiments was larger than the actual exposure dose of the population. According to a previous study, the levels of TiO_2 NPs in air and water ranged from 0.7 to 16 μg/L.[182] It is estimated that children have an intake of TiO_2 NPs of about 2–3 mg/kg/day, while adults have a TiO_2 NPs intake of about 1 mg/kg/day.[2] Human exposure to TiO_2 NPs is mainly through dietary intake and air inhalation. Although the exposure methods selected in animal studies attempted to mimic human exposure closely, there are some gaps. For example, the system for intranasal administration is simple compared to inhalation administration. Furthermore, intranasal administration is significantly affected by the inhalational dose.[183] The intranasal administration volumes in rodents at a given time should be limited to approximately 5 μL per nostril since volumes greater than this are likely to become wasted.[184,185] Furthermore, ingested TiO_2 NPs first interacts with the oral mucosa. However, intragastric administration does not interact with the oral mucosa and is thus associated with significant differences in absorption, bioavailability, and metabolism with implications for assumptions and models of toxicity kinetics.[81] In addition, the exposure period and duration also influence the neurotoxic effects of TiO_2 NPs.[45,94] However, the exposure duration in experiments tends to be shorter than that in humans. Species differences are often unavoidable. Therefore, there is a need to conduct epidemiological studies exploring the neurotoxic effects of TiO_2 NPs on humans.

Furthermore, the physical and chemical properties of TiO_2 NPs can affect their neurotoxicity. Particle size is key. In general, small particles are more likely to be absorbed and thus exert toxic effects.[186] According to some previous studies, the neurotoxic effects of TiO_2 NPs depend on particle size.[60,168] The hydrodynamic diameter or secondary particle sizes of TiO_2 NPs are important with respect to neurotoxicity. While smaller NPs may seem more neurotoxic, they are also more likely to clump together and form aggregates.[186] Theoretically, the particle aggregation would increase the effective particle size thus reducing the neurotoxic potential. Several studies have used dynamic light scattering (DLS) to determine the effects of hydrodynamics or secondary particle size of TiO_2 NPs on neurotoxicity. However, no studies have explored the effect of aggregate particle size on the neurotoxicity of TiO_2 NPs. The zeta potential of TiO_2 NPs has also been investigated in most neurotoxicological studies. Since most cell membranes are negatively charged, the zeta potential affects the tendency of NPs to penetrate the membrane, with cationic particles generally exhibiting higher toxicity associated with cell wall damage.[187] Furthermore, the surface charge of the nanoparticles can determine the degree of aggregation.[122,162,164] However, further studies are needed to investigate whether the zeta potential affects

the neurotoxicity of TiO_2 NPs. In addition, the toxicity of TiO_2 NPs is dependent on crystalline phases. The anatase form of TiO_2 NPs is more neurotoxic than that of rutile TiO_2 NPs and P25 TiO_2 NPs since anatase has a higher ability to induce oxidative stress.[152,166,188]

Taken together, various factors can affect the neurotoxic potential of TiO_2 NPs, including physical and chemical properties of TiO_2 NPs, and exposure dose, exposure duration, exposed species. However, the specific effects of these factors on the neurotoxic effects of TiO_2 NPs still need to be systematically compared.

Reflections on Neurotoxicity Induced by TiO_2 NPs

Most studies to date have focused on rodents, and most experimental exposures used are not very realistic for human exposure. In addition, there is currently limited information on the levels of TiO_2 NPs in the environment, consumer goods, and food products. For humans, more accurate monitoring is needed to determine daily exposure levels, particle characteristics and exposure route, all of which affect the neurotoxic potential of TiO_2 NPs. Evaluating and availing data on TiO_2 NPs levels in different environmental media helps to reliably estimate human exposure and thus assess the risk of TiO_2 NPs. Furthermore, the degree of uptake through the digestive system, respiratory system, potential BBB crossing, and potential translocation to or even accumulation in nervous system should be further investigated. This information will indicate which route of exposure mitigation is most valuable for human health protection. However, apart from the recommended exposure limits (REL) established by the National Institute for Occupational Safety and Health (NIOSH), no other regulatory agencies have set occupational or environmental exposure limits for TiO_2 NPs.[9] There are limitations in the monitoring methods of TiO_2 NPs. There is an urgent need to develop appropriate methods for reducing TiO_2 NPs in environmental media and food to prevent their potentially harmful health effects.[189]

The specific mechanisms behind the neurotoxic effects of TiO_2 NPs have only been explored through animal and cell experiments. TiO_2 NPs increase the formation of reactive oxygen species (ROS) in the brain, thus inducing oxidative stress. Ze et al reported that TiO_2 NPs induced oxidative stress thus causing brain damage through overactivation of the p38-Nrf-2 signaling pathway.[78] Oxidative stress can induce neuroinflammation, thus further aggravating cell damage.[63,73,92] Cell damage, including structural and functional damage, is associated with increased onset and development of neurodevelopmental or neurodegenerative diseases, such as autism spectrum disorder (ASD) and PD.[87,100] Cell damage is also linked to behavioral deficits.[46,57] Abnormal motor ability could be caused by a decrease in the axon length of motor neurons.[120] In addition, changes in hippocampal synaptic plasticity could lead to decreased spatial recognition.[80] The development of axons, dendrites and synapses is regulated by various signaling pathways. TiO_2 NPs impair the growth of axons and dendrites through excessive activation of the ERK1/2/MAPK signaling pathway.[84] In addition, impairment of dendritic growth by TiO_2 NPs is also related to inhibition of the Wnt/β-catenin signaling pathway.[147] Moreover, suppression of the neuronal synaptic outgrowth by TiO_2 NPs is linked to the inhibition of the BDNF-TrkB signaling pathway.[148] Furthermore, the accumulation of TiO_2 NPs in the brain could cause alterations in brain biochemistry and changes in neurotransmitter levels, contributing to behavioral changes.[45,57,117] Although all of these studies confirm that TiO_2 NPs cause neurotoxic effects through different mechanisms, most of the evidence on the neurotoxic effects of TiO_2 NPs is fragmentary and is obtained from different species. Furthermore, few of these mechanism studies have explored whether the neurotoxic effects of TiO_2 NPs are mediated through synergistic interactions of multiple brain regions, organs, and systems. Whether TiO_2 NPs with different characteristics cause different degrees of toxic effects through different mechanisms remains be further explored. Extensive systematic studies are needed to fully elucidate the neurotoxic mechanisms of TiO_2 NPs, which will be helpful for the prevention and treatment of neurotoxic effects of TiO_2 NPs.

Conclusion

Animals and humans can be exposed to TiO_2 NPs through different exposure pathways, thus posing health hazards. At present, the neurotoxic effects of TiO_2 NPs have only been evaluated through animal models, including rats, mice, and zebrafish, and cell studies, including primary neurons, PC12, and SH-SY5Y cell lines. TiO_2 NPs can induce oxidative stress, promote neuroinflammation, alter brain biochemistry, or damage neurons. Neuronal damage can further lead to various behavioral disorders and is closely associated with increased onset and development of neurodevelopmental or

neurodegenerative diseases. However, due to the lack of relevant epidemiological studies, whether TiO_2 NPs are linked to neurodevelopmental or neurodegenerative diseases in humans remains unknown. Furthermore, the neurotoxic potential of TiO_2 NPs can be affected by various factors. There is a need for researchers to understand the neurotoxic effects of TiO_2 NPs on humans and develop strategies for mitigating the effects of TiO_2 NPs on human health.

Abbreviations

NMs, nanomaterials; TiO_2 NPs, titanium dioxide nanoparticles; FPs, fine particles; PB, placental barrier; BBB, blood-brain barrier; *C. elegans, Caenorhabditis elegans*; SD rats, Sprague-Dawley rats; PND, postnatal day; BDNF, brain-derived neurotrophic factor; BW, body weight; BDE-209, decabromodiphenyl oxide; NMJ, neuromuscular junction; AchE, acetylcholinesterase; MDA, malondialdehyde; CNS, central nervous system; NMDA, N-methyl-D-aspartic acid; ER, endoplasmic reticulum; HBMECs, human brain microvascular endothelial cells; DRG, dorsal root ganglion; HCECs, human cerebral endothelial cell line; HNSCs, human neural stem cell line; 1C11, neuroectodermal stem cell line; DLS, Dynamic light scattering; REL, recommended exposure limit; NIOSH, National Institute for Occupational Safety and Health; ROS, reactive oxygen species; ASD, autism spectrum disorder; PD, Parkinson's disease.

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