Chapter

In-Utero Neurotoxicity of Nanoparticles

Nikhat J. Siddiqi, Sabiha Fatima, Bechan Sharma and Mohamed Samir Elrobh

Abstract

The unique physicochemical properties of nanoparticles (NPs) make them widely used in cosmetics, medicines, food additives, and antibacterial and antiviral compounds. NPs are also used in therapy and diagnostic applications. Depending on their origin, the NPs are commonly classified as naturally occurring and synthetic or anthropogenic NPs. Naturally occurring nanoparticles can be formed by many physical, chemical, and biological processes occurring in all spheres of the earth. However, synthetic NPs are specifically designed or unintentionally produced by different human activities. Owing to their nano size and special properties, the engineered NPs can enter the human body through different routes such as dermal penetration, intravenous injection and inhalation. NPs may accumulate in various tissues and organs including the brain. Indiscriminate use of NP is a matter concern due to the dangers of NP exposure to living organisms. It is possible for NPs to cross the placental barrier, and adversely affect the developing fetus, posing a health hazard in them by causing neurodevelopmental toxicity. Thus, NP-induced neurotoxicity is a topic that demands attention at the maternal-fetal interface. This chapter summarizes the routes by which NPs circumvent the blood-brain barrier, including recent investigations about NPs' neurotoxicity as well as possible mechanisms involved in neural fetotoxicity.

Keywords: nanoparticle, neurotoxicity, placental barrier, blood-brain barrier

1. Introduction

The term nanoparticle (NP) refers to particles with at least one dimension less than 100 nanometers [1]. NPs are an essential part of earth's biogeochemical system, produced by many physical and chemical processes including different natural and human activities. They are commonly classified as naturally occurring and synthetic or anthropogenic NPs, depending on their origin. Synthetic or anthropogenic NPs can be further categorized into two types: incidental and engineered nanoparticles [2]. Naturally occurring nanoparticles can be formed by chemical, photochemical, mechanical, thermal, and biological processes occurring in all spheres of the Earth. NPs such as alumina, iron oxide, gold, sulfur manganese oxide, and so on derived from natural sources can be found in volcanic ash, fine sand, ocean spray, and even some biological matter [1]. Incidental nanoparticles are unintentionally produced as a byproduct of human day-to-day activities involving combustion process such as running diesel engines, large-scale mining, and even starting a fire.

On the other hand, the engineered or manufactured NPs such as silver, gold, zinc, metal oxides like manganese dioxide (MnO_2), aluminum oxide Al_2O_3, titanium oxide (TiO_2) of controlled shape, sizes, and compositions are specifically designed and deliberately synthesized by human beings [3]. Engineered NP include nonmetals like carbon nanotubes and quantum dots, polymers like chitosan, alginate, lipids like stearic acid, and metal sulfide like CuS, AgS, ZnS and so on [4]. Another classification of NP is their grouping into organic nanoparticles and inorganic nanoparticles. Organic nanoparticles include liposomes, dendrimers, micelles and so on. Examples of some of inorganic NP include metallic NP like gold, iron, silver, aluminum, titanium oxide (TiO_2), and zinc oxide (ZnO). Nanomaterials can also be classified based on their size for example zero-dimension, one dimension, two dimension, and three dimensions [5]. Silver, gold, copper, and platinum are some of the most commonly used metals NP. Metal-based NPs can be easily conjugated with various functional groups, like polylysine, polyethylene glycol (PEG) or bovine serum albumin [6, 7].

The technological advancements of human society as well as progress in the field of nanotechnology have shown a sharp rise in consumer products that deliberately include synthetic nanoparticles [8]. This has resulted in high levels of exposure to many types of synthetic NPs, and it is likely that this trend will continue in future. The easiest place to find these nano-enabled products in our own homes is in health care products, cosmetics, and food additives. In the past decade, many companies have used ZnO and TiO_2 NPs as sun block materials because these materials are very effective at absorbing UV radiation [9]. Some commonly used nanomaterials as food additives include silver, silicon dioxide (SiO_2), titanium TiO_2, and iron oxide (Fe_2O_3) [10]. Silver NPs are also commonly used as antibacterial and antiviral agents, while gold NPs are used for drug delivery, photothermal therapy and diagnostic applications, and polymeric NPs are used for controlled and targeted drug delivery [11].

Extensive use of engineered NP poses risk to human health. The health hazards are cause of concern in pregnant women and their unborn children. Therefore, it is important to study the toxic effect of NP on developing fetuses. In this chapter, we summarize the developmental toxicity of NP on the nervous system.

2. Factors affecting the toxicity of nanoparticles

The embryonic toxicity of nanoparticles depends on their bioaccumulation, which in turn depends on the following [12]:

- Chemical composition, particle size, shape, surface modification, and degree of agglomeration. Smaller NPs have been shown to induce more pronounced blood brain barrier (BBB) breakdown, brain edema and neuronal injuries, glial fibrillary acidic protein upregulation, and myelin vesiculation in young animals [13]. Similarly, different shapes of the same NP have been shown to induce different cellular responses by nonspecific uptake into cells [14]. In vivo animal studies have demonstrated that administration of higher doses of smaller particles NP caused their increased accumulation in placental and embryonic/fetal tissues [15].

- Type of coating, concentration of particles, surface charge of the particles, zeta potential, and crystal form. Unmodified fullerene NPs can generate reactive oxygen species (ROS) to damage cells, whereas surface-modified

fullerene NPs have been demonstrated to enter cerebral microvessel endothelial cells and protect these cells by attenuating ROS-induced cellular damage, such as F-actin depolymerization [16].

- Other factors include the pH of the solution, salt concentration and the temperature [17], "protein corona," chemical characteristics, metal impurities, and degradation properties [18].

- Particle dissolution also alters the particle presence [15].

- Routes of exposure in in vivo studies. Inhalation is the main route of exposure in occupational and environmental settings. Experimental studies commonly use intravenous and intra peritoneal routes [15].

- The anatomical and functional state of the placenta [19, 20] and the critical period of exposure during gestation [15].

- Zeta potential of the NP. The charges on the NP determine their interactions with the biological system. Also, the zeta potential determines the stability of the NP in colloidal systems [21].

3. Entry of nanoparticles

The exogenous entry of engineered NP is mainly from hand-to-mouth contact in the workplaces. Nanoparticles enter the body through food, drinking water, drugs, or exposure during medical procedures. Inhalation of airborne nanoparticles is also an important point of entry into the body [22]. Larger particles are trapped in the nasopharyngeal region (5–30 μm), while the smaller particles (1–5 μm) get deposited in the tracheobronchial region. These particles can be removed by mucociliary clearance. Finally, the remaining submicron particles (< 1 μm) and nanoparticles (< 100 nm) with the smallest size distribution penetrate deeply into the alveolar region, where removal mechanisms may be insufficient. Nanosized particles can reach the alveolar region of the lungs where they get in contact with the alveolar epithelium. From the alveolar epithelium these particles can cross the blood-air-tissue barrier and enter the bloodstream to reach various organs [22]. Inhaled ultrafine particles may get deposited in the olfactory mucosa from where they can translocate in the central nervous system (CNS), which in turn might cause neurotoxicity. Studies have shown that the CNS may be a crucial target for nanoparticle inhalation or intranasal installation exposure [23, 24]. The third route of entry of NP into the body is through dermal penetration [22, 25].

The NPs enter the CNS through three main routes: (1) Transport through the lymphatic and circulatory system; (2) Activity of the mucocilliary escalator followed by oral exposure; and (3) Transport through the olfactory and trigeminal nerves [18, 26]. This pathway involves the passage of nanoparticles through the olfactory epithelium and the neurons associated with it to the brain [18]. Carbonaceous nanomaterials have been reported to show increased access to the brain via the facilitation of olfactory mucosa and olfactory nerve [23]. After uptake, NPs can permeate into other parts of the brain by simple diffusion and then travel along the direction of the convection of the interstitial fluid and the cerebrospinal fluid flow [27].

4. Barriers that restrict the entry of substances into the brain

4.1 Blood: Brain barrier (BBB)

The blood-brain barrier (BBB) is a term used to describe the unique properties of the microvasculature of the central nervous system (CNS). CNS is made of continuous and non-fenestrated vessels. These blood vessels function to regulate the movement of molecules, ions, and cells between the blood and the CNS [28, 29]. The central nervous system of vertebrates is isolated from the rest of the body by BBB. Normal functioning of BBB is essential for homeostasis. The BBB is made of two main types of cells, that is, endothelial cells (EC) and mural cells. ECs function to regulate the movement of ions, molecules, and cells between the blood and the brain. ECs are held together by tight junctions (TJs), which greatly restrict the paracellular movement of solutes [30]. The tight junctions hold CNS ECs in place forming a paracellular barrier to molecules and ions [30].

Mural cells are the cells surrounding the large vessels and pericytes, which are present on the abluminal surface of the endothelium [31]. Pericytes and astrocytes are considered the key cell types involved in BBB regulation through their interactions with brain endothelial cells. Astrocytes interact with brain endothelium and are thought to be involved in the maintenance of BBB endothelial cell properties [32] and regulate BBB permeability [33]. The BBB restricts the movement of molecules by forming a physical barrier, which is represented by tight junctions between the endothelial cells. The endothelial cells express two main types of transporters: the efflux transporters, which transport lipophilic substances toward the blood [34] and nutrient transporters, which transport nutrients into the CNS and remove waste products from the CNS to the blood [35]. The EC cells of the CNS are characterized by a higher number of mitochondria [36]. These mitochondria supply the BBB with Adenosine triphosphate to carry out their transport processes.

Other cell types of the BBB are astrocytes and immune cells, mainly macrophages and microglial cells [30]. Pericytes, astrocyte end-feet, and a discontinuous basal membrane support the functions of the BBB. The highly selective functionality of the BBB is due to endothelial tight junctions that are assisted by astrocytes and pericytes. The tight influx control is complemented by the efflux transport system, which rapidly eliminates classic xenobiotics and NMs buildup in the brain [37]. However, nanomaterials have been reported to cross the BBB via a transcytosis-mediated route [38].

4.2 Metabolic barrier

A second barrier observed in the nervous system is the metabolic barrier. The metabolic barrier is composed of enzymes and transport systems [39]. The metabolism of endothelial cells plays an important role in the function of BBB. L-Dihydroxyphenylalanine is the precursor of dopamine which enters the brain through the neutral amino acid-transport system. However, its entry is restricted due to L-Dihydroxyphenylalanine decarboxylase and monoamine oxidase inside the endothelial cells of the brain capillaries. This "enzymatic blood-brain barrier" limits the passage of L-Dihydroxyphenylalanine into the brain (https://nba.uth.tmc.edu/neuroscience/m/s4/chapter11.html). The brain capillaries contain enzymes that metabolize neurotransmitters. These enzymes include endopeptidases, cholinesterases, aminopeptidases, and Gamma-Aminobutyric acidtransaminases. The brain capillaries also contain drug and toxin-metabolizing enzymes found in the liver [40].

The endothelium of the BBB lacks pinocytic vesicles. This limits pinocytosis by the cells of BBB. The cells of BBB express many enzymes on the intra and

extracellular surfaces, which restrict the movement of substances through the BBB. P-glycoproteins, and similar substances present on the endothelial cells also help to eliminate various endogenous and exogenous toxins [18]. P-glycoproteins cause multi-drug-resistant cancer cells to pump out the drugs. The endothelial cells have P-proteins, which help to pump some hydrophobic substances like cyclosporin A, domperidone, digoxin and so on into the blood.

4.3 Blood-Cerebrospinal fluid barrier

A third barrier represented by the blood-Cerebrospinal fluid barrier also serves to prevent indiscriminate entry of substances in the CNS [41]. This barrier is made up of choroid plexus epithelial cells. The blood-Cerebrospinal fluid barrier is made up of choroid plexus epithelial cells, which have smaller tight junctions than the BBB endothelia. The blood-Cerebrospinal fluid barrier prevents the entry of macromolecules into the Cerebrospinal fluid. The active transport systems of the BBB actively remove therapeutic organic acids from the Cerebrospinal fluid [42].

5. Circumvention of the blood-brain barrier by NPs

Some of the ways by which NP can circumvent the blood brain barrier include the following (**Figure 1**):

- Transcellular diffusion—Low molecular weight solid lipid nanoparticles [43].
- Paracellular diffusion—this route is taken by silica and reduced graphene oxide NP [44, 45].
- Receptor-mediated transcytosis—Engineered nanomaterials with ligands such as transferrin, insulin, ApoE can avoid the BBB by this route [46].

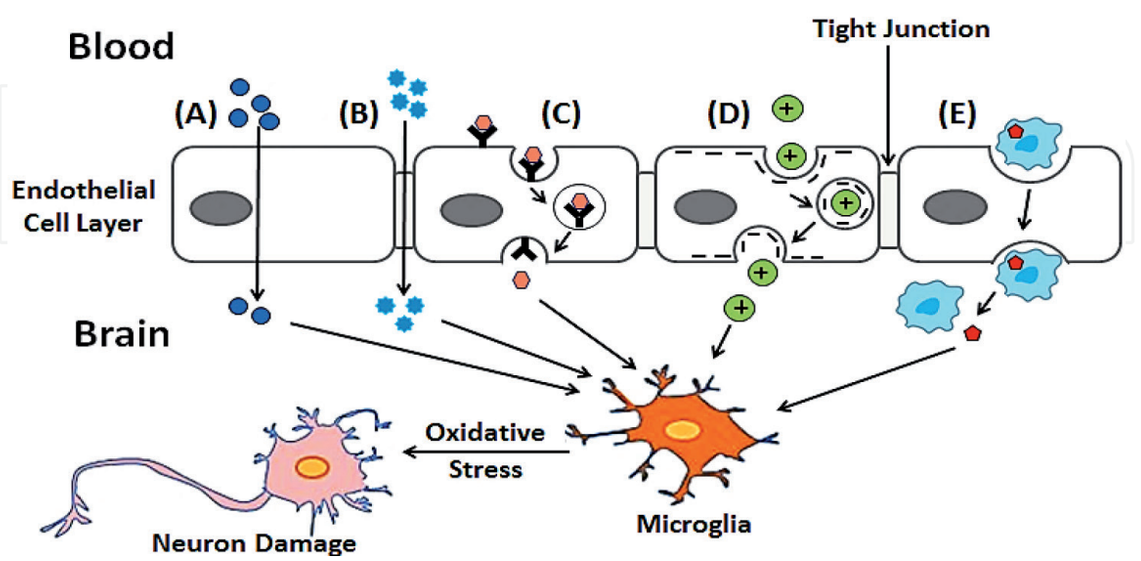

Figure 1.
Possible pathways through which nanoparticles cross the blood-brain barrier (BBB) and damage the neurons. Engineered nanomaterials with specific physicochemical properties can cross the BBB through various transport pathways such as (A) transcellular diffusion; (B) paracellular diffusion; (C) receptor-mediated transcytosis; (D) adsorptive-mediated transcytosis; and (E) cell mediated transcytosis. Nanoparticles interact directly with neuronal cells and cause neurotoxicity.

- Adsorptive-mediated transcytosis—Cationic albumin-conjugated pegylated NPs enter the brain by adsorptive-mediated transcytosis [47].

- Cell mediated transcytosis—Macrophages take up engineered nanomaterials and release them into the CNS [48].

6. Translocation of nanoparticles through the placenta

Exposure of pregnant mice to different NPs has been reported to induce pregnancy complications or damage to the fetus. Placenta is the maternal-fetal interface, which is formed of both maternal and fetal tissues that protects the embryo from harmful substances in the maternal blood. Placenta functions to exchange oxygen, nutrients, metabolic waste, and other molecules between the maternal and fetal bloodstream [49]. Factors that control the transfer of substances between maternal and fetal circulation include membrane surface area and thickness, blood flow, hydrostatic pressure in the intervillous chamber and the difference between fetal and maternal osmotic pressure [50]. Beside the placenta, amnion, chorion and parietal decidua also surround the fetus. These membranes are impervious to most of the xenobiotics in the maternal blood [51].

The brains from the fetuses of rats and mice have shown the presence of NP when the pregnant mothers were exposed to NP [52, 53]. Nano-silica and nano-TiO_2 have been reported to accumulate in the placenta, fetal liver, and fetal brain when injected to pregnant mice [54]. The extent of transfer of nanoparticle across the placenta depends on the characteristics and functionalization of the particles [55, 56]. NPs with diameters 1–100 nm have been shown to transverse the placental barrier and were detected in the brain of the offspring [57, 58]. Gestational age is an important factor affecting the toxicity of NP on the fetus [50]. Fennell et al. [59] have demonstrated that AgNP administered through oral and IV route on gestational day 18 resulted in placental accumulation after 48 h. Campagnolo et al. [60] demonstrated that inhalation of Ag NP during the first gestational day until the fifteenth gestational day in female rats caused fetal resorption. This was accompanied with an increased expression of pregnancy-relevant inflammatory cytokines in the placentas. Zhang et al. [19] have shown that maternal exposure of mice to TiO_2 NP decreased in angiogenesis in placental tissue and activated apoptotic pathways through caspase-3 in placental tissue.

Studies have demonstrated that various NPs can cross the BBB and placental barrier [61, 62]. Titanium dioxide nanomaterials ($nTiO_2$) have been reported to cross the placental barrier in pregnant mice and cause neurotoxicity in their offspring. Toxicity to the brain cells was reported to be caused due to necrosis (**Figure 2**) [63].

6.1 Mammalian embryonic model

Rodents, primarily mice and rats have been commonly used for gestational translocation of NPs [15]. Mice have been commonly used for mammalian embryo toxicity studies [64–66]. Although rabbits have been used in fewer studies, rabbit placentae bear closer resemblance to human placentae than that of other rodents. Therefore, rabbits should be the preferable animal model to study gestational particle exposure [15]. Other nonmammalian species like drosophila and zebrafish have also been used in *in vivo* studies [67].

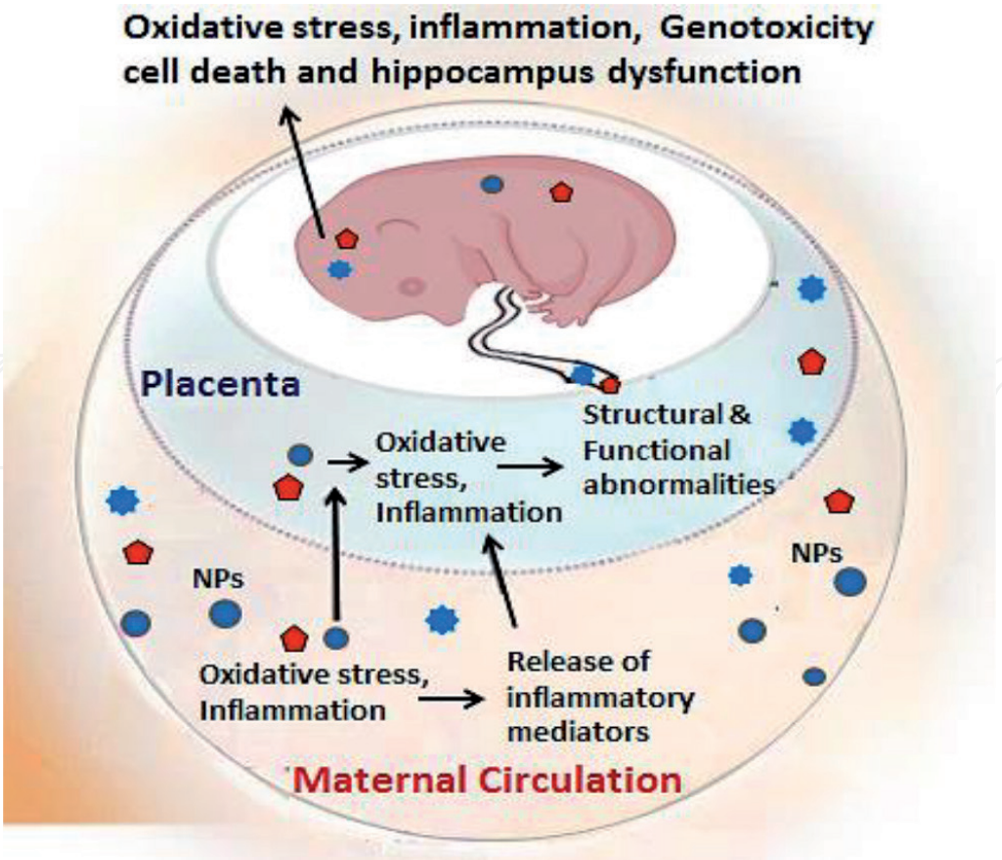

Figure 2.
Maternal exposure of nanoparticles (NPs) results in neural fetotoxicity and developmental abnormalities. Direct translocation of NPs from maternal circulation across the placental barrier into growing fetus has been recognized as the major factor involved in NP-induced fetotoxicity. Accumulation of NPs in the fetus can cause structural and functional abnormalities in various fetal tissues, including the central nervous system (CNS) which is the main target of metallic NPs. Oxidative stress, induction of inflammatory responses, alterations in gene expression, DNA damage, necrosis, and apoptosis are the mechanisms associated with NP-induced neural fetotoxicity.

6.2 Effects of nanoparticles on fetal brain

The developing brain is highly vulnerable to nanomaterials [18] due to the incomplete development of BBB in the fetus [68]. The CNS shows considerable plasticity in the early stages of development and therefore highly susceptible to the toxic effects of NP [69]. The placenta is a multifunctional organ forming a barrier between maternal and fetal tissues. In utero exposure to NPs is one of main routes of exposure during the development of the nervous system [70]. Neurodevelopmental studies have shown that both male and female offspring show differential phenotypes after prenatal insults by NPs [18].

Among various NPs, many studies have been reported on the neurotoxicity of TiO_2 NP. Injection of TiO_2 NP into pregnant mice resulted in altered expression of genes associated with brain development and function of the central nervous system in embryos [71]. The effects of TiO_2 seem to continue on the developing brain even during lactation [72]. The effects of titanium dioxide nanomaterials in pregnant mice include reduced size of the placenta and disrupted anatomical structure of the fetal brain and liver. Toxicity to the brain cells was reported to be caused due to necrosis [63]. One study showed that TiO_2 NPs administered subcutaneously to pregnant mice resulted in an increased number of apoptotic cells in the olfactory bulb of the brain and damage to cranial nerves [58]. A subsequent study showed that

the mice fetuses that were exposed to TiO_2 NPs prenatally exhibited an increased level of dopamine and its metabolites in the prefrontal cortex and neostriatum. This demonstrates that prenatal exposure to TiO_2 NPs might affect the development of the central dopaminergic system in mouse offspring [73]. In utero exposure of mice to TiO_2, NP has been shown to cause changes in the genes associated with the brain development and functions of central nervous system in the embryo [71]. Accumulation of TiO_2 NP in the placenta may interfere with the development of nervous system of the fetus by impairing the transport of nutrients to the fetus [74].

Injection of silica (Si) NPs to pregnant mice resulted in their accumulation in the brain of the embryo [54]. Other studies have reported that ZnO and TiO_2 NPs causes neurotoxic effects in fetus after passing through the placenta [71, 75]. Injection of cobalt-chrome (CoCr) NPs into pregnant mice has been reported to cause neurodevelopmental abnormalities, like reactive astrogliosis and increased DNA damage in the fetal hippocampus [76].

6.3 Effects of prenatal exposure to NP on the offspring

Here, we briefly enumerate some of the effects of NPs in offspring associated with prenatal exposure. The effects of prenatal exposure to nanoparticles include neurobehavioral alterations in the offspring [77]. Other effects of prenatal exposure include accumulation of NP in the hippocampus [58, 78, 79]. These NPs in the fetal brain cause disturbances in the CNS homeostasis. The accumulated NP has been reported to cause psychiatric disorders such as autism, schizophrenia, and depression in offspring [80]. Exposure of pregnant mice to aluminum NP has been shown to induce neurodevelopmental changes which persisted during adulthood. This was accompanied by an anxiety-like behavior and impairment of cognitive function in offspring exposed to aluminum nanoparticles during in utero life [20]. Prenatal exposure to TiO_2 NPs has been shown to impair the antioxidant status, cause oxidative damage to nucleic acids and lipids in the brain of newborn pups and enhanced the depressive-like behaviors during adulthood. Prenatal exposure to TiO_2 NP has been associated with depressive behavior in adults [81]. In the case of ZnO NP, the depressive behavior has been attributed to their neurotoxic effects on neural development [82].

Pups from mice exposed to Al2O3 before and during pregnancy have been shown to have higher levels of Al accumulation in the hippocampus [20]. Similarly, in the case of Sprague Dawley rats the pups of dams exposed to silver NP showed the accumulation of silver in the brain, lung, liver, and kidneys [78]. Subcutaneous injection of TiO_2 NP to CD-1 pregnant mice caused the accumulation of TiO_2 NPs in the brain and testis of offspring [58]. However, exposure of Sprague Dawley rats to Zn NPs before mating and during lactation caused no accumulation of these NPs in the brain of offspring [83]. Prenatal exposure of mice to TiO_2 NPs causes anatomical alterations in cerebral cortex, olfactory bulb and regions associated with the dopamine systems in the offspring [84].

Studies of Mohammadipour et al. [85] and Gao et al. [72] showed that in pregnant rats treated with TiO_2 NPs significantly decreased hippocampal cell proliferation, impaired learning, and memory, and affected synaptic plasticity in the hippocampal dentate gyrus area in newborn rats. Similarly, the study of Zhou and his collogues [86] showed that maternal exposure to TiO_2 NP results in inhibition of hippocampal and dysfunction of the rho/NMDAR signaling pathway in offspring. Maternal CB-NP exposure induced the long-term activation of astrocytes resulting in reactive astrogliosis in the brains of young mice [87]. TiO_2 NP injection to pregnant mice has been reported to cause symptoms akin to autism spectrum disorder (ASD) and neurodevelopment disorders in neonates, without the detectable

presence of NP in the placenta [88]. Another study indicated that nano-TiO_2 can cross the blood-fetal barrier and placental barrier, thereby delaying the development of fetal mice and inducing skeletal malformation [89].

7. Mechanisms of nanoparticle toxicity

Various hypothesizes have been proposed from time to time regarding the toxicity of NP. Nanoparticles can directly cross the placenta and cause damage to the fetus because of their high surface reactivity. Because of their small size, NPs can easily reach the brain and are taken up by the brain cells, such as neurons and glia. Mechanisms of NP uptake by cells include pinocytosis, endocytosis dependent on caveolae and lipid raft composition, clathrin-dependent endocytosis, and phagocytosis [90]. Due to their high surface reactivity, the nanoparticles can cause the generation of reactive oxygen species [91] and inflammation [92]. The metal ions of the NP have been proposed to contribute to their toxicity [93, 94]. The neurotoxic effects can either result in the direct alteration of the structure or activity of the neural system or lead to subsequent effects due to glial activations and glial-neuronal interactions [95]. The nanoparticles may also exert their toxic effects due to their limited elimination/excretion from the brain.

Oxidative stress has been implicated as one of the major mechanisms of NP toxicity. Consequences of oxidative stress include mitochondrial membrane damage and dysfunction, which in turn leads to cell death [96]. Inflammation caused by the production of cytokines appear to be a second mechanism by which the NP exerts their cytotoxic effects [97]. ZnO NPs have been shown to induce the production of pro-inflammatory cytokines in the brain of mice, accompanied by an impairment of cAMP/CREB signaling pathway. The degree of inflammation correlated with the age of the mice [56]. NPs interact with enzymes, potential apoptotic, or necrotic factors and induces inflammatory processes [12]. NP show properties similar to that of viruses and cause damage to DNA affecting cell proliferation [90]. NP can reduce mitochondrial function [98] and generate cellular morphological abnormalities [99] Cui et al. [81] postulated that prenatal exposure to NP resulted in an impairment of antioxidant capabilities in the brain of newborn pups.

Accumulation of NPs along the endosomal pathway may affect the morphology and functioning of the BBB. The interaction of the NP with biological macromolecules like DNA, lipids, and proteins may lead to the generation of oxidative stress, conformational changes in the macromolecules, mutations, alterations in membrane permeability, activation of various signaling pathways, alterations in the functions of enzymes, and exposure of new protein epitopes [100]. Genotoxic effects of NP include chromosomal aberrations, DNA strand breaks, oxidative DNA damage, DNA adducts, and micronucleus formation [101, 102]. Interactions of NP with microglia and astrocyte may activate NF-κB signaling and result in the release of mediators of inflammation and apoptosis [103]. On the other hand, oxidative stress induced mitochondrial DNA damage results in Nod-like receptor protein 3 (NLRP3) inflammasome activation, which subsequently regulates inflammatory responses by activating caspase-1 and interleukin-1β (IL-1β) release [104].

Most of the resulting damage of the nervous tissue is usually irreversible [18]. NPs have been reported to disrupt the cytoskeleton of cells of the CNS and thus cause cell death. NPs been shown to regulate the expression of neuronal channels and other proteins involved in excitability and neurotransmission [105]. Microglia, account for ~20% of the glial cells in the brain. They are a type of glial cells, which are the resident innate immune cells in the brain and regulate

neuroinflammation [106]. Choi et al. [107] demonstrated that low levels of SiNPs can alter microglial function by changing the expression of proinflammatory genes and cytokine release. Excessively activated or uncontrollable microglia can cause nerve toxicity by inducing proinflammatory factors, such as interleukin-1β, tumor necrosis factor (TNF)-α, prostaglandin E2, and interferon-γ (**Figure 3**) [18].

Autophagy (autophagic flux) is a highly regulated cellular process which by eliminating long-lived proteins and damaged organelle components through the lysosomal mechanism maintains cellular homeostasis [18]. NPs have been demonstrated to be autophagic inducers [108]. Autophagy has been found to be correlated with increased DNA strand breaks and other defensive mechanisms [109]. NPs have been reported to induce autophagy through the generation of ROS and lysosomal-dependent mechanism [18]. Autophagy induced by NPs can have protective or detrimental effect on cells. During intracellular oxidative stress, imbalance and excessive ROS generation decline in autophagy-lysosome degradation function results in autophagic flux impairment, which leads to significant accumulation of the substrate of autophagy within the cell and may even trigger cell death through mitochondrial pathway [110].

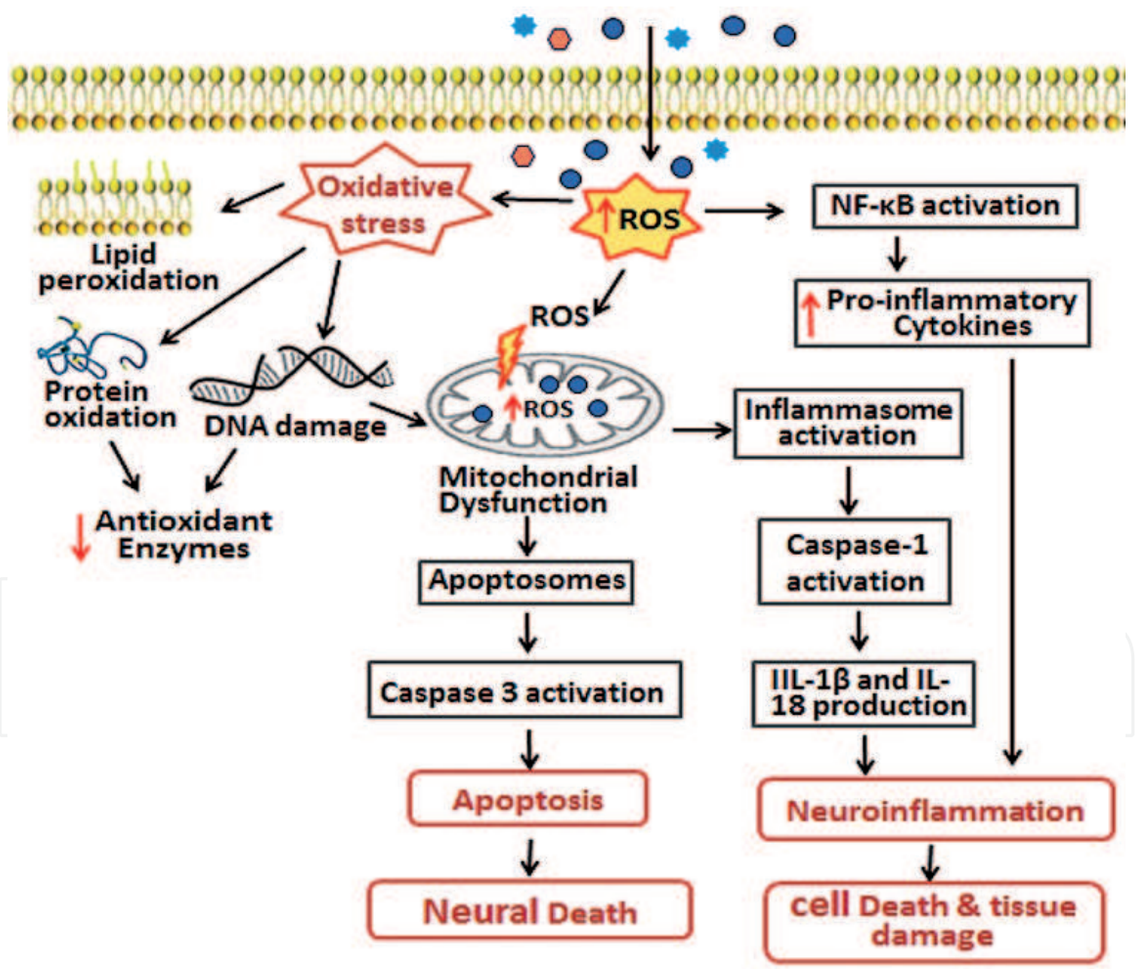

Figure 3.
Mechanism of nanoparticles (NPs)-induced neurotoxicity. Supraphysiological levels of reactive oxygen species (ROS) induce oxidative damage to the cellular macromolecules such as lipids, protein, and both mitochondrial and nuclear DNA. ROS-induced protein peroxidation may result in loss of catalytic activity of many enzymes including the antioxidant enzymes. NPs-mediated genotoxic stress in turn, can drive apoptosis mainly through the intrinsic mitochondrial apoptotic cell death pathway in neuronal cells. Mitochondrial dysfunction activates inflammasomes, which triggers the release of proinflammatory cytokines IL-1β and IL-18 via caspase-1 activation. Moreover, ROS-induced activation of nuclear factor kappa B (NF-κB) pathway may trigger proinflammatory responses, which is one of the key factors associated with NPs-induced neurological inflammation.

8. Conclusion

The brain has a limited capacity to excrete NPs [111]. Therefore, NPs that bypass the blood brain barrier and reach the fetal brain during embryonic development result in neurodevelopmental toxicity in growing fetus and psychiatric disorders in offspring. Compelling evidence from animal studies on nanotoxicity during pregnancy shows that cautions must be taken by pregnant women when using NP-based products or medicine. Deeper understanding of interaction of NPS with the biological system and the underlying mechanism on neurotoxicity will help in the development of safety guidelines on the use of engineered NPs in medicine and commercial products without health hazard. However, there is a need to study the effects of long-term exposure to NP with realistic routes and levels of exposure to identify the chronic effects of NP to fetal nervous system.

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References

[1] Griffin S, Masood MI, Nasim MJ, Sarfraz M, Ebokaiwe AP, Schäfer KH, et al. Natural nanoparticles: A particular matter inspired by nature. Antioxidants (Basel). 2017;**7**(1):3. DOI: 10.3390/antiox7010003

[2] Lespes G, Faucher S, Slaveykova VI. Natural nanoparticles, anthropogenic nanoparticles, where is the frontier? Frontiers in Environmental Science. 2020;**8**(71). DOI: 10.3389/fenvs.2020.00071

[3] Hochella MF, Mogk DW, Ranville J, Allen IC, et al. Natural, incidental, and engineered nanomaterials and their impacts on the earth system. Science. 2019;**363**(6434):eaau8299. DOI: 10.1126/science.aau8299

[4] Sharma VK, Filip J, Zboril R, Varma RS. Natural inorganic nanoparticles—Formation, fate, and toxicity in the environment. Chemical Society Reviews. 2015;**44**(23):8410-8423. DOI: 10.1039/c5cs00236b

[5] Teleanu DM, Chircov C, Grumezescu AM, Teleanu RI. Neurotoxicity of nanomaterials: An up-to-date overview. Nanomaterials (Basel). 2019;**9**(1):96. DOI: 10.3390/nano9010096

[6] Asharani PV, Lian WY, Gong Z, Valiyaveettil S. Toxicity of silver nanoparticles in zebrafish models. Nanotechnology. 2008;**19**(25):255102. DOI: 10.1088/0957-4484/19/25/255102

[7] King-Heiden TC, Wiecinski PN, Mangham AN, Metz KM, Nesbit D, Pedersen JA, et al. Quantum dot nanotoxicity assessment using the zebrafish embryo. Environmental Science and Technology. 2009;**43**(5):1605-1611. DOI: 10.1021/es801925c

[8] Contado C. Nanomaterials in consumer products: A challenging analytical problem. Frontiers in Chemistry. 2015;**3**:48. DOI: 10.3389/fchem.2015.00048

[9] Smijs TG, Pavel S. Titanium dioxide and zinc oxide nanoparticles in sunscreens: Focus on their safety and effectiveness. Nanotechnology, Science and Applications. 2011;**4**:95-112. DOI: 10.2147/NSA.S19419

[10] Ghebretatios M, Schaly S, Prakash S. Nanoparticles in the food industry and their impact on human gut microbiome and diseases. International Journal of Molecular Sciences. 2021;**22**(4):1942. DOI: 10.3390/ijms22041942

[11] Gupta R, Xie H. Nanoparticles in daily life: Applications, toxicity and regulations. Journal of Environmental Pathology, Toxicology and Oncology. 2018;**37**(3):209-230. DOI: 10.1615/JEnviron Pathol Toxicol Oncol. 201802 6009

[12] Celá P, Veselá B, Matalová E, Večeřa Z, Buchtová M. Embryonic toxicity of nanoparticles. Cells, Tissues, Organs. 2014;**199**(1):1-23. DOI: 10.1159/000362163

[13] Sharma A, Muresanu DF, Patnaik R, Sharma HS. Size- and age-dependent neurotoxicity of engineered metal nanoparticles in rats. Molecular Neurobiology. 2013;**48**(2):386-396. DOI: 10.1007/s12035-013-8500-0

[14] Gratton SE, Ropp PA, Pohlhaus PD, Luft JC, Madden VJ, Napier ME, et al. The effect of particle design on cellular internalization pathways. Proceedings of the National Academy of Sciences of the United States of America. 2008;**105**(33):11613-11618. DOI: 10.1073/pnas.0801763105

[15] Bongaerts E, Nawrot TS, Van Pee T, Ameloot M, Bové H. Translocation of (ultra)fine particles and nanoparticles

across the placenta: A systematic review on the evidence of in vitro, ex vivo, and in vivo studies. Particle and Fibre Toxicology. 2020;**17**(1):56. DOI: 10.1186/s12989-020-00386-8

[16] Lao F, Chen L, Li W, Ge C, Qu Y, Sun Q, et al. Fullerene nanoparticles selectively enter oxidation-damaged cerebral microvessel endothelial cells and inhibit JNK-related apoptosis. ACS Nano. 2009;**3**(11):3358-3368. DOI: 10.1021/nn900912n

[17] Rogers NJ, Franklin NM, Apte SC, Batley GE. The importance of physical and chemical characterization in nanoparticle toxicity studies. Integrated Environmental Assessment and Management. 2007;**3**(2):303-304. DOI: 10.1002/ieam.5630030219

[18] Feng X, Chen A, Zhang Y, Wang J, Shao L, Wei L. Central nervous system toxicity of metallic nanoparticles. International Journal of Nanomedicine. 2015;**10**:4321-4340. DOI: 10.2147/IJN.S78308

[19] Zhang L, Xie X, Zhou Y, Yu D, Deng Y, Ouyang J, et al. Gestational exposure to titanium dioxide nanoparticles impairs the placentation through dysregulation of vascularization, proliferation and apoptosis in mice. International Journal of Nanomedicine. 2018;**13**:777-789. DOI: 10.2147/IJN.S152400

[20] Zhang Q, Ding Y, He K, Li H, Gao F, Moehling TJ, et al. Exposure to alumina nanoparticles in female mice during pregnancy induces neurodevelopmental toxicity in the offspring. Frontiers in Pharmacology. 2018;**9**:253. DOI: 10.3389/fphar.2018.00253

[21] Frank LA, Onzi GR, Morawski AS, Pohlmann AR, Guterres SS, Contri RV. Chitosan as a coating material for nanoparticles intended for biomedical applications. Reactive and Functional Polymers. 2020;**147**. DOI: org/10.1016/j.reactfunctpolym.2019.104459

[22] Oberdörster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, et al. ILSI research foundation/risk science institute nanomaterial toxicity screening working group. Principles for characterizing the potential human health effects from exposure to nanomaterials: Elements of a screening strategy. Particle and Fibre Toxicology. 2005;**2**:8. DOI: 10.1186/1743-8977-2-8

[23] Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W, et al. Translocation of inhaled ultrafine particles to the brain. Inhalation Toxicology. 2004;**16**(6-7):437-445. DOI: 10.1080/08958370490439597

[24] Elder A, Gelein R, Silva V, Feikert T, Opanashuk L, Carter J, et al. Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. Environmental Health Perspectives. 2006;**114**(8):1172-1178. DOI: 10.1289/ehp.9030

[25] Borm PJ, Robbins D, Haubold S, Kuhlbusch T, Fissan H, Donaldson K, et al. The potential risks of nanomaterials: A review carried out for ECETOC. Particle and Fibre Toxicology. 2006;**3**:11. DOI: 10.1186/1743-8977-3-11

[26] Geraets L, Oomen AG, Schroeter JD, Coleman VA, Cassee FR. Tissue distribution of inhaled micro- and nano-sized cerium oxide particles in rats: Results from a 28-day exposure study. Toxicological Sciences. 2012;**127**(2):463-473. DOI: 10.1093/toxsci/kfs113

[27] Wolak DJ, Thorne RG. Diffusion of macromolecules in the brain: Implications for drug delivery. Molecular Pharmaceutics. 2013;**10**(5):1492-1504. DOI: 10.1021/mp300495e

[28] Zlokovic BV. The blood-brain barrier in health and chronic neurodegenerative disorders. Neuron. 2008;**57**(2):178-201. DOI: 10.1016/j.neuron.2008.01.003

[29] Daneman R. The blood-brain barrier in health and disease. Annals of Neurology. 2012;**72**(5):648-672. DOI: 10.1002/ana.23648

[30] Daneman R, Prat A. The blood-brain barrier. Cold Spring Harbor Perspectives in Biology. 2015;**7**(1):a020412. DOI: 10.1101/cshperspect.a020412

[31] Sims DE. The pericyte—A review. Tissue and Cell. 1986;**18**(2):153-174. DOI: 10.1016/0040-8166(86)90026-1

[32] Abbott NJ, Rönnbäck L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. Nature Reviews Neuroscience. 2006;**7**(1):41-53. DOI: 10.1038/nrn1824

[33] O'Brown NM, Pfau SJ, Gu C. Bridging barriers: A comparative look at the blood-brain barrier across organisms. Genes and Development. 2018;**32**(7-8): 466-478. DOI: 10.1101/gad.309823.117

[34] Löscher W, Potschka H. Blood-brain barrier active efflux transporters: ATP-binding cassette gene family. NeuroRx. 2005;**2**(1):86-98. DOI: 10.1602/neurorx.2.1.86

[35] Mittapalli RK, Manda VK, Adkins CE, Geldenhuys WJ, Lockman PR. Exploiting nutrient transporters at the blood-brain barrier to improve brain distribution of small molecules. Therapeutic Delivery. 2010;**1**(6):775-784. DOI: 10.4155/tde.10.76

[36] Kluge MA, Fetterman JL, Vita JA. Mitochondria and endothelial function. Circulation Research. 2013;**112**(8):1171-1188. DOI: 10.1161/CIRCRESAHA.111.300233

[37] Wohlfart S, Gelperina S, Kreuter J. Transport of drugs across the blood-brain barrier by nanoparticles. Journal of Controlled Release. 2012;**161**(2):264-273. DOI: 10.1016/j.jconrel.2011.08.017

[38] Mc Carthy DJ, Malhotra M, O'Mahony AM, Cryan JF, O'Driscoll CM. Nanoparticles and the blood-brain barrier: Advancing from in-vitro models towards therapeutic significance. Pharmaceutical Research. 2015;**32**(4): 1161-1185. DOI: 10.1007/s11095-014-1545-6

[39] Persidsky Y, Ramirez SH, Haorah J, Kanmogne GD. Blood-brain barrier: Structural components and function under physiologic and pathologic conditions. Journal of Neuroimmune Pharmacology. 2006;**1**(3):223-236. DOI: 10.1007/s11481-006-9025-3

[40] Minn A, Ghersi-Egea JF, Perrin R, Leininger B, Siest G. Drug metabolizing enzymes in the brain and cerebral microvessels. Brain Research Reviews. 1991;**16**(1):65-82. DOI: 10.1016/0165-0173(91)90020-9

[41] Béduneau A, Saulnier P, Benoit JP. Active targeting of brain tumors using nanocarriers. Biomaterials. 2007;**28**(33): 4947-4967. DOI: 10.1016/j.biomaterials.2007.06.011

[42] Spector R. Nature and consequences of mammalian brain and Cerebrospinal fluid efflux transporters: Four decades of progress. Journal of Neurochemistry. 2010;**112**(1):13-23. DOI: 10.1111/j.1471-4159.2009.06451.x

[43] Neves AR, Queiroz JF, Lima SAC, Reis S. Apo E-functionalization of solid lipid nanoparticles enhances brain drug delivery: Uptake mechanism and transport pathways. Bioconjugate Chemistry. 2017;**28**(4):995-1004. DOI: 10.1021/acs.bioconjchem.6b00705

[44] Mendonça MC, Soares ES, de Jesus MB, Ceragioli HJ, Ferreira MS, Catharino RR, et al. Reduced graphene oxide induces transient blood-brain barrier opening: An in vivo study. Journal of Nanobiotechnology. 2015;**13**:78. DOI: 10.1186/s12951-015-0143-z

[45] Liu X, Sui B, Sun J. Blood-brain barrier dysfunction induced by silica

NPs in vitro and in vivo: Involvement of oxidative stress and Rho-kinase/JNK signaling pathways. Biomaterials. 2017;**121**:64-82. DOI: 10.1016/j.biomaterials.2017.01.006

[46] Kafa H, Wang JT, Rubio N, Klippstein R, Costa PM, Hassan HA, et al. Translocation of LRP1 targeted carbon nanotubes of different diameters across the blood-brain barrier in vitro and in vivo. Journal of Controlled Release. 2016;**225**:217-229. DOI: 10.1016/j.jconrel.2016.01.031

[47] Lu W, Sun Q, Wan J, She Z, Jiang XG. Cationic albumin-conjugated pegylated nanoparticles allow gene delivery into brain tumors via intravenous administration. Cancer Research. 2006;**66**(24):11878-11887. DOI: 10.1158/0008-5472.CAN-06-2354

[48] Ge D, Du Q, Ran B, Liu X, Wang X, Ma X, et al. The neurotoxicity induced by engineered nanomaterials. International Journal of Nanomedicine. 2019;**14**:4167-4186. DOI: 10.2147/IJN.S203352

[49] Maltepe E, Fisher SJ. Placenta: The forgotten organ. Annual Review of Cell and Developmental Biology. 2015;**31**:523-552. DOI: 10.1146/annurev-cellbio-100814-125620

[50] Pereira KV, Giacomeli R, Gomes de Gomes M, Haas SE. The challenge of using nanotherapy during pregnancy: Technological aspects and biomedical implications. Placenta. 2020;**100**(75-80). DOI: 10.1016/j.placenta.2020.08.005

[51] Menezes V, Malek A, Keelan JA. Nanoparticulate drug delivery in pregnancy: Placental passage and fetal exposure. Current Pharmaceutical Biotechnology. 2011;**12**(5):731-742. DOI: 10.2174/138920111795471010

[52] Di Bona KR, Xu Y, Ramirez PA, DeLaine J, Parker C, Bao Y, et al. Surface charge and dosage dependent potential developmental toxicity and biodistribution of iron oxide nanoparticles in pregnant CD-1 mice. Reproductive Toxicology. 2014;**50**:36-42. DOI: 10.1016/j.reprotox.2014.09.010

[53] Semmler-Behnke M, Lipka J, Wenk A, Hirn S, Schäffler M, Tian F, et al. Size dependent translocation and fetal accumulation of gold nanoparticles from maternal blood in the rat. Particle and Fibre Toxicology. 2014;**11**:33. DOI: 10.1186/s12989-014-0033-9

[54] Yamashita K, Yoshioka Y, Higashisaka K, Mimura K, Morishita Y, Nozaki M, et al. Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. Nature Nanotechnology. 2011;**6**(5):321-328. DOI: 10.1038/nnano.2011.41

[55] Yang H, Sun C, Fan Z, Tian X, Yan L, Du L, et al. Effects of gestational age and surface modification on materno-fetal transfer of nanoparticles in murine pregnancy. Scientific Reports. 2012;**2**:847. DOI: 10.1038/srep00847

[56] Tian L, Lin B, Wu L, Li K, Liu H, Yan J, et al. Neurotoxicity induced by zinc oxide nanoparticles: Age-related differences and interaction. Scientific Reports. 2015;**5**:16117. DOI: 10.1038/srep16117

[57] Wick P, Malek A, Manser P, Meili D, Maeder-Althaus X, Diener L, et al. Barrier capacity of human placenta for nanosized materials. Environmental Health Perspectives. 2010;**118**(3):432-436. DOI: 10.1289/ehp.0901200

[58] Takeda K, Suzuki K, Ishihara A, Kubo-Irie M, Fujimoto R, Tabata M, et al. Nanoparticles transferred from pregnant mice to their offspring can damage the genital and cranial nerve systems. Journal of Health Science. 2009;**55**:95-102. DOI: 10.1248/jhs.55.95

[59] Fennell TR, Mortensen NP, Black SR, Snyder RW, Levine KE, Poitras E, et al. Disposition of

intravenously or orally administered silver nanoparticles in pregnant rats and the effect on the biochemical profile in urine. Journal of Applied Toxicology. 2017;**37**(5):530-544. DOI: 10.1002/jat.3387

[60] Campagnolo L, Massimiani M, Vecchione L, Piccirilli D, Toschi N, Magrini A, et al. Silver nanoparticles inhaled during pregnancy reach and affect the placenta and the foetus. Nanotoxicology. 2017;**11**(5):687-698. DOI: 10.1080/17435390.2017.1343875

[61] Keelan JA. Nanotoxicology: Nanoparticles versus the placenta. Nature Nanotechnology. 2011;**6**(5):263-264. DOI: 10.1038/nnano.2011.65

[62] Muoth C, Aengenheister L, Kucki M, Wick P, Buerki-Thurnherr T. Nanoparticle transport across the placental barrier: Pushing the field forward! Nanomedicine (London, England). 2016;**11**(8):941-957. DOI: 10.2217/nnm-2015-0012

[63] Naserzadeh P, Ghanbary F, Ashtari P, Seydi E, Ashtari K, Akbari M. Biocompatibility assessment of titanium dioxide nanoparticles in mice fetoplacental unit. Journal of Biomedical Materials Research. Part A. 2018;**106**(2):580-589. DOI: 10.1002/jbm.a.36221

[64] Bailey GP, Wise LD, Buschmann J, Hurtt M, Fisher JE. Pre- and postnatal developmental toxicity study design for pharmaceuticals. Birth Defects Research. Part B, Developmental and Reproductive Toxicology. 2009;**86**(6):437-445. DOI: 10.1002/bdrb.20217

[65] Wise LD, Buschmann J, Feuston MH, Fisher JE, Hew KW, Hoberman AM, et al. Embryo-fetal developmental toxicity study design for pharmaceuticals. Birth Defects Research. Part B, Developmental and Reproductive Toxicology. 2009;**86**(6):418-428. DOI: 10.1002/bdrb.20214

[66] Khan Z, Wolff HS, Fredrickson JR, Walker DL, Daftary GS, Morbeck DE. Mouse strain and quality control testing: Improved sensitivity of the mouse embryo assay with embryos from outbred mice. Fertility and Sterility. 2013;**99**(3):847-854. DOI: 10.1016/j.fertnstert.2012.10.046

[67] Haque E, Ward AC. Zebrafish as a model to evaluate nanoparticle toxicity. Nanomaterials (Basel). 2018;**8**(7):561. DOI: 10.3390/nano8070561

[68] Watson RE, Desesso JM, Hurtt ME, Cappon GD. Postnatal growth and morphological development of the brain: A species comparison. Birth Defects Research. Part B, Developmental and Reproductive Toxicology. 2006;**77**(5):471-484. DOI: 10.1002/bdrb.20090

[69] Siddiqui MA, Alhadlaq HA, Ahmad J, Al-Khedhairy AA, Musarrat J, Ahamed M. Copper oxide nanoparticles induced mitochondria mediated apoptosis in human hepatocarcinoma cells. PLoS One. 2013;**8**(8):e69534. DOI: 10.1371/journal.pone.0069534

[70] Rice D, Barone S Jr. Critical periods of vulnerability for the developing nervous system: Evidence from humans and animal models. Environmental Health Perspectives. 2000;**108**(Suppl. 3):511-533. DOI: 10.1289/ehp.00108s3511

[71] Shimizu M, Tainaka H, Oba T, Mizuo K, Umezawa M, Takeda K. Maternal exposure to nanoparticulate titanium dioxide during the prenatal period alters gene expression related to brain development in the mouse. Particle and Fibre Toxicology. 2009;**6**:20. DOI: 10.1186/1743-8977-6-20

[72] Gao X, Yin S, Tang M, Chen J, Yang Z, Zhang W, et al. Effects of developmental exposure to TiO_2 nanoparticles on synaptic plasticity in hippocampal dentate gyrus area: An in vivo study in anesthetized rats. Biological Trace Element Research.

2011;**143**(3):1616-1628. DOI: 10.1007/s12011-011-8990-4

[73] Takahashi Y, Mizuo K, Shinkai Y, Oshio S, Takeda K. Prenatal exposure to titanium dioxide nanoparticles increases dopamine levels in the prefrontal cortex and neostriatum of mice. The Journal of Toxicological Sciences. 2010;**35**(5):749-756. DOI: 10.2131/jts.35.749

[74] Wu Y, Chen L, Chen F, Zou H, Wang Z. A key moment for TiO_2: Prenatal exposure to TiO_2 nanoparticles may inhibit the development of offspring. Ecotoxicology and Environmental Safety. 2020;**202**:110911. DOI: 10.1016/j.ecoenv.2020.110911

[75] Okada Y, Tachibana K, Yanagita S, Takeda K. Prenatal exposure to zinc oxide particles alters monoaminergic neurotransmitter levels in the brain of mouse offspring. The Journal of Toxicological Sciences. 2013;**38**(3):363-370. DOI: 10.2131/jts.38.363

[76] Hawkins SJ, Crompton LA, Sood A, Saunders M, Boyle NT, Buckley A, et al. Nanoparticle-induced neuronal toxicity across placental barriers is mediated by autophagy and dependent on astrocytes. Nature Nanotechnology. 2018;**13**(5):427-433. DOI: 10.1038/s41565-018-0085-3

[77] Hougaard KS, Jackson P, Jensen KA, Sloth JJ, Löschner K, Larsen EH, et al. Effects of prenatal exposure to surface-coated nanosized titanium dioxide (UV-Titan). A study in mice. Particle and Fibre Toxicology. 2010;**7**:16. DOI: 10.1186/1743-8977-7-16

[78] Lee Y, Choi J, Kim P, Choi K, Kim S, Shon W, et al. A transfer of silver nanoparticles from pregnant rat to offspring. Toxicology Research. 2012;**28**(3):139-141. DOI: 10.5487/TR.2012.28.3.139

[79] Zhang Y, Wu J, Feng X, Wang R, Chen A, Shao L. Current understanding of the toxicological risk posed to the fetus following maternal exposure to nanoparticles. Expert Opinion on Drug Metabolism and Toxicology. 2017;**13**(12):1251-1263. DOI: 10.1080/17425255.2018.1397131

[80] O'Connor MJ, Paley B. Psychiatric conditions associated with prenatal alcohol exposure. Developmental Disabilities Research Reviews. 2009;**15**(3):225-234. DOI: 10.1002/ddrr.74

[81] Cui Y, Chen X, Zhou Z, Lei Y, Ma M, Cao R, et al. Prenatal exposure to nanoparticulate titanium dioxide enhances depressive-like behaviors in adult rats. Chemosphere. 2014;**96**:99-104. DOI: 10.1016/j.chemosphere.2013.07.051

[82] Alimohammadi S, Hosseini MS, Behbood L. Prenatal exposure to zinc oxide nanoparticles can induce depressive-like behaviors in mice offspring. International Journal of Peptide Research and Therapeutics. 2019;**25**:401-409. DOI: 10.1007/s10989-018-9686-9

[83] Jo E, Seo G, Kwon JT, Lee M, Bc L, Eom I, et al. Exposure to zinc oxide nanoparticles affects reproductive development and biodistribution in offspring rats. The Journal of Toxicological Sciences. 2013;**38**(4):525-530. DOI: 10.2131/jts.38.525

[84] Umezawa M, Tainaka H, Kawashima N, Shimizu M, Takeda K. Effect of fetal exposure to titanium dioxide nanoparticle on brain development—Brain region information. The Journal of Toxicological Sciences. 2012;**37**(6):1247-1252. DOI: 10.2131/jts.37.1247

[85] Mohammadipour A, Fazel A, Haghir H, Motejaded F, Rafatpanah H, Zabihi H, et al. Maternal exposure to titanium dioxide nanoparticles during pregnancy; impaired memory and decreased hippocampal cell proliferation in rat offspring. Environmental Toxicology and Pharmacology. 2014;**37**(2):617-625. DOI: 10.1016/j.etap.2014.01.014

[86] Zhou Y, Ji J, Hong F, Zhuang J, Wang L. Maternal exposure to nanoparticulate titanium dioxide causes inhibition of hippocampal development involving dysfunction of the rho/NMDAR signaling pathway in offspring. Journal of Biomedical Nanotechnology. 2019;**15**(4):839-847. DOI: 10.1166/jbn.2019.2723

[87] Onoda A, Takeda K, Umezawa M. Dose-dependent induction of astrocyte activation and reactive astrogliosis in mouse brain following maternal exposure to carbon black nanoparticle. Particle and Fibre Toxicology. 2017;**14**(1):4. DOI: 10.1186/s12989-017-0184-6

[88] Notter T, Aengenheister L, Weber-Stadlbauer U, Naegeli H, Wick P, Meyer U, et al. Prenatal exposure to TiO_2 nanoparticles in mice causes behavioral deficits with relevance to autism spectrum disorder and beyond. Translational Psychiatry. 2018;**8**(1):193. DOI: 10.1038/s41398-018-0251-2

[89] Hong F, Zhou Y, Zhao X, Sheng L, Wang L. Maternal exposure to nanosized titanium dioxide suppresses embryonic development in mice. International Journal of Nanomedicine. 2017;**12**:6197-6204. DOI: 10.2147/IJN.S143598

[90] Asharani PV, Hande MP, Valiyaveettil S. Anti-proliferative activity of silver nanoparticles. BMC Cell Biology. 2009;**10**:65. DOI: 10.1186/1471-2121-10-65

[91] Fu PP, Xia Q, Hwang HM, Ray PC, Yu H. Mechanisms of nanotoxicity: Generation of reactive oxygen species. Journal of Food and Drug Analysis. 2014;**22**(1):64-75. DOI: 10.1016/j.jfda.2014.01.005

[92] Shirasuna K, Usui F, Karasawa T, Kimura H, Kawashima A, Mizukami H, et al. Nanosilica-induced placental inflammation and pregnancy complications: Different roles of the inflammasome components NLRP3 and ASC. Nanotoxicology. 2015;**9**(5):554-567. DOI: 10.3109/17435390.2014.956156

[93] Katsumiti A, Gilliland D, Arostegui I, Cajaraville MP. Mechanisms of toxicity of Ag nanoparticles in comparison to bulk and ionic ag on mussel hemocytes and gill cells. PLoS One. 2015;**10**(6):e0129039. DOI: 10.1371/journal.pone.0129039

[94] Xiao Y, Vijver MG, Chen G, Peijnenburg WJ. Toxicity and accumulation of Cu and ZnO nanoparticles in Daphnia magna. Environmental Science and Technology. 2015;**49**(7):4657-4664. DOI: 10.1021/acs.est.5b00538

[95] Li J, Martin FL. Chapter 4—Current perspective on nanomaterial-induced adverse effects: Neurotoxicity as a case example. In: Jiang X, Gao H, editors. Neurotoxicity of Nanomaterials and Nanomedicine. Cambridge, Massachusetts: Academic Press; 2017. pp. 75-98

[96] Sayes CM, Gobin AM, Ausman KD, Mendez J, West JL, Colvin VL. Nano-C60 cytotoxicity is due to lipid peroxidation. Biomaterials. 2005;**26**(36):7587-7595. DOI: 10.1016/j.biomaterials.2005.05.027

[97] Trickler WJ, Lantz SM, Murdock RC, Schrand AM, Robinson BL, Newport GD, et al. Silver nanoparticle induced blood-brain barrier inflammation and increased permeability in primary rat brain microvessel endothelial cells. Toxicological Sciences. 2010;**118**(1):160-170. DOI: 10.1093/toxsci/kfq244

[98] Chen X, Schluesener HJ. Nanosilver: A nanoproduct in medical application. Toxicology Letters. 2008;**176**(1):1-12. DOI: 10.1016/j.toxlet.2007.10.004

[99] Hussain SM, Hess KL, Gearhart JM, Geiss KT, Schlager JJ. In vitro toxicity of nanoparticles in BRL 3A rat liver cells. Toxicology in Vitro. 2005;**19**(7):975-983. DOI: 10.1016/j.tiv.2005.06.034

[100] Xia T, Li N, Nel AE. Potential health impact of nanoparticles. Annual Review of Public Health. 2009;**30**:137-150. DOI: 10.1146/annurev.publhealth.031308.100155

[101] Landsiedel R, Kapp MD, Schulz M, Wiench K, Oesch F. Genotoxicity investigations on nanomaterials: Methods, preparation and characterization of test material, potential artifacts and limitations—Many questions, some answers. Mutation Research. 2009;**681**(2-3):241-258. DOI: 10.1016/j.mrrev.2008.10.002

[102] Xie H, Mason MM, Wise JP Sr. Genotoxicity of metal nanoparticles. Reviews on Environmental Health. 2011;**26**(4):251-268. DOI: 10.1515/reveh.2011.033

[103] Albensi BC. What is nuclear factor kappa B (NF-κB) doing in and to the mitochondrion? Frontiers in Cell and Development Biology. 2019;**7**:154. DOI: 10.3389/fcell.2019.00154

[104] Gómez DM, Urcuqui-Inchima S, Hernandez JC. Silica nanoparticles induce NLRP3 inflammasome activation in human primary immune cells. Innate Immunity. 2017;**23**(8):697-708. DOI: 10.1177/1753425917738331

[105] Kang Y, Liu J, Song B, Feng X, Ou L, Wei L, et al. Potential links between cytoskeletal disturbances and electroneurophysiological dysfunctions induced in the central nervous system by inorganic nanoparticles. Cellular Physiology and Biochemistry. 2016;**40**(6):1487-1505. DOI: 10.1159/000453200

[106] Block ML, Elder A, Auten RL, Bilbo SD, Chen H, Chen JC, et al. The outdoor air pollution and brain health workshop. Neurotoxicology. 2012;**33**(5):972-984. DOI: 10.1016/j.neuro.2012.08.014

[107] Choi J, Zheng Q, Katz HE, Guilarte TR. Silica-based nanoparticle uptake and cellular response by primary microglia. Environmental Health Perspectives. 2010;**118**(5):589-595. DOI: 10.1289/ehp.0901534

[108] Podila R, Brown JM. Toxicity of engineered nanomaterials: A physicochemical perspective. Journal of Biochemical and Molecular Toxicology. 2013;**27**(1):50-55. DOI: 10.1002/jbt.21442

[109] Halamoda Kenzaoui B, Chapuis Bernasconi C, Guney-Ayra S, Juillerat-Jeanneret L. Induction of oxidative stress, lysosome activation and autophagy by nanoparticles in human brain-derived endothelial cells. The Biochemical Journal. 2012;**441**(3):813-821. DOI: 10.1042/BJ20111252

[110] Feng X, Zhang Y, Zhang C, Lai X, Zhang Y, Wu J, et al. Nanomaterial-mediated autophagy: Coexisting hazard and health benefits in biomedicine. Particle and Fibre Toxicology. 2020;**17**(1):53. DOI: 10.1186/s12989-020-00372-0

[111] Song B, Zhang Y, Liu J, Feng X, Zhou T, Shao L. Unraveling the neurotoxicity of titanium dioxide nanoparticles: focusing on molecular mechanisms. Beilstein Journals Nanotechnol. 2016 Apr **29**;7:645-654. DOI: 10.3762/bjnano.7.57. PMID: 27335754; PMCID: PMC4901937.