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# Titanium dioxide nanoparticles: some aspects of toxicity/focus on the development

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Nanosized titanium dioxide ($TiO_2$) particles belong to the most widely manufactured nanoparticles (NPs) on a global scale because of their photocatalytic properties and the related surface effects. $TiO_2$ NPs are in the top five NPs used in consumer products. Ultrafine $TiO_2$ is widely used in the number of applications, including white pigment in paint, ceramics, food additive, food packaging material, sunscreens, cosmetic creams, and, component of surgical implants. Data evidencing rapid distribution, slow or ineffective elimination, and potential long-time tissue accumulation are especially important for the human risk assessment of ultrafine $TiO_2$ and represent new challenges to more responsibly investigate potential adverse effects by the action of $TiO_2$ NPs considering their ubiquitous exposure in various doses. Transport of ultrafine $TiO_2$ particles in systemic circulation and further transition through barriers, especially the placental and blood-brain ones, are well documented. Therefore, from the developmental point of view, there is a raising concern in the exposure to $TiO_2$ NPs during critical windows, in the pregnancy or the lactation period, and the fact that human mothers, women and men in fertile age and last but not least children may be exposed to high cumulative doses. In this review, toxicokinetics and particularly toxicity of $TiO_2$ NPs in relation to the developing processes, oriented mainly on the development of the central nervous system, are discussed

**Key words:** nanoparticles, nanotoxicity, nanomaterials, titanium dioxide, reproductive toxicity, developmental toxicity, blood brain barrier, placental barrier

## Introduction

Nanoparticles (NPs) represent a class of organic and inorganic substances between 1 and 100 nm in size, which may be of an natural origin, engineered for specific purposes or produced as a waste product of the human civilizing activity (Lin et al. 2014).

A rapid development of nanoscience and nanotechnologies has given a rise to wide range applications of man-made nanomaterials (NMs) in specific biomedicine fields for treating, diagnosing, monitoring, controlling, and repairing biological systems at the molecular level (Moghini et al. 2005; Oberdoster 2010; Shvedova and Kagan 2010). Engineered NMs, such as nanosilica particles, silver, titanium dioxide ($TiO_2$) NPs, and nanowires have been already widely applied in electronics, antibacterial materials, food industry, paints, and cosmetics (Taylor et al. 2012). The recent inventory of NMs in food, feed,

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and agriculture has shown that the nano-encapsulates, silver, and $TiO_2$ NPs are the most often used NMs at present (Peters et al. 2014; Smolkova et al. 2015).

On the other hand, many incidental NPs can be derived from the industrial activities, mainly originated in power plants by coal, oil, and natural gas. Complex mixture of NPs can be derived from traffic emissions, incineration of waste, and combustion of fossil fuel. However, they can also be formed in military shooting ranges or freed from conventional welding sets (Zoroddu et al. 2014).

Despite the many advantages which nanomaterials afford, increasing concern on their potential adverse effects to human health as well as environmental pollution has been expressed. Unique properties of NPs predestine their advantages, such as intermixing, diffusion, sensoric response, and ultrafast kinetics within the frame of a local process at nanoscale (Jiao et al. 2014). On the contrary, the expansion of NP applications, ultra small size, penetration via biological membrane barriers, long-term retention in tissues and organs, interaction with biological macromolecules, and subsequent toxic effects, have forced the scientists to investigate the potential hazard of these unique materials within the scope of a new toxicology branch named nanotoxicology (Zhao and Castranova 2011; Dusinska et al. 2013; Zoroddu et al. 2014). Moreover, within the frame of the nanotoxicology, the research focused on the embryonic development and reproductive systems have stimulated the formation of a nanotoxicology subbranch, referred as a nanoreprotoxicology (Campagnolo et al. 2012).

## Titanium dioxide NPs

$TiO_2$ bulk form, is a food additive approved by European Union as E171 primarily used as a pigment in variety of consumer and personal care products (FAO/WHO 2010). It is a fine, white, crystalline, odorless powder, which exhibits relatively low toxicity (Zhang et al. 2010). In 2005, the United States National Institute for Occupational Safety and Health (NIOSH), proposed a recommended 0.3 $mg/m^3$ exposure limit (REL) for $TiO_2$ NPs, a value 10 times lower than the REL for $TiO_2$ fine particles (FPs). Though $TiO_2$ (bulk material) has been considered as an inert mineral, almost non-toxic to human; in 2006, the IARC classified it as a possible carcinogen for human when inhaled (Group 2B) (IARC 2010). Identification of the carcinogenicity of $TiO_2$ NPs by different routes of exposure is of high priority (IARC 2010).

Nanosized $TiO_2$ particles belong to the most widely manufactured NPs on a global scale because of its photocatalytic properties and the related surface effects. $TiO_2$ NPs are in the top five NPs used in consumer products (Hashimoto et al. 2005; Shukla et al. 2011). Their clear different optical, catalytic, and electronic characteristics compared to $TiO_2$ fine particles are determined by the variation in size, structure, shape, by the surface to volume ratio, the charge, agglomerate and aggregate formation, together with their insolubility in aqueous solutions (Fabian et al. 2008; Zorodu et al. 2014).

$TiO_2$ NPs are widely used in number of applications, such as a white pigment in paint, ceramics, as a food additive, in food packaging material, in sunscreens, in cosmetic creams, and as a component of surgical implants. Recently, the nano-form of $TiO_2$ has been also applied in paints as an antimicrobial agent, due to its hydroxyl radical generative property (Kaiser et al. 2013). They are also broadly used in the environmental decontamination of air, water, and soil by destruction of pesticides (Choi et al. 2006; Tran and Webster 2009; Besov et al. 2010; Shi et al. 2013). Self-cleaning and anti-fogging materials as well as coatings and paints for sanitization and disinfection products used in hospitals against a variety of different microbes are in progressive commertionalization (Krystek et al. 2014). $TiO_2$ NPs are also under investigation as potential photosensitizers for use in photodynamic therapy (PDT) (Szacilowski et al. 2005). Similarly, nanopreparations with $TiO_2$ NPs are intensively evaluated as a novel therapy for dermatologic diseases, including acne vulgaris, recurrent condyloma accuminata, atopic dermatitis, hyperpigmented skin lesions, and other non-dermatologic diseases (Wiesenthal et al. 2011).

$TiO_2$ occurs naturally as anatase, rutile, and brookite mineral forms, of which rutile and anatase are most common. Rutile is considered as a more inert form, whereas anatase is an active form of $TiO_2$ with the greater toxic potential than $TiO_2$ rutile (Nemmar et al. 2013; Shi et al. 2013). Their characteristics can be modified by several methods to improve their functionality and stability. In the connection, $TiO_2$ nanorods can be doped with iron to increase their photocatalytic activity (Nemmar et al. 2008). Further, surface modification such as coating, influences the activity of $TiO_2$ NPs (Liang et al. 2006). Tedja et al. (2012) demonstrated diminished cytotoxicity in human lung cells when the surface of $TiO_2$ NPs was modified by a grafting-to polymer technique, combining catalytic chain transfer and thiol-ene click chemistry. On the other hand, the exposure to $SiO_2$-coated rutile $TiO_2$ NPs caused pulmonary neutrophilia, increased

expression of tumor necrosis factor-alpha (TNF-α), and neutrophil attracting chemokine CXCL1 in lung tissue. Interestingly, uncoated rutile and anatase did not induce significant inflammation (Rossi et al. 2009).

## Routes of exposure to $TiO_2$ NPs

There are three main ways how NPs may enter the body: by inhalation through the respiratory tract, permeation through the skin, and ingestion through the digestive tract.

Human exposure to $TiO_2$ NPs may occur during manufacturing as well as a current use. In the workplace, the particular routes of exposures of toxicological relevance are inhalation and dermal exposures. For consumers, dermal application of personal care products and oral exposure to food colorants and nutritional supplements are the most frequent. Regarding the oral exposure and considering the particle size as crucial factor for adverse potential of NPs, it is noteworthy to highlight the study by Weir et al. (2012) who have recorded that candies, sweets, and chewing gums contain the highest amount of $TiO_2$ in scale of < 100 nm. This fact should be a serious warning because children population, as frequent consumers of sweets, is in a higher risk when compared to adults. Consumer inhalation is also possible during application of antimicrobial spray with $TiO_2$ NPs (Shi et al. 2013; Zoroddu et al. 2014). In nanomedicine, intravenous and subcutaneous injection of $TiO_2$ nanocarriers represents unique way of intentional exposure to $TiO_2$ NPs, avoiding the normal absorption process (Zhao and Castranova 2011). Potential exposure to $TiO_2$ nanotubes, as drug carriers on orthopedic implants for the prevention of periprosthetic joint infections, might also be expected (Chennella et al. 2013). Moreover, internal exposure to $TiO_2$ NPs derived from the mechanical wear of surgical implants has been already described (Tran and Webster 2009).

## Toxicokinetics of $TiO_2$ NPs

Absorption, tissue distribution, metabolism, accumulation, and elimination of NPs may be affected by routes of exposures, chemical composition, particle size, shape and charge, agglomeration and aggregation status, as well as solubility.

### Absorption

The absorption of NPs from *gastrointestinal tract* (GIT) is generally influenced by their size and morphological properties. The absorption is greater for the smaller than the larger NPs; moreover, the negatively charged particles are spread in the negatively charged mucus layer unlike the positively charged NPs which are there trapped (Zorrodu et al. 2014). $TiO_2$ NPs have been shown to be absorbed from the GIT through the surrounding lymphoid tissues. As food products and beverages may contain $TiO_2$ NPs, GIT may be very important route for $TiO_2$ NPs (Hagens et al. 2007). Their absorption is estimated to be approximately 15- to 25-fold higher for NPs when compared to $TiO_2$ fine particles of (Desai et al. 1996). Very slow absorption of $TiO_2$ NPs after oral administration (Geraets et al. 2014) and obvious particle size dependency with the pronounced absorption from GIT for 150 to 500 nm sized $TiO_2$ NPs into the liver, spleen, and lymph have been described (Jani et al. 1990).

*Dermal absorption* of $TiO_2$ NPs is also of interest because of many consumer products, such as cosmetics and sunscreens with $TiO_2$ NPs content. Several studies have concluded that $TiO_2$ NPs does not penetrate the intact human skin, they are not systematically available to a significant extent after dermal exposure (for review see Shi et al. 2013) and they remain in the uppermost layers of the stratum cornea, in intact skin, compromised skin or skin exposed to simulated solar radiation (Miquel-Jeanjean et al. 2012). In summary, dermal absorption $TiO_2$ NPs *in vivo* and *in vitro* was recorded to be very low resulting in values bellow the detection levels (reviewed in Geraets et al. 2014), except the systemic bioavailability observed after subchronic dermal absorption in hairless mice and porcine skin (Wu et al. 2009).

*Pulmonary absorption* of $TiO_2$ NPs following inhalation represents very important entry gate of $TiO_2$ NPs into human body in occupational environment. Although no human data are available, inhalation, intratracheal instillation, and intranasal studies in rats have suggested that $TiO_2$ NPs can translocate from the airway lumen to interstitial tissue and subsequently through the systemic circulation to systemic tissues or from nasal cavity into sensory nerves and to the nervous system (Sager et al. 2008; Wang et al. 2008). Translocation of $TiO_2$ NPs in alveolar region across air-blood barrier has been described to be size dependent in range of 5-100 nm (Geiser and Kreyling 2010).

### Distribution and accumulation

Nanomaterials rapidly distribute from blood to tissues, particularly to a highly perfused reticuloendothelial

system (RES)-containing tissues, such as liver and spleen (Geraets et al. 2014). In systemic circulation, $TiO_2$ NPs can interact with plasma components (Deng et al. 2009), contribute to disturbances in the corona environment (Mikkelsen et al. 2011) and penetrate human red blood cells (anatase, 20-30 nm, 5 μg/ml; Rothen-Rutishauser et al. 2006). Within only 30 min after intratracheal administration of $TiO_2$ NPs (20 nm), they have been also found in platelets inside of pulmonary capillaries of rats forming platelets aggregations (Zoroddu et al. 2014). In tracheal explants system, $TiO_2$ NPs (21 nm; 5 mg/h; 1 h) entered epithelium and translocated to the subepithelial space (Churg et al. 1998). Acute instillation and sub-chronic studies with $TiO_2$ NPs (20 nm) have even showed an access to the pulmonary interstitium (Ferin et al. 1992). Moreover, other studies have demonstrated that intranasally instilled $TiO_2$ NPs could be also translocated into the central nervous system via the olfactory nerves and cause brain lesions (Wang et al. 2008; Li et al. 2010).

In regard to most extensive occupational exposures to $TiO_2$ NPs by inhalation, most studies to date, concerning $TiO_2$ NPs toxicology, have been focused on pulmonary inflammation (Inoue et al. 2008; Sager et al. 2008). Following intratracheal instillation of 1 mg/kg b.w. of $TiO_2$ NPs (20 nm) in rats, the nanosized $TiO_2$ was mainly accumulated in lungs with high persistence and slow clearance after 3 months of exposure (Zhang et al. 2013). Beside of the pulmonary inflammation, the inhalation exposure to $TiO_2$ NPs was coincided with increased likelihood of arrhythmic events. Ultrafine $TiO_2$ NPs (2 mg/kg b.w.; <50 nm) were able to reach the heart via pulmonary barrier and acutely alter the cardiac excitability even after a single intratracheal administration (Savi et al. 2014).

Many other reports have clearly shown that $TiO_2$ NPs administered in different ways migrate through systemic circulation to different organs, accumulate there, and cause a serious injury (Arora et al. 2012). $TiO_2$ NPs were found in liver, spleen, kidney, lung, heart, and brain of animals in which they have induced oxidative stress, inflammatory reactions, DNA damage, and apoptosis (reviewed in Gao et al. 2011). In acute toxicity study, the highest accumulation of $TiO_2$ NPs (80-110 nm) was found in spleen after a single intraperitoneal injection, but it was also deposited in liver, kidney, and lung (Chen et al. 2009). Severe spleen lesion, hepatocellular necrosis and apoptosis, hepatic fibrosis, thrombosis in the pulmonary vascular system, and renal glomerulus swelling have been observed in high-dose group (2592 mg/kg b.w.). On the other hand, Huggins and Froehlich (1966) have reported that after intravenous injection of $TiO_2$ (0.2-0.4 μm; 250 mg/kg b.w.) to rats, the highest $TiO_2$ NPs levels were found in liver, followed by the spleen, lung, and kidneys, with no detectable levels of $TiO_2$ NPs in blood cells, plasma, brain, and lymph nodes. Any remarkable toxic effects have been observed in the study of Fabian et al. (2008) after a single intravenous injection of $TiO_2$ (5 mg/kg b.w., 20-30 nm) to experimental animals. Similarly, no clinical and hematologic signs of a systemic inflammatory reaction were observed after intravenous administration with more than hundredfold higher dose (560 mg/kg b.w.) of $TiO_2$ NPs (4.7 nm) (Umbreit et al. 2007). In contrast, a much higher dose of $TiO_2$ NPs (5 g/kg b.w., 80 nm) administered by a single application of oral gavage to mice of both gender have been demonstrated to cause hepatic injury represented by an elevated ALT/AST (aspartate aminotransferase /alanine aminotransferase) enzyme ratio and serum LDH (lactate dehydrogenase) in female rats only. Moreover, increased serum BUN (blood urea nitrogen) and CR (creatinine) levels also indicated kidney dysfunction in females (Wang et al. 2007).

Studying long-term (90-day) exposure to $TiO_2$ NPs in rats, spleen injury induced by intragastric administration (2.5, 5, 10 mg/kg b.w.) resulted in histopathological changes and reduction of immune capacity as a consequence of significant alteration of inflammatory and apoptotic cytokines expression (Sang et al. 2012). Another long-lasting (90-day) oral toxicity study in mice showed $TiO_2$ NPs (10 mg/kg b.w.) accumulation in the ovary and ovarian damage, oxidative stress, imbalance of sex hormones, and mineral element distribution, followed by decrease of fertility and pregnancy rates. In male mice, long-time retention of $TiO_2$ NPs resulted in testicular lesions, sperm malformations, alterations in gene expression profiles, and serum sex hormone levels (Gao et al. 2012; Gao et al. 2013). More recently, the sex-related effects after only 5-day oral exposure to $TiO_2$ NPs (anatase; 284 nm; 2 mg/kg b.w.) have been recorded in the study of Tassinari et al (2013). Deposition of $TiO_2$ NPs was significantly increased in spleen and ovaries. Endocrine-active tissues such as thyroid (both sexes), adrenal cortex (females only), adrenal medulla (both sexes), and ovarian granulose cells, as demonstrated by histopathological findings, have been shown to be a target of $TiO_2$ NPs. Changes in serum levels of testosterone (both sexes) and T3 (males only) were concurrently present (Tassinari et al. 2013). The data are reviewed in Table 1.

**Table 1**
**Effects of titanium dioxide ($TiO_2$) nanoparticles after different ways of exposure**

| Animal/ Model | Concentration and size | Exposure | Effects | References |
|---|---|---|---|---|
| Mice | 1 mg/kg b.w. 20 nm | Intratracheal instillation | Accumulation in lung | Zhang et al. 2013 |
| Rats | 2 mg/kg b.w. <50 nm | Intratracheal administration; a single dose | Pulmonary inflammation, arrhythmic events | Savi et al. 2014 |
| Rats | 100 mg/kg b.w. < 20 nm, anatase | Oral administration; PND 2-21 | Oxidative stress, inflammatory reactions, DNA damage and apoptosis | Gao et al. 2011 |
| Mice | 324-2592 mg/kg b.w. 80-110 nm | Intraperitoneal injection; a single dose | Spleen lesion, hepatocellular necrosis and apoptosis, hepatic fibrosis, trombosis in the pulmonary vascular system, renal glomerulus swelling | Chen et al. 2009 |
| Rats | 250 mg/kg b.w. 0.2-0.4 μm | Intravenous injection | Highest hepatic accumulation | Huggins and Froehlich 1966 |
| Rats | 5 mg/kg b.w., 20-30 nm | Intravenous injection; a single dose | No toxic effects | Fabian et al. 2008 |
| Mice | 560 mg/kg b.w. 4.7 nm | Intravenous administration | No clinical and hematologic signs of a systemic inflammatory reaction | Umbreit et al. 2007 |
| Mice | 5 g/kg b.w. 80 nm | Oral gavage, a single dose | Hepatic injury, kidney dysfunction in females | Wang et al. 2007 |
| Rats | 2.5, 5, 10 mg/kg b.w. | Intragastric administration; for 90 days | Spleen injury, alteration of cytokines expression | Sang et al. 2012 |
| Mice | 10 mg/kg b.w. | Intragastric administration; for 90 days | Ovarian damage, oxidative stress, imbalance of sex hormones and mineral element distribution | Gao et al. 2012 |
| Mice | 2.5, 5, 10 mg/kg b.w. | Intragastric administration; for 90 days | Testicular lesions, sperm malformations, alterations in gene expression profiles and serum sex hormone levels | Gao et al. 2013 |
| Spraque-Dawley rats | 2 mg/kg b.w. 284 nm; anatase | Oral administration; for 5 days | Increased deposition in spleen and ovaries; pathological changes in thyroid, ovaries, adrenal cortex and medulla; altered serum levels of testosterone and T3 | Tassinari et al (2013) |

b.w.=body weight; GD=gestation day; PND=postnatal day

## Elimination/Excretion

Elimination of NPs is, in general, a quite slow and in the case of metal oxide NPs may rather be related to the dissolution. Nanosized $TiO_2$ in systemic circulation has two potential pathways for clearance, by urine from kidneys and via the bile into feces from the liver.

Interesting kinetic input for human risk assessment of $TiO_2$ NPs offered the recent study by Geraets et al. (2014) with long time investigation of titan elimination. After intravenous administration of single or five repeated doses of $TiO_2$ NPs, similar titan levels to those in vehicle control rats were observed in feces as well as in urine consistently with the negligible elimination. At day 90 post-exposure,

redistribution of titan levels was found between liver and spleen (higher levels in spleen). Furthermore, disappearance of $TiO_2$ from the reproductive organs was different, with no detectable titan concentrations in testes at day 30 after the exposure and still detectable levels in ovaries at day 90 (Geraets et al. 2014).

Inhaled $TiO_2$ NPs deposited in respiratory tract and phagocytized by alveolar macrophages can be transported to the larynx by mucociliary action and cleared *via* expiration of sputum. Further, they can be swallowed and thereafter moved into the GIT (Shi et al. 2013). Thought, *in vitro* studies with $TiO_2$ NPs (anatase/rutile; 80/20; 25 nm) have shown rapid alveolar macrophages uptake by Fcγ receptor II (Scherbart et al. 2011), Ambalavanan et al. (2013) have suggested that exposure of developing lung to nanosized $TiO_2$ may lead to an ineffective clearance by macrophages and persistent inflammation followed by respiratory failure in neonatal rats. Similarly, highly toxic particles, $TiO_2$ nano-belts longer than 15 μm, are not able to be sequestered into the cell lysosomes and their persistence in the lung induces inflammatory response and release of inflammatory cytokines with detrimental effects (Hamilton et al. 2009). Ineffective macrophage clearance of inhaled $TiO_2$ NPs and their bio-persistence might be followed by translocation into the lung interstitium and through the vasculature induce adverse systemic effects (Geiser et al. 2008). On the other hand, the agglomerates formed at high doses of nanosized $TiO_2$ may hardly migrate to systemic organs (Shi et al. 2013).

Generally, data evidencing the rapid distribution, slow or ineffective elimination, and potential long-time tissue accumulation, are especially important for the human risk assessment of $TiO_2$ NPs and represent new challenges to investigate in more details potential adverse effects of ultrafine $TiO_2$ considering their ubiquitous exposure in various doses.

## Transport of $TiO_2$ NPs through blood brain barrier (BBB)

The BBB is a highly specialized system that separates peripheral circulation from the cerebrospinal fluid to restrict the access of large or hydrophilic compounds to the brain. The endothelial cell monolayer connected by a complex of tight junctions creates a physical barrier which severely limits the paracellular transport across the BBB. In addition to physical barrier, the BBB also contains several metabolic barriers to drug delivery. Additionally, array of intra- and extracellular enzymes inactivates many compounds that attempt to cross the BBB (Patel et al. 2012).

The establishment of the BBB is dependent on the specialized endothelial tight junction cells, particular pattern of enzymatic activity, a distinct electrochemical gradient, and specific BBB transporters. The development of the BBB is a gradual process, it starts *in utero* shortly after intraneural neovascularization (Bauer and Bauer 2000) and it acquires capabilities comparable to those in adulthood at 6 months of the human age

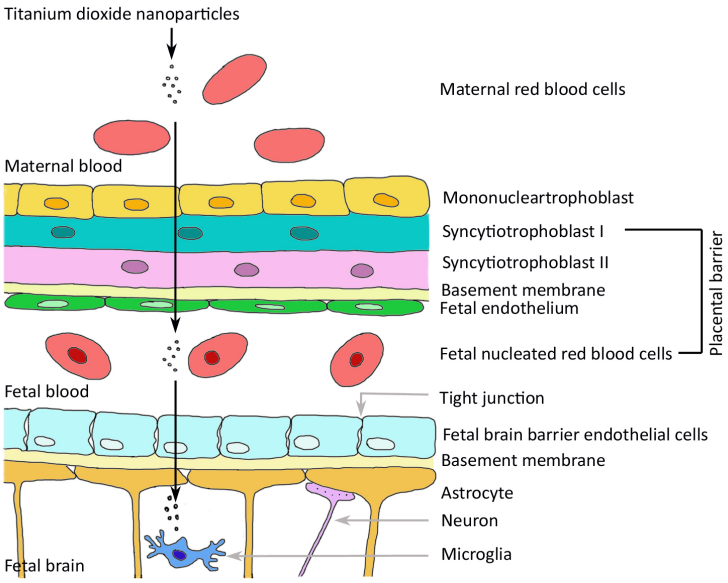


**Fig. 1. Transition of $TiO_2$ nanoparticles across placental and fetal blood brain barrier after mother`s exposure to ultrafine $TiO_2$ during pregnancy.**

(Costa et al. 2004). The formation of the tight junction associated transmembrane proteins, occludin and claudin-5, occurs also during gestation (Virgintino et al. 2004). Generally, despite species-species differences, it is thought that structural and functional aspects of the BBB are similar in various species (Cserr and Bundgaard 1984) and that the rat brain appears notably to be an appropriate model for the risk evaluation of toxic effects on the postnatal brain development as most of the brain structures that develop postnatally in humans also develop postnatally in rats (Watson et al. 2006).

The evidence exists that NPs, whose diameter is smaller than 200 nm, are able to cross the BBB (Tsuji et al. 2006). Specific mechanisms of the most NPs, targeting the brain structures, are largely unknown. Two different pathways have been proposed to reach this organ: uptake of NPs by sensory nerve endings in airway epithelia, followed by axonal translocation to the CNS or by the nerve endings of the olfactory bulb and subsequent translocation to brain structures (Fig. 1). Another pathway is uptake of NPs through BBB via systemic distribution (Medina et al. 2007; Hu et al. 2010).

The brain is especially vulnerable to oxidative stress damage because of its high content of easily peroxidizable unsaturated fatty acids, high oxygen consumption rate, and relative deficiency of antioxidant enzymes compared with other organs (Skaper et al. 1999). In this connection, the mechanisms of brain neuron injury are diverse; however, a common mechanism of oxidative stress caused by NPs has been identified (Oberdoster et al. 2004). Oxidative stress is *inter alia* extensively mediated by the microglia, a macrophage-like, normally inactive phagocytic cell unless exposed to exogenous stimuli, such as NPs. The response known as the oxidative burst is induced which includes an increase in metabolic activity, a change in cell shape and size, and cytoplasmic engulfment of the stimuli (Long et al. 2007). From developmental point of view, compromised BBB function has been shown to occur as a result of oxidative stress (Haorah et al. 2005) and inflammation (Stolp et al. 2005) during critical periods when the BBB is particularly sensitive to external stimuli.

Beside of an increasing number of studies, showing that $TiO_2$ NPs may have negative effects on the respiratory and metabolic system of organisms, very few studies have been focused on the central nervous system (CNS). $TiO_2$ NPs have also capacity to penetrate the BBB and subsequently influence the BBB function, brain physiology, and cause serious adverse effects.

The study of Ma et al. (2009) have shown that daily intraperitoneal injection of $TiO_2$ NPs (150 mg/kg) for 14 days resulted in NPs accumulation in the brain with subsequent organ injury due to the oxidative stress. Lipid peroxidation, the decrease of the total anti-oxidation capacity and activity of antioxidative enzymes, the reduction of glutamic acid levels and acetylcholinesterase activity, and excessive release of nitric oxide, have been described. Moreover, it was also found that $TiO_2$ NPs content was significantly higher than those of $TiO_2$ fine particles (FP) of the same concentration (Ma et al. 2009). Li et al. (2010) have suggested that intratracheally administered $TiO_2$ NPs (3 nm; 13.2 mg/kg; once a week for 4 weeks) might also pass through the BBB in mice. Interesting findings were described by the action of intra-nasally instilled $TiO_2$ NPs (25 nm, 80 nm, 155 nm; 50 mg/ kg; 30 days). Translocated $TiO_2$ NPs (80 nm, 155 nm) to and accumulated in murine brain caused imbalance of monoaminergic neurotransmitters: significantly increased norepinephrine and 5-hydroxytriptamine levels, while levels of dopamine (DA), 3,4-dihydrophenylacetic acid, homovanillic, and 5-hydroxyindole acetic acid were decreased (Wang et al. 2008). Mice hippocampal neuroinflammation, due to $TiO_2$ NPs (208-330 nm; 2.5, 5, 10 mg/kg b.w.; intranasal administration for 90 days), occurred as evidenced by altered expression levels of the genes and their proteins in TLRs/TNF-α/NF-κB signaling pathway and by a reduction in immune capacity (Ze et al. 2014). Over-proliferation of the glial cells, neuron necrosis, and abscission of perikaryon, nuclear irregularity, and cellular degeneration in mouse hippocampus have caused the impairment of spatial memory following exposure to nanosized $TiO_2$ (Ze et al. 2014). Strong oxidative stress, mitochondrial damage in human glial cells, and an increase in the mitochondrial membrane potential also suggested $TiO_2$ NPs toxicity to the hippocampus region (Huerta-Garcia et al. 2014).

The evidence for perturbation of ionic homeostasis in the mouse brain provided significantly decreased Na/K-ATPase activity and $K^+$ content and significantly increased $Na^+$ content measured after $TiO_2$ NPs exposure in study by Hu et al. (2010). Additionally, decreased zinc content in the brain by the action of the $TiO_2$ NPs suggested the impairment of cognition and spatial memory as zinc functions as a neuronal messenger and modulator of synaptic transmission (Hu et al. 2010). The data are reviewed in Table 2.

The findings recorded should focus attention on the effects of $TiO_2$ NPs application and exposure, especially on long-term exposure effects in the human CNS, having regard also to next generation implication. Further-

more, neurotoxicity of nanosized $TiO_2$ damaging CNS should provide a base for the design and development of the drug delivery systems using NPs, because until now, the evaluation of neurotoxicity has been limited and the mechanisms of NP effects are poorly understood (Hu and Gao 2010).

## Transport of $TiO_2$ NPs through placental barrier

As placental barrier limits applications of some drugs during pregnancy, nanotechnology might offer solutions to this problem by developing drug-NP conjugates accumulating in specific target tissues but not in

**Table 2**
**Effects of translocation of titanium dioxide ($TiO_2$) nanoparticles through blood-brain and placental barriers**

| Animal/ Model | Concentration and size | Exposure | Effects | References |
|---|---|---|---|---|
| ICR mice | 150 mg/kg b.w. 5 nm | Intraperitoneal injection; daily for 14 days | Accumulation of NPs in brain; brain injury and oxidative stress | Ma et al. 2009 |
| Mice | 13,2 mg/kg 3 nm | Intratracheal administration; once per week for 4 weeks | Crossed through BBB; brain injury and oxidative stress | Li et al. 2010 |
| ICR female mice | 2.5, 5, 10 mg/kg b.w. 280-330 nm | Intranasal administration; 90 days | Hippocampal neuroinflammation, impairment of spatial memory | Ze et al. 2014 |
| ICR mice | 5, 10, 50 mg/kg b.w. | Intragastric administration; every day for 60 days | Impairment of spatial memory, disturbance of the homeostasis of trace elements, enzymes, neurotransmitter system in the brain | Hu et al. 2010 |
| Rat (C6) and human (U373) glial cells | | | Strong oxidative stress; damage of mitochondria | Huerta-Garcia et al. 2014 |
| Pregnant ICR mice | 1 mg/ml 25-70 nm | Subcutaneous administration; GD 6, 9, 12, 15, 18 | Defects in development of the central dopaminergic system in offspring | Takahashi et al. 2010 |
| Pregnant ICR mice | 1 μg/ μl-100 μl 2570 nm | Subcutaneous injection; GD 6, 9, 12, 15 | Alteration of genes expression related to the development and function of CNS | Shimizu et al. 2009 |
| Pregnant mice | 0.8 mg/animal 35 nm | Intravenous injection | NPs accumulation in the placenta, fetal liver and brain; pregnancy complications; smaller fetuses | Yamashita et al. 2011 |
| Pregnant rats | 100 mg/kg b.w. < 20 nm, anatase | Oral administration; PD 21 to PND 2 | Decreased hippocampal synaptogenesis due to oxidative stress and inflammation | Gao et al. 2011 |
| Pregnant Wistar rats | 100 mg/kg b.w. 10 nm, anatase | Oral administration; daily, GD 2-21 | Reduced cell proliferation in the hippocampus and impaired learning and memory of offspring | Mohammadipour et al. 2014 |
| Pregnant Wistar rats | 5 g/kg b.w. 21 nm | Oral administration; once a day, GD 6-12 | Detection of NPs in maternal and neonatal lungs; inflammatory lesions in lung and delayed saccular development in neonates | Elbastawisy et al. 2014 |
| Mice | 100, 1000 mg/kg b.w. 50 nm | Oral administration; GD 9 | Increase in fetal deformities and mortality | Philbrook et al. 2011 |

b.w.=body weight; GD=gestation day; PN=prenatal day; PND=postnatal day

developing fetus (Menjoge et al. 2011). Beside of drugs, commercially used NMs are also able to cross placental barrier and cause various disturbances in developing organism (Fig. 1). The formation of the placenta since implantation and during the first trimester causes that the embryo is not separated by a fully mature barrier from maternal circulation during placentation. For that, the grater possibility for NPs to enter the fetal tissues exists what leads to greater risk of the embryo mortality and different congenital malformations (Bevilacqua et al. 2010; Kulvietis et al. 2011).

Within the scope of nanotoxicology, the study of developmental abnormalities and dysfunctions after NPs exposure should be seriously considered especially during formation of the embryo. The potential toxicity of $TiO_2$ in next generation has been evaluated in several studies which have focused on developmental toxicity using *in vivo* rodent models (Shimizu et al. 2009; Takeda et al. 2009; Hougaard et al. 2010; Takahashi et al. 2010; Yamashita et al. 2011). Although experimental data show that nanosize $TiO_2$ may move across the placenta into fetus, it has not yet been evidenced, whether human exposure to $TiO_2$ NPs can cause developmental toxicity.

Studies exploring embryo/developmental toxicity of $TiO_2$ NPs in mammals are not very abundant in comparison with those for chickens and fish (reviewed in Taylor et al. 2012), but a sufficient amount of evidence highlighted effects of nanosized $TiO_2$ on the development of the CNS.

Interesting findings by the action of prenatal exposure (subcutaneous administration) to $TiO_2$ NPs presented Takahashi et al. (2010), who have determined a deleterious effect on dopaminergic system of developing mouse brain. Anatas form of $TiO_2$ NPs [25-70 nm; 1 mg/ml; gestation days (GD) 6, 9, 12, 15] significantly increased the levels of DA and DA metabolites in the striatal and prefrontal areas exposed rats compared to the control animals. As the increase in DA levels might affect motor and cognitive functions and, moreover, defects in the dopaminergic system are associated with psychiatric pathologies such as ADHD (attention deficit hyperactivity disorder) and schizophrenia, prenatal exposure to $TiO_2$ NPs appears to be critical. The subcutaneous administration of nanosized $TiO_2$ (25 and 70 nm; 16 mg/kg b.w.; GD 3, 7, 10, 14) to pregnant mice also affected genital and cranial nerve systems in male offspring, when $TiO_2$ NPs were found in the testes as well as brain of 6-week-old male mice (Takeda et al. 2009). The alteration of gene expression related to mice brain development and function were observed by Shimizu et al. (2009) as a consequence of a subcutaneous injection to pregnant mice (anatase; 2570 nm; 100 μl $TiO_2$ NPs suspended at 1 μg/μl; GD 6, 9, 12, 15). Furthermore, in fetal (GD 16) as well as pup brains [postnatal days (PND) 2, 7, 14, 21], altered genes associated with response to oxidative stress, cell death, and mitochondrial activity have been observed. Yamashita et al. (2011) have recorded serious pregnancy complications after intravenous injection of $TiO_2$ NPs (0.8 mg per mouse; 35 nm). Lower uterine weights, significantly higher fetal resorption rates, and smaller fetuses along with decreased maternal body weight were revealed. Accumulated $TiO_2$ NPs have been found in the placenta, fetal liver, and fetal brain. In study of Gao et al. (2011), nanopowder $TiO_2$ (anatase, < 25 nm; final dosage 100 mg/kg b.w.) administered orally to female adult rats during pregnancy (from prenatal day 21 to PND 2) or in the lactation period (from PND 2 to 21) decreased hippocampal synaptogenesis, impaired the input/output (*I/O*) function and paired-pulse reaction (PPR) of hippocampus as potential result of oxidative stress, and inflammation. The adverse effects of $TiO_2$ NPs exposure on hippocampus being mainly responsible for learning and memory suggested that developmental brains are undoubtedly target organs of ultrafine $TiO_2$. Support for such conclusion have presented findings recorded by Mohammadipour et al. (2014) who have indicated that the oral exposure of pregnant mothers to $TiO_2$ NPs (anatase; 10 nm; 100 mg/kg b.w.; GD 2-21) can impair hippocampal cell proliferation in newborn offspring followed by memory and learning impairment in adulthood due to neurogenesis damage in developmental period. Adverse effect of $TiO_2$ NPs prenatal exposure on emotional behaviors in adulthood have been caused by significant oxidative damage to nucleic acids and lipids in the neonatal rat brains being manifested as depressive-like behaviors in adulthood (Cui et al. 2014).

Recent data have proven the transition of $TiO_2$ NPs from the systemic circulation after the maternal intake by mouth to pulmonary tissue of adult as well as neonatal lungs (Elbastawisy et al. 2014). Maternal oral gavage of nanosize $TiO_2$ (21 nm; 5 g/kg b.w.; 0.1 ml/10 g b.w. GD 6-12) resulted in histomorphometric alterations (abnormal thinning of the alveolar septa, changes of the lamellar inclusion ultrastructure), epithelial apoptosis, interstitial particle-laden macrophages and neonatal saccular maldevelopment (Elbastawisy et al. 2014). Only a single maternal oral dose of $TiO_2$ NPs (50 nm; 100 or

1000 mg/kg b.w.; GD 9) was able to cause harmful effect on developing CD-1 mouse tissues as assessed by a significant increase in the exencephaly, open eyelids, leg and tail defects, and mortality (Philbrook et al. 2011). The data are reviewed in Table 2.

Transport of ultrafine $TiO_2$ particles in systemic circulation and further transition through barriers, specifically placental and blood-brain bariers are well documented. From developmental point of view, there is a raising the concern in the exposure during critical windows, due to ubiquity of $TiO_2$ NPs in consumer goods and the fact that human mothers may be exposed to high cumulative doses.

## Molecular mechanisms of $TiO_2$ NPs action

Several *in vitro* and *in vivo* studies have explored possible mechanisms through which $TiO_2$ NPs may exert their toxic effect. Three possible pathogenetic mechanisms have been suggested: oxidative stress, inflammation, and apoptosis, which could or could not be linked together.

The overproduction of reactive oxygen species (ROS) such as $O_2^{\cdot-}$ (the superoxide anion), .OH (the hydroxyl radicals) and $H_2O_2$ (the non-radical hydrogen peroxide) and high levels of lipid, protein and DNA peroxidation in the mouse brain, spleen, liver, and kidney by the action of $TiO_2$ NPs exposure, as main pathogenetic mechanisms, have been described in many experiments (Ma et al. 2009; Li et al. 2010; Zhao et al. 2010; Ze et al. 2013). At the molecular level, nuclear factor kappa B (NF-κB), the major stress response transcription factor and Nrf-2, the critical regulator of the cellular antioxidant response, have been identified as the target transcriptional factors of $TiO_2$ NPs toxicity significantly increased in gene as well as protein expression levels (Ze et al. 2013). Furthermore, the markedly increased levels of anti-inflammatory protein heme oxigenase 1 (HO-1) were determined, through which p38-Nrf-2 signal transduction pathway activation following $TiO_2$ NPs exposure may act as a cellular adaptive response to oxidative stress.

Hippocampal neuroinflammation, due to $TiO_2$ NPs administration, occurred as it was evidenced by altered expression levels of the genes and their proteins in TLRs/TNF-α/NF-κB (tool like receptors/tumor necrosis factor α/nuclear factor kappa B) signaling pathway and reduction in immune capacity (Ze et al. 2014). Another study has shown increased expression of IL-1ß, IL-2, IL-4, IL-6, IL-10, and IL-18 in nephritic inflammation caused by $TiO_2$ NPs. In addition, $TiO_2$ NPs activated NF-κB, and subsequent increase in the expression of TNF-α, macrophage migration inhibitory factor (MMIF), cross-reaction protein, transformation growth factor ß (TGF-ß), interferon-γ, and CYP1A1 and decrease in the heat shock protein 70 (Hsp 70) expression have been associated with ultrafine $TiO_2$ oral administration (Gui et al. 2011). Post-exposure elevated levels of pro-inflammatory mediators, such as IL-1ß, TNF-α and macrophage inflammatory protein (MIP)-2, in bronchoalveolar lavage fluid (BALF) and mRNA expression of TNF-α and IL-1ß in lung tissue have been recorded after intraperitoneal application of nanosized $TiO_2$ (Moon et al. 2010). Interestingly, *in vitro* study in mass cells (RBL-$_2H_3$) has revealed directly triggered inflammatory mediators without traditional immuno-stimulation by allergens as result of membrane L-type $Ca^{2+}$ channel activation (Chen et al. 2012).

From developmental point of view, it is worth to highlight that maternal (lung) inflammation following nanosized UV-Titan exposure, may result in a cross-placental transfer of inflammatory cytokines and adversely interfere with fetal neurodevelopment inducing structural and functional abnormalities in the adult offspring (Meyer et al. 2009; Hougaard et al. 2010).

Finally, sufficient research evidence for generation of ROS following $TiO_2$ NPs exposure has been assembled in many *in vitro* and *in vivo* studies. ROS-induced signaling and activation of the IL family of cytokines, Bax, caspases 3 and 9, NF-κB, and p53 and phosphorylation of p38 and $G_2$M phase cell cycle arrest were described to be common findings (Meena et al. 2012; Shi et al. 2013). In future studies, mapping of the molecular pathways altered in organs of the descendants might reveal the molecular targets of the exposure and unmask the potential relevance to human health (Hougaard et al. 2010).

## Conclusion

Thirty three years ago, the International Program on Chemical Safety (1982) has shown that most of the ingested titanium is excreted by urine and not absorbed by organism. The recent studies have indicated that nano-$TiO_2$ particles have stronger toxicity. In addition, histopathological and functional changes in different organs have been described (Chen et al. 2009).

The human or environmental data for $TiO_2$ NPs exposure are very limited. Several findings have suggested the need for a caution in consumers as well as workers

handling NMs. Recommendation on exposure hazard assessment made throughout the life cycle of products containing $TiO_2$ NPs as well as the effective proceedings as a proper local exhaust ventilation, filtration, containment, and good work practices could contribute to decrease of human risk (Methner et al. 2010). To assure worker as well as consumer safety, development of a framework enabling risk management for all commercial $TiO_2$ NPs including bio-safety evaluation of $TiO_2$ nanoparticulate carriers for drug delivery, should be stated (Shi et al. 2013).

As it is evident from many studies, different exposure routes may cause different toxic effects and pattern of adverse effects on the retention sites which appears to be specific to specific NPs. In the future, particularly long-term low level effects by nanoscale $TiO_2$ focused on potentially susceptible life-stages and sex-related susceptibility should draw attention. Especially, the assessment of the reproductive and developmental toxicity of NPs is at the beginning. Moreover, the legislative measures aimed to control the exposure of pregnant women as well as women and men in fertile age to NPs are missing (Campagnolo et al. 2012).

With respect to the exposure during pregnancy and postnatal development it should be strictly considered the barrier development such as placental as well as blood brain barriers. Complex biological status, such as pregnancy and the early stage of life, and finely regulated processes of proliferation, differentiation, maturation, receptor imprinting, and apoptosis included within them, highlight the urgent need to investigate and to understand the interaction of engineered nanomaterials (ENMs) with the cell machinery and to help in designing of ENMs aimed to avoid the adverse impact on organism during critical developmental windows. In the connection, children's exposure to some toxicants have more pronounced adverse effects or act only on processes occurring during development. Mitotically inheritable epigenetic patterns which have a key role in embryogenesis and also later in development can be long-lasting or passed to the next generation. Epigenetic pathological effects of $TiO_2$ NPs/ENMs were already described, but their ability to induce diseases remains unclear (Smolkova et al. 2015). In this sense, it is important to understand that young children and pregnant/lactating women are continuously exposed to varying doses of ENMs which might exert potential toxicity to developing systems and cause many not easily or not immediately observable diseases (Campagnolo et al. 2012).

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