Titanium Dioxide Nanoparticles: A Review of Toxicological Effects

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Abstract

There is an increased interest in nanotechnology applications in various fields e.g. medicine, pharmacy, environmental protection and agriculture. Due to an increasing scope of applications, the exposure of humans to nanoparticles (NPs) is inevitable. In a wide group of nanoparticles currently used on an industrial scale, titanium dioxide nanoparticles—TiO2NPs—are particularly popular. They are commonly used as a food additive (E 171). The possible risk to health after consuming food containing nanoparticles has been poorly explored but it is supposed that the toxicity of nanoparticles depends on their size, morphology, rate of migration and amount consumed. TiO2NPs can induce inflammation due to oxidative stress. They can also have a genotoxic effect leading to apoptosis or chromosomal instability. This article provides a review of previous studies concerning the effects of exposure to TiO2NPs on a living organism (human, animal) in order to demonstrate potential toxicity of inorganic nanoparticles on human health.
Keywords: Nanoparticles; Titanium; Oxidative stress; Neurotoxicity; Lung

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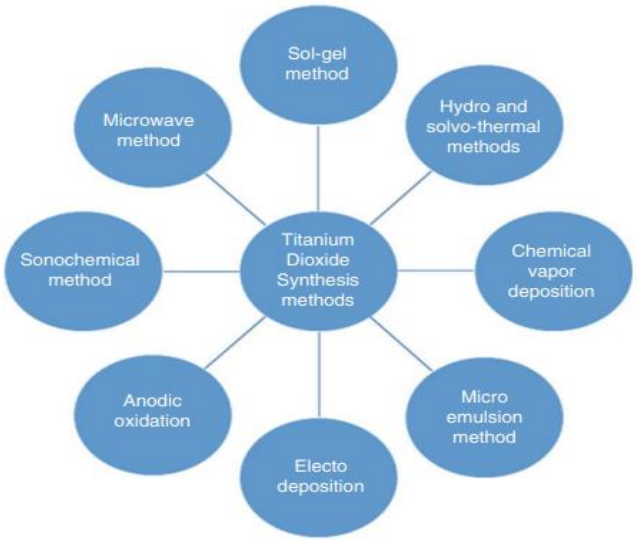
Introduction

Nanomaterials are defined as materials where at least one of their dimensions (length, height, and width) is in the nanoscale, i.e. smaller than 100 nm **(Paras et al., 2022).** According to The International Organization for Standardization (ISO), nanoparticles (NPs) are defined as "nano-objects with all external dimensions in the nanoscale i.e. entirely nano-sized **(Barhoum et al., 2022).**

NPs have attracted huge attention due to their small size and high surface to volume ratio that give rise to their reactivity and extraordinary chemical, electronic, optical, magnetic, and mechanic properties **(Selmani et al., 2022).** Titanium is the 9th most common element in the earth's crust. Titanium dioxide (TiO_2), oxide of the element titanium, is found naturally in several kinds of rock and mineral sands. It appears red or reddish brown to black in color due to the presence of other metals contamination such as iron and chromium. However, TiO_2 can also be obtained from different synthetic sources



(Fig. 1) (Sungur, 2021).

Figure (1): Different methods of TiO_2NPs synthesis **(Sungur, 2021).**

Titanium dioxide nanoparticles (TiO_2NPs) are being manufactured in large quantities for use in a wide range of applications. More than 10,000 tons of TiO_2NPs were produced worldwide in 2010 **(Paramo et al., 2020).** Titanium dioxide is a non-combustible and odorless white powder. It has the molecular weight of 79.9 g/mol, the relative density of 4.26 g/cm^3 at 25°C, the boiling point of 2972**℃**, and the melting point of 1843**℃ (Dosawada et al., 2023).** The unique characters of TiO_2NPs are directly related to its crystal structure which depends on the method and temperature of preparation process. TiO_2NPs exist in three main crystalline forms; anatase, rutile and brookite. Anatase and rutile forms have many commercial values compared to brookite **(Irshad et al., 2021).** TiO_2 has been approved by the European Food Safety Authority (EFSA) as food colorant under the number E171 **(EFSA, 2021).** According to the Food and Drug Administration (FDA), the permissible amount of E171 in food products shouldn't exceed 1% by food's weight **(Blaznik et al., 2021).** The average daily intake of E171 was estimated at 0.5-2 mg/kg body weight **(Sungur, 2021).**

Pharmacokinetics of Titanium Dioxide Nanoparticles:

Absorption: The pharmacokinetics of TiO_2NPs depends on many factors, including particle type, size, surface charge, surface coating, dose and exposure route **(Rolo et al., 2022).** Human exposure to TiO_2NPs occurs during both manufacture and use of TiO_2NPs and of products containing it **(Rashid et al., 2021).** Inhalation is the primary route of occupational exposure to TiO_2NPs. When TiO_2NPs are inhaled, they are transported to lung tissues, capillaries, airways and alveoli **(Dar et al., 2020).** Generally, there are three possible pathways for the retained NPs in lung tissues to leave the lungs: (i) mucociliary transport to the larynx and translocation to the gastrointestinal tract (GIT); (ii) macrophage mediated transport to lung-associated lymph nodes; (iii) translocation to the systemic blood circulation which may be facilitated by inflammation processes of the lung tissues **(Zeman et al., 2018).** Oral exposure to TiO_2NPs has increased as a result of a dramatic increase in food products containing them. Ingestion may also occur as a result of consumption of food contaminated by TiO_2NPs released into the environment for example; TiO_2NPs in sunscreens or other products can be released into the environment, particularly sea or river water, resulting in significant TiO_2NPs accumulation in animal species intended for food consumption **(Qian et al., 2020).** TiO_2NPs ingestion could be also related to consumption of pharmaceuticals as TiO_2NPs is a component of the protective coatings of pharmaceuticals, adding whiteness and enhancing their stability owing to its ability to scatter light and absorb ultra-violet (UV) rays. Upon ingestion, TiO_2NPs undergo transcytosis in which both the enterocytes (the epithelial cells lining the inner surface of the small and large intestines) and the intestinal Microfold cells (M cells of the Peyer's patches, important for the initiation of the mucosal immunity response) transport NPs across the GIT **(Fiordaliso et al., 2022).** Human skin has unique barrier characteristics that stand against the penetration of TiO_2NPs into the skin. Several studies have found that TiO_2NPs, even with particle size less than 100 nm, can't penetrate through the intact human dermis. Moreover, other studies have revealed that the TiO_2NPs that do manage to penetrate the skin do not exhibit toxicity under certain conditions **(Rashid et al., 2021).** Tattooing is an emerging source of TiO_2NPs especially among young people. TiO_2 is present in white inks but is also used to change the color strength of other tattoo pigments. After injection of the tattoo inks it can reach the systemic circulation and be transported to other organs **(Weiß et al., 2021).**

Distribution: The biodistribution of TiO_2NPs occurs via two kinetic processes; penetration through the blood vessels to different organs and phagocytosis of NPs by the mononuclear phagocytic system **(Ziental et al., 2020).** TiO_2NPs have the potential to cross different biological barriers such as the blood brain barrier (BBB) and the blood–placental barrier. TiO_2NPs accumulate in different organs mainly in the liver, kidneys, spleen, lymph nodes, heart and lungs, and then leave the body nearly 2 weeks after administration **(Al-Doaiss et al., 2021).** The limited elimination of TiO_2NPs from the internal organs, related to their low solubility, may lead to their accumulation in tissues upon repeated exposure **(Chen et al., 2020).**

Excretion: TiO_2NPs are cleared from the systemic circulation by two routes; kidneys/urine or bile/feces, with renal excretion as the primary route of TiO_2NPs elimination **(Ziental et al., 2020).** The International Program on Chemical Safety for TiO_2 declared that ingested TiO_2 is eliminated mainly through urine **(Abdel-Halim et al., 2022).** Although dissolution of NPs was found to be negligible, translocated TiO_2NPs are predominantly excreted in the urine as smaller agglomerates and/or disagglomerated primary particles **(Kreyling et al., 2019).**

Applications of Titanium Dioxide Nanoparticles:

Titanium dioxide NPs have found usage in numerous applications in different fields such as medical implants, drug delivery and antibacterial applications **(Al-Nemrawi et al., 2022).** TiO_2NPs are also used in the production of a wide range of goods; as a food colorant and as a pigment in paints and cosmetics (Pigment White 6 or CI 77891) **(Kirkland et al., 2022).** In treatment of cancer, TiO_2NPs act as vehicle for chemotherapeutic drugs. This allows targeted delivery of the drug to specific sites i.e. healthy normal cells remain unaffected, and also controlling the drug release **(Kawassaki et al., 2021).** TiO_2NPs have been successfully used in orthopedic and dental implants because of flexibility, high corrosion resistance in biological media and high tensile strength **(Celesti et al., 2022).** TiO_2NPs coating of metal pins and wires and of dental implants offers better protection against infection and thus promotes healing of bone fractures and more durability of the dental implants **(Jafari et al., 2020).** They are used in pharmaceuticals industry, food and food packaging, and environmental disinfection **(Fatima et al., 2021).** They are added in many consumer care products; oral hygiene products, sunscreens, ointments and creams that are applied to the skin to delay skin ageing and avoid sunburns **(Bwatanglang et al., 2022).** TiO_2NPs are highly efficient in photo-conversion so they are commonly used in dye-sensitized solar cells for the conversion of solar energy into electrical energy **(Ramakrishnan et al., 2018).** TiO_2NPs are used for improving the purification of air as for example; Ti mesh filters are used to remove the smoke generated by cigarette smoking **(Jassal et al., 2022).**

Mechanism of toxicity of Titanium Dioxide Nanoparticles:

Reactive oxygen species (ROS) are generated by aerobic organisms within the cell and are normally in equilibrium with antioxidant molecules. The imbalance between ROS and antioxidants, caused by excessive production of ROS and/or depletion of antioxidant molecules, leads to the development of oxidative stress **(Yao et al., 2021).** One of the major mechanisms of TiO_2NPs is its ability to disturb the oxidant/antioxidant redox balance in the body inducing oxidative stress **(Salman et al., 2021).** ROS interact with biologically important molecules e.g. nucleic acids, proteins and lipids **(Dvorakova et al., 2021).** TiO_2NPs-induced ROS and lipid peroxidation cause damage to the integrity of the cell membrane, resulting in increased permeability which allows TiO_2NPs to enter the cell **(Rashid et al., 2021).** Upon entrance into the cell, the internalized TiO_2NPs are transported to lysosomes, where they generate lysosomal stress and release cytosol that reacts with cellular components, resulting in DNA damage, DNA rearrangement, altered gene expression, oxidative stress, and inflammation **(Carriere et al., 2020), (Kansara et al., 2020) & (Luo et al., 2020).** Increased ROS generation can

also affect the cellular signaling cascade that controls processes such as cell proliferation, inflammation, and cell death **(Shabbir et al., 2021).** Exposure to TiO_2NPs leads also to endoplasmic reticulum (ER) stress. ER stress intermediates either activation or deactivation of critical genes involved in ROS generation such as the arachidonic acid pathway and the mitochondrial respiratory chain. The TiO_2NPs might also bind to the mitochondrial membrane, where they increase the electron transport chain within the mitochondria, thereby activating the mitochondria-mediated apoptotic pathway **(Abdel-Halim et al., 2022).** In summary, the mechanism of action of TiO_2NPs is: (i) production of ROS and formation of energy-rich electron-hole pairs in the presence of UV illumination; (ii) binding to the cell membrane, resulting in cell wall damage and peroxidation of lipids in the cell membrane; and (iii) binding to intracellular organelles and biological macromolecules **(Rashid et al., 2021).**

Toxic Effects of Titanium Dioxide Nanoparticles:

Neurotoxicity: The brain is particularly vulnerable to damage from oxidative stress due to its high rate of oxygen consumption, high content of readily peroxidizable unsaturated fatty acids, and the relatively low content of antioxidant enzymes compared to other organs **(Jelinek et al., 2021).** Various factors can influence the neurotoxic potential of TiO_2NPs, including exposure route, dose, and duration and its physical and chemical properties **(Prüst et al., 2020).** Following oral exposure, TiO_2NPs can readily accumulate in the brain due to its ability to cross the BBB **(Grissa et al., 2020).** Oral TiO_2NPs have been shown to induce impairment of neurotransmitter metabolism, oxidative stress, neuroinflammation as well as hippocampal, cortical, and cerebellar neuron apoptosis **(Halawa et al., 2022).** When TiO_2NPs are inhaled, they are deposited in all regions of the respiratory tract and by diffusion and translocation they pass across cell barriers from the portal of entry to secondary organs such as the central nervous system. Inhaled TiO_2NPs can enter directly to the brain through the olfactory nerve, circumventing the BBB, and are deposited particularly in the hippocampal region **(Márquez-Ramírez et al., 2012).**

Cardiovascular toxicity: The cardiovascular system (CVS) is highly susceptible to TiO_2NPs toxicity for several reasons. TiO_2NPs toxicity is dependent on exposure load (concentration and exposure time), NPs physiochemical characteristics (such as surface potential, size and phase) and tissue properties (such as lipid content, metabolism and antioxidant capacity) **(Mehran et al., 2022).** After administration, TiO_2NPs reach and penetrate the cardiac tissue and mediate direct cardiac injury. TiO_2NPs get internalized into the cardiomyoblasts via actin-mediated endocytosis **(Huerta-García et al., 2019). El-Bestawy and Tolba (2020)** reported that exposure to TiO_2NPs for only 2 weeks was sufficient to cause cardiac damage. Long-term oral exposure to food grade TiO_2NPs exerts toxic damage on myocardial tissues **(Shaltout et al., 2022)** and aggravates progression of atherosclerosis **(Zhu et al., 2022).**

Pulmonary toxicity: Acute exposure to TiO_2NPs caused oxidative stress, inflammatory changes and DNA damage in the lungs **(El-Shamy, 2022). Papp et al. (2020)** reported that subacute intratracheal installation of TiO_2NPs resulted in mild atelectasis or emphysema. **Smallcombe et al. (2020)**

proposed that TiO_2NPs exacerbate respiratory syncytial virus (RSV) infection. Several studies have linked TiO_2NPs exposure with the development of allergic airway diseases, such as asthma. **Harfoush et al. (2020)** reported that inhalation of TiO_2NPs enhanced eosinophil infiltration in the lungs, increased neutrophil production and cytokines release, caused goblet cell hyperplasia and mucus hyper secretion which are major characteristics of asthma. Similar results were obtained by **(Lim et al., 2021). Yamano et al. (2022)** noted development of alveolar inflammatory spots in response to TiO_2NPs inhalation. They suggested that it may represent early pneumoconiosis. "In 2006, the International Agency for Research on Cancer (IARC) classified TiO_2 as a possible inhalation carcinogen (Group 2B) for humans" **(Guseva Canu et al., 2020).**

Gastrointestinal toxicity: **Shaltout et al. (2022)** recorded that TiO_2NPs caused blunting of the intestinal surface with a reduction in the number of intestinal villi. As a result, there was marked affection of absorption and transport of important micronutrients as iron, zinc and fatty acids across the intestines. Chronic ingestion of TiO_2NPs may result in disruption of gut microbiota composition, contributing to the onset or exacerbation of intestinal inflammatory diseases such as inflammatory bowel disease **(Barreau et al., 2021).**

Hepatotoxicity: The liver is the primary target organ for accumulation of TiO_2NPs, followed by the spleen and the lung. **Chen et al. (2019)** suggested that liver damage may not be due to direct accumulation of TiO_2NPs in the liver, but rather an indirect effect through the gut–liver axis. **Mohammed and Safwat (2020)** demonstrated that TiO_2NPs-induced hepatotoxicity is mediated by oxidative stress which triggered the pro-inflammatory signaling cascades by the upregulation of expression levels of toll-like receptor 4 (TLR-4), nuclear factor-κβ (NF-κβ), and the subsequent increased tumor necrosis factor-α (TNF-α) expression levels leading to inflammation and tissue injury. Although elevated levels of TLR, known activators of the immune cell response, and elevated amounts of NF-κβ and TNF-α, together with infiltration of inflammatory cells in the liver support the role of inflammation on the onset of TiO_2NPs-induced hepatotoxicity, the identity of the initial trigger of hepatotoxicity is still unclear because there is no explanation, yet, of how TiO_2NPs activate the TLRs in the first place **(Vilas-Boas and Vinken, 2021).**

Nephrotoxicity: Kidneys are highly susceptible to toxic materials due to its high blood supply (it receives about 20-25% of the cardiac output) and are concerned with elimination of harmful substances. So, kidneys are considered to be one of the vital organs vulnerable to the effect of TiO_2NPs **(Abdel-Halim et al., 2022).** According to **Mousa (2020),** TiO_2NPs administration increased the NF-κβ and TNF-α level in the renal tissues. NF-κβ is a necessary transcriptional mediator for production of the pro-inflammatory cytokines that plays a major role in the injury of renal tubular epithelial cells. Significant reduction in the renal B-cell lymphoma-2 (BCL-2) (the anti-apoptotic family protein) level and a significant increase in the renal caspase-3 activity were detected, indicating the enhancement of renal apoptosis. Inflammation and apoptosis in renal tubules following exposure to TiO_2NPs may be due to excess of ROS production.

Reproductive toxicity: The testicular tissue is highly susceptible to the effect of free radicals and oxidative stress for several reasons, including high levels of unsaturated fatty acids, high rate of cell division, cell competition for oxygen rate and low oxygen pressure due to weakened blood vessels. So, oxidative stress is considered a significant factor for the development of male infertility **(Said et al., 2022).** According to **Dantas et al. (2022),** exposure to inorganic NPs, as TiO_2NPs, can disrupt the blood-testis barrier and affect both sperm production and morphology due to ROS generation and/or induction of inflammatory response. Accumulation of TiO_2NPs caused disturbed equilibrium of ovarian mineral elements which plays integral role in ovarian physiology. Reported alteration of sex hormones caused atresia of primary and second follicle development and reduction of fertility. Apoptosis in the ovaries was also observed **(Dianová et al., 2022).**

Metabolic toxicity: **Bakour et al. (2021)** reported disturbance of lipid profile that was attributed to TiO_2NPs-induced disruption of genes responsible for lipid metabolism and inhibition of lipoprotein lipase enzyme activity (a main enzyme involved in the metabolism, transport and tissue uptake of lipids). **Hu et al. (2020)** attributed TiO_2NPs-induced hyperglycemia to insulin resistance (IR) meanwhile insulin secretion from the pancreas wasn't affected. On the contrary, **Abdel Aal et al. (2020)** suggested that TiO_2NPs caused structural damage of the pancreas leading to hyperglycemia.

Genotoxicity: Genotoxicity of TiO_2NPs depends on crystal form; anatase being more toxic than rutile, particle size; smaller particles are more toxic as they easily penetrate into the nucleus, the time, and the concentration of TiO_2NPs exposure; the lower the concentration, the weaker the toxicity **(Shi et al., 2022).** Genotoxicity occurs via direct and indirect mechanisms; TiO_2NPs can enter the nucleus, inducing direct DNA damage through direct contact with DNA and chromosome, while indirect genotoxicity of TiO_2NPs results from the increased lysosomal release of DNases, the formation of nanoaggregates that can extrude nucleus or by ROS accumulation. TiO_2NPs can also negatively influence the DNA repair process **(Ling et al., 2021).**

Immunotoxicity: **Dhupal et al. (2018)** showed that TiO_2NPs can induce immune cell apoptosis through p38 mitogen activated-protein kinase (MAPK) pathway, resulting in a decrease in immune cells. TiO_2NPs can also induce apoptosis by causing nuclear pyknosis, activating caspase-3, increasing Bax (pro-apoptosis), and inhibiting Bcl-2 (anti-apoptosis) **(He et al., 2018) & (Zhang et al., 2018). Hong et al. (2017)** also recorded reduction in lymphocyte subpopulations such as CD3+, CD4+, CD8+, and natural killer (NK) cells via activation of the NF-κβ-mediated MAPK signaling pathways indicating toxic effects of TiO_2NPs on lymphoid organs, T cells and innate immune cells.

Conclusions: Along with global economic growth, our direct or indirect exposure to metallic NPs has been increasing. With regard to new properties offered by their small size, NPs are incorporated in more and more commercial products. Regular supply of TiO_2NPs at small doses can affect the brain, the heart and other internal organs, which can lead to an increased risk of developing many diseases, tumors or progress of existing cancer processes. The mechanism behind the nanotoxicity is attributed to oxidative stress.

No Conflict of interest.

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