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Nanotoxicity – Biological and Environmental Toxicity

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Outline

Introduction

While assessing the applications of nanotechnology in the medical domain, nanomedicines have emerged as groundbreaking solutions for treating previously deemed incurable diseases. Despite the notable advancements, there remains a substantial journey for nanomedicines to establish their definitive role in mainstream pharmaceuticals. Nanomedicines entail the utilization of materials, such as metals, carbon, and polymers, characterized by at least one dimension within the nanoscale range. In recent years, a pronounced interest has been observed among nanotechnologists and pharmacists regarding the exploration of metal and metal oxide, carbon-based, and polymeric nanoparticles. This exploration aims to assess their potential in treating diverse diseases, including but not limited to liver cancer, pancreatic cancer, lung cancer, and various neurodegenerative conditions. Additionally, researchers are diligently investigating the toxicological impacts of nanomedicines on both living organisms and the environment.

Initially, nanoparticles were predominantly synthesized through chemical methods. However, as the evaluation of their toxic by-products progressed, scientists have redirected their attention towards bio-chemical synthesis. Subsequently, there is an increasing emphasis on the utilization of polymeric nanoparticles for their potential application in nanomedicine. Unlike metal oxide nanoparticles, which are known to induce various forms of cellular damage through interactions with the cell membrane, genetic material, and the generation of reactive oxygen species (ROS), polymeric nanoparticles exhibit a lower propensity for such adverse effects [1]. To mitigate this limitation, polymeric nanoparticles are loaded with specific drug molecules to induce cellular damage selectively. The choice of the drug-loaded onto these nanoparticles is contingent upon the intended applications for which the nanoparticles will be studied. An illustrative example is the synthesis of iron oxide nanoparticles, where the use of naked metal oxide particles is employed. Hassan, D. et al., along with their research group, conducted a study on the synthesis of iron oxide (IO) nanoparticles utilizing plant extracts derived from the tentacles of the bottlebrush plant flower. The research team systematically synthesized IO nanoparticles and explored the influence of annealing temperature on both the physical and magnetic properties of these nanoparticles. Their investigations revealed that an increase in temperature led to a reduction in the size of the nanoparticles, attributed to temperature-induced stress. Subsequently, the research group focused on the smallest-sized IO nanoparticles and delved into their biomedical potential. They discovered that these nanoparticles exhibited notable toxicity against twelve water-borne pathogenic bacterial cultures. Furthermore, the nanoparticles demonstrated antioxidant properties. In the context of biomedical applications, the IO nanoparticles displayed significant toxicity against HepG2 (liver) cancer cell lines, exhibiting an impressive 80% inhibition of cancer cells at higher concentrations. Recognizing the importance of evaluating potential adverse effects, the group conducted toxicity assessments on human blood-isolated macrophages through a Hemolytic assay. The results indicated that the nanoparticles were deemed safe for use, particularly at lower concentrations. Even at higher concentrations, specifically 500µg/mL, the nanoparticles exhibited a lysis rate of less than 28%. This comprehensive evaluation underscores the potential therapeutic benefits of IO nanoparticles while ensuring their safety for practical applications [2].

Indeed, it is crucial to acknowledge that the safety of nanoparticles, including the IO nanoparticles discussed earlier, cannot be assumed universally. The dynamic nature of the human body, influenced by factors such as temperature, pH, various biochemicals, acids, and bodily fluids, may lead to different behaviours and interactions with nanoparticles. Even if deemed safe for human use,

predicting their toxicity on animals (both terrestrial and aquatic) and their potential impact on the broader environment poses a significant challenge.
Consequently, a thorough evaluation of nanoparticle toxicities on living organisms and the environment becomes imperative. This evaluation aims to provide clarity on whether nanomedicines contribute to overall betterment and, if so, what risks are associated with their use. This chapter will comprehensively explore and discuss potential toxicities associated with different types of nanoparticles and nanomedicines. By addressing these concerns, we can better understand the potential benefits and risks involved in the application of nanotechnologies in medicine and other fields.

Environmental Toxicity

Plants, animals, soil, water, and aquatic organisms, alongside microorganisms, constitute integral components of our environment. A sustainable environment is indispensable for life to thrive unless organisms adapt to new living conditions. Human existence is intricately interconnected with the environment, and the production of our food is fundamentally reliant on the health of these ecological elements. The profound impacts of climate change have demonstrated that even minor alterations in the environment can disrupt entire ecosystems. Hence, it becomes imperative to rigorously investigate the repercussions of nanomaterials on our environment. Understanding these effects is crucial to ensure the sustainability and equilibrium of our ecosystems, safeguarding not only the diverse forms of life but also the resources upon which human survival hinges.
The elemental composition and physicochemical properties of Nanomaterials (NMs) can serve as disruptive factors in the development of organisms, impeding their regular physiological functions and potentially leading to malformations that pose a lethal threat to embryos and growing animals. It is not only the chemical structure of NMs that impacts normal biological mechanisms, but also the size of these particles introduces properties that interfere with the chemical, physical, and biological activities within living organisms [45]. Cells exhibit diverse reactions based on the sizes of Nanomaterials (NMs). Small nanomaterials can easily penetrate cell membranes, gaining access to organelles within the cell. For instance, titanium dioxide (TiO2) nanoparticles have the ability to target mitochondria, leading to a disruption in mitochondrial dynamics [206].
Moreover, the positive electric charges of Nanomaterials (NMs) can lead to the destruction of membrane lipid bilayers. Additionally, the surface coating of nanomaterials has been identified as a factor causing disturbance in cell structure [207]. In a comprehensive study by Handy et al., investigating ecotoxicity effects on various organisms such as fish species, algae, bacteria, mollusks, and mammals, it was revealed that nanomaterials can exert toxic effects on these diverse species [45].
Recent studies employ a range of living models to assess the potential impacts of NMs on organisms. Investigations using mammalian models (mice) or bony fishes (zebrafish) have demonstrated that nanomaterials, such as cadmium oxide nanoparticles in mice and silica nanoparticles in zebrafish, can induce harmful effects on the embryonic and reproductive systems [208, 209]. Furthermore, experiments conducted with rat models have unveiled the hazardous effects of nanomaterials on the brain. For instance, exposure to silver nanoparticles (Ag-NMs) with diameters of 25, 40, and 80 nm for 24 hours affected the blood-brain barrier, triggering a pro-inflammatory reaction that later

evolved into brain inflammation accompanied by neurotoxic effects [211]. These findings highlight the potential risks associated with nanomaterial exposure across different biological systems.
In studies focusing on the impact of nanomaterials on plants, treating maize seedlings with a concentration of 1000 mgL-1 of silicon dioxide (SiO2) nanoparticles (NPs) resulted in reduced shoot fresh weight and shoot length. Similarly, treatment with titanium dioxide (TiO2) NPs at the same concentration caused a decrease in pigment content, affecting total chlorophyll content and the chlorophyll/carotenoid ratio [246]. Hydroponic cultures of Lolium perenne exposed to zinc oxide (ZnO) nanoparticles and Zn2+ ions demonstrated that ZnO-NMs were capable of penetrating cells, crossing root cell walls, and reaching vascular tissue through the endodermis. This uptake damaged cortical and epidermal cells, ultimately leading to a reduction in plant biomass [249].

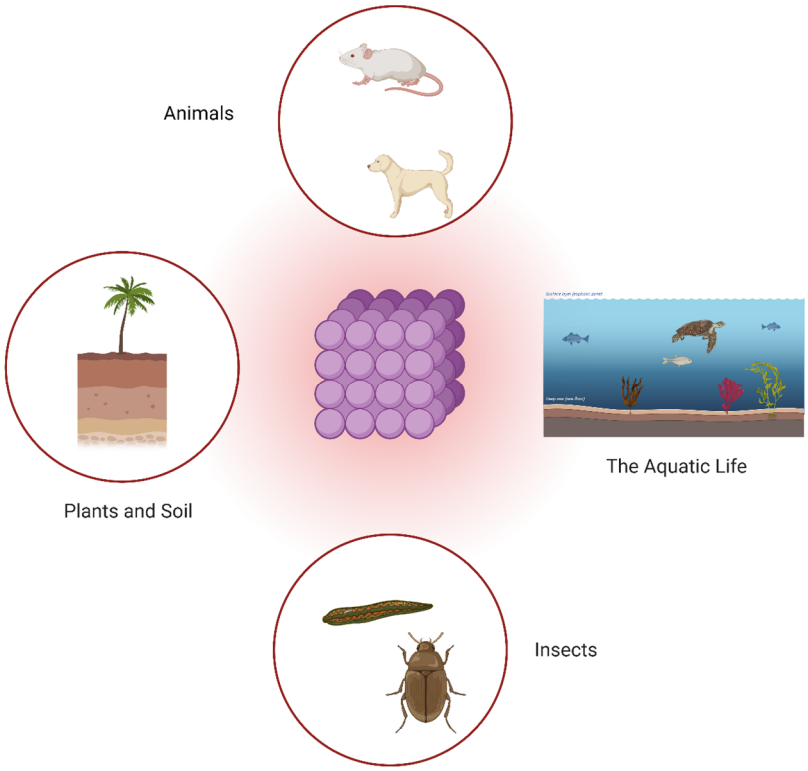

FIGURE 7.1
Nanoparticles and their Possible Targets in the Environment.

The impact of nanomaterials on active mitotic tissues, specifically root meristem, was assessed in various plant species. Roots of Allium cepa, Nicotiana tabacum, and Glycine max exhibited vulnerability to silver (Ag), ZnO, cerium dioxide (CeO2), and titanium dioxide (TiO2) NMs in cytological analyses, measured through techniques such as random amplified polymorphic DNA (RAPD) and DNA laddering. The observed root growth suppression was linked to cell division errors and chromosome abnormalities, including bridges, multiple breaks, micronuclei release, early chromosome separation, and DNA damage. Similar results were observed in Allium cepa plants treated with aluminum oxide

(Al2O3) nanoparticles at different concentrations, ranging from 1.25 to 5 µM, resulting in micronuclei and DNA damage with increased concentrations [250, 251]. These findings emphasize the need for a thorough understanding of the potential adverse effects of nanomaterials on plant growth and development. **Figure 7.1** shows the nanoparticles and their possible targets in the environment if they escape into the environment.

Furthermore, nanoparticles have the potential to be released into the environment during their transportation or mishandling in experimental applications. This unintended release can occur without awareness, leading to changes in the chemical composition of soil and water. A study by Lowry et al. highlighted that if silver (Ag) nanoparticles are leached into the environment, over an 18-month period, the silver ions can react with sulfur present in the environment, forming AgS. This reaction results in a reduction of sulfur's presence in biological matter and alters its availability in the environment. This underscores the importance of carefully managing and monitoring the use of nanoparticles to mitigate their unintended environmental impacts. Whereas, Changes in pH can significantly impact the colloidal stability and dissolution of Nanomaterials (NMs), primarily due to alterations in the surface charge of the NMs, impacting their fate in the environment. When an NM carries a positive charge, it tends to bind more readily to soil, as many soils possess a negative charge resulting from electrostatic forces. The presence of Natural Organic Matter (NOM) can further stabilize NMs by adhering to their surfaces, forming a steric coating around the particles. This phenomenon enhances the mobility of NMs, especially in soils with a high content of NOM. Understanding the processes of adsorption and desorption of NMs on various solid surfaces is crucial for comprehending their fate and transport in the environment. For example, NMs may adsorb onto particles of suspended solids present in water, soil, and sediments, influencing their transport and mobility. If organic matter becomes adsorbed onto the surface of NMs in the environment, the NM effectively functions as a sorbent, further impacting its behaviour in the environmental matrix.

Biological Toxicities

Aside from escaping into the environment and cause damage to the various parts of our ecosystem, nanoparticles may cause various types of toxicities inside the humans and the tested animals also. The following explained are various types of possible toxicities that nanoparticles may induce inside a living body.

Cellular toxicity of nanocarriers

A cell is a fundamental unit of life, all life originates on a cell or cells [3]. All living bodies have cells, as running fuel in them, as their responsibility revolves around carrying food and energy, and oxygen around the body and delivering it to organs that need it [4]. There are two major classified types of cells, Red Blood cells (RBCs) and White Blood Cells (WBCs). The major responsibility of RBCs is to transport and deliver oxygen and food throughout the body [5] while WBCs are a defensive shield of the body, their work revolves around trapping and killing the pathogenic entities that enter the body be it bacteria, fungi, or virus [6]. If a higher dose of pathogenic entities enter in such a way that WBCs cannot tackle all or destroy all, the pathogens attack the cells and organs and multiply to cause illness [7]. Hence, to address these illnesses, diverse medications are introduced that exhibit toxicity towards invading bacterial or fungal cells and/or virally altered cells. Generally, anti-bacterial and

anti-fungal drugs tend to have minimal toxicity on human cells, but their misuse can result in severe and potentially fatal side effects, such as liver mutations. It is widely acknowledged that contemporary bacteria are developing resistance to numerous existing anti-bacterial drugs, as they possess a natural ability to develop resistance against foreign entities. Consequently, either potent dosages (e.g., up to 2g per dose) or the administration of a new generation of antimicrobial drugs is now recommended. Several countries have enacted stringent regulations regarding the prescription of antibacterial drugs; however, in many nations, particularly those in the developing world, access to antibacterial drugs can be obtained without the need for a prescription. Conversely, anti-viral drugs, including those targeting cancer (i.e., anti-cancer), display toxicity towards both mutated and normal healthy cells. Their primary purpose is to induce cell death, irrespective of the cell's health status.

As a result, a novel category of pharmaceuticals, known as Nanomedicines, is currently under extensive exploration, revolutionizing the field of pharmacy and pharmacology. Nanomedicines employ nanomaterials for the diagnosis, prognosis, and treatment of various diseases. These nanomaterials can either be utilized directly to induce toxicity in bacterial, fungal, and cancerous cells, or they can serve as carriers for delivering drugs to fungal, cancerous, and/or bacterial cells. When nanomaterials are chosen as drug carriers, they require surface functionalization, or the drug can be loaded inside the nanomaterial. Such nanomaterials acting as carriers for drugs are termed as nanocarriers.

Nanoparticles and nanocarriers employed for their biomedical potential may belong to various categories, including metals and metal oxides or polymeric materials. There exist diverse mechanisms through which a nanomaterial or nanocarrier can induce cell death. In the case of bacterial cells, a nanoparticle may follow one or more paths to eliminate it. Potential pathways include the generation of reactive oxygen species (ROS), damage to the electron transport chain, disruption of peptidoglycan, disruption of the cell membrane and/or cell wall, inactivation of enzymes, denaturation of proteins, and damage to DNA/RNA or nuclear membrane. When compared to unaltered nanoparticles, those modified with transferrin have demonstrated heightened efficiency in cellular uptake and improved delivery of drugs inside cells [8]. Furthermore, evidence suggests that transferrin-linked polymeric nanoparticles play a significant role in overcoming resistance to chemotherapy drugs [9]. In a study Sani A., et al., reported bioinspired synthesis of Cobalt Oxide (Co_3O_4) nanoparticles by using plant extracts of Rosmarinus officinalis as reducing agent for cobalt salt and as stablizing agent for syntheszied nanoparticles, to keep them in nanosize range. Furthemroe, the group evulated the toxicty of the syntheszied nanoparticles against bacterial cells and found them to be quite potent against Streptococcus pneumoniae, while teir toxicty on human RBCs was also evilate as part of the study and they group found that their syntheszied nanoparticles were raturing 28% of humna blood driven macrophages, which shows that nanoparticles show toxicty against the human blood cells, even though it is quite small[10]. Furthermroe, Sani. A., an group also worked on biosynthesis of NiO nanopartiles and used them for their biomedcial applications and potential against HepG2 cancer cell lines and pathogenic bacterial strains. The results were also promissing and the group also measured the toxicty of biosyntheszied NiO nanoparticles, and found that nanomaterials were safer to use, at lower concentrations [11].

Iron Oxide has been most widely used for their potential potency against the cancer cell lines. Hassan. D., et. al., and group syntheszied IO nanoparticles using greener approach and used them for their potential cellular toxicty against the bacterial strains and against the Leishmania. Furtherore, the group studied their potential against liver cancer and found them to be toxic. It was proposed that

the major reason for killing was generation of ROS for the cell death [2]. In a further study, Khan. I., and group worked on synthesis Doxoribucin (DOX) loaded PLGA nanoparticles and studies their toxicty against the cells, i.e., T47D and MDA-MB-231 breats cancer cell lines and found that nanoparticles delivered the drug as well as generated the ROS for degrading the cells. Although the group did not revealed the exact mechnaism, if the ROS were distroying the Mitochondria and attackin the memerabes of DNA, but they showed extreme toxicity against the studeis cell lines [12]. Furthermore, Vaz-Ramos, J., et. al., worked on the sysnthesis of Silica (SiO_2) shelled IO cored nanoparticles and laoded the core with anti-cancer drug Epirubicin (EPI) to explore their cellular toxicity on HepG2 cell lines and also evaluated the EPI release over the 2 days period. The group found that Nanoparticles inhibited the HepG2 cells growth by more than 97% and the drug release was arround 38% at the end of 2 days [13].

Above mentioned studies have shown their potential of treating cancer and bacteria related to infectious diseas, but still the question about toxicity remains intact. The studies have mentioned in the haemolytic assyas that the nanoparticles i.e., NiO, Co_3O_4 and IO, were safe to use at lower concnetrations, but still they showed toxicity. Now it is better to further carry out the toxicity studies in relation to the concentration sof nanopaticles and define, which ceoncentrations would be better to use. Furthermore, aside the cell toxicity, annoparticles have other ways also to cause various other types of toxicites. These types of toxicites must be idenfied also.

Muscle Toxicity

Aside from cellular toxicity, nanomaterials can cause various other types of toxicities also, including Muscle toxicity. Nanoparticles can accumulate inside the muscles and cause toxicity. The bioaccumulation can cause denaturing of muscles, resulting in muscle toxicity. There have been studies that have shown the muscle toxicity of nanoparticles. For instance, Sayed, A. E.-D. H. and group conducted an investigation into the impact of silver nanoparticles (AgNPs) and silver nitrate (AgNO3) on the gills and muscles of African catfish (Clarias garepinus). Biomarkers such as changes in bioaccumulation, histopathology, and histochemistry were utilized in the study. The research focused on the size-dependent toxicity of AgNPs, specifically with reported sizes of 20 and 40 nm, and concentrations of 10 and 100 µg/L. Significant variations in silver (Ag) concentrations were observed in gill and muscle tissues across all exposed groups compared to the control group. At both tissue and cellular levels, histopathological alterations were identified, including aneurysms, collapse, subepithelial edema, lifting and hyperplasia of interlamellar epithelium, curling of secondary lamellae, epithelial cell necrosis, hypertrophy, and erythrocyte proliferation. Furthermore, there were bifurcation and fusion of filaments, an increase in the number and size of mucous cells in the gill tissues, and degeneration of muscle bundles, inflammatory cell infiltration, focal necrotic areas, splitting of muscle fibers, broken myofibrils, thickening of muscle bundles, dislocated striated muscles, and shortening of muscle bundles in fish exposed to AgNPs. Observations also included depletion of carbohydrates in gill tissues, and muscle fibers with an increase in the number and size of mucous cells that condensed in the secondary lamellae. A 15-day recovery period resulted in improvements in most histopathological and histochemical parameters affected by AgNPs and AgNO3. In conclusion, the study suggests the sensitivity of the gills and muscles of *C. garepinus* to AgNPs, emphasizing the necessity of a recovery strategy [14]. While, In Hassan's research, the potential toxic effects of biomedical nanoparticles (specifically polyacrylic acid and polyethylenimine coated magnetic NPs) and two industrial nanoparticles (SiO2 and TiO2) were investigated. The study

employed various short-term and long-term exposure protocols on two physiologically distinct in vitro models capable of differentiation: the L6 rat skeletal muscle cell line and biomimetic normal porcine urothelial (NPU) cells. The findings indicated that L6 cells exhibited greater sensitivity to nanoparticle exposure compared to NPU cells. Transmission electron microscopy revealed NP uptake in L6 cells but not in NPU cells. **Figure 7.2** shows images of Control, TiO2, PAA, SiO2 and PEI exposed L6 cells after 96 hours of exposure (figure adopted from [15], published under open access, Creative Commons (CC) License). In L6 cells, a dose-dependent reduction in cell viability and an increase in reactive oxygen species (ROS) formation were observed after 24 hours of exposure. Continuous exposure to more stable TiO2 and polyacrylic acid (PAA) nanoparticles led to elevated levels of nuclear factor Nrf2 mRNA, suggesting an oxidative damage-associated response. Additionally, internalized magnetic PAA and TiO2 nanoparticles impeded the differentiation of L6 cells. The research proposes the utilization of L6 skeletal muscle cells and NPU cells as a novel approach for assessing the potential long-term toxicity of relevant nanoparticles present in the blood and/or potentially secreted into the urine [15].

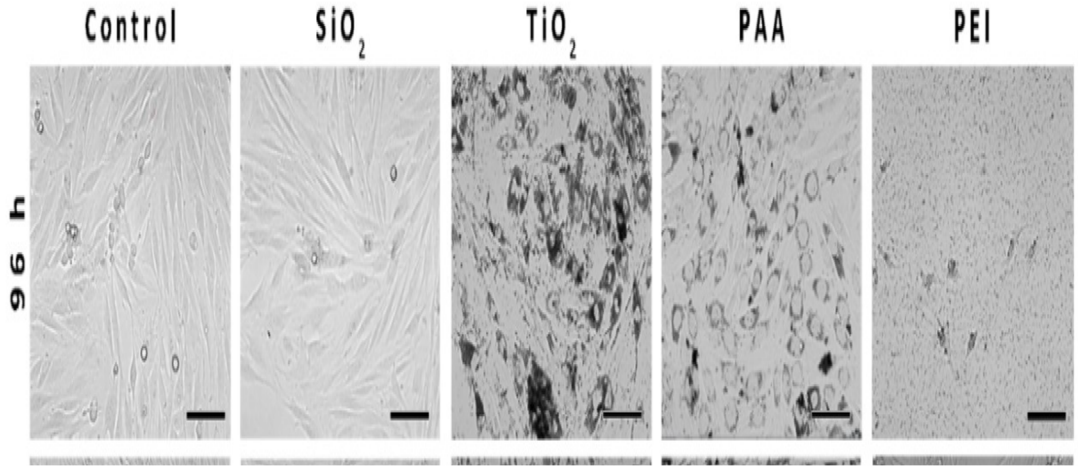

FIGURE 7.2
Contrast Micropscopic images of L6 cells, after 96h of exposure with SiO2, TiO2, PAA and PEI and control cells (figure adopted from [15], published under open access, Creative Commons (CC) License).

With that another group led by Carmo, T. L. L., studied muscle toxicity of TiO2 NPs on *Prochilodus lineatus* – a neotropical fish's white muscle after exposing it to 5, 10 and 50 mg/L concentration of NPs. The research group noted that the inhibition of acetylcholinesterase (AChE) took place in white muscles following acute exposure. The group also mentioned that TiO2 NPs were accumulating inside the white Muscle. However, despite the accumulation of TiO2-NP in this tissue during subchronic exposure, homeostasis appeared to be restored in this period [16].

Hence, despite numerous studies, it remains the responsibility of scientists and researchers to thoroughly investigate the muscle toxicity of nanoparticles (NPs). This comprehensive evaluation is essential before these NPs can be considered for potential applications in treating patients with acute and chronic infections, as well as individuals combating various life-threatening diseases such as cancer.

Gene toxicity

Another potential mechanism through which nanocarriers and nanoparticles may induce cell death, whether in cancerous or healthy cells, is by targeting genes and causing genotoxicity. It is well-established that nanoparticles have the capacity to generate Reactive Oxygen and Nitrogen Species (RONS). Therefore, during treatment, it is imperative to monitor and regulate the concentrations of RONS being generated to ensure the safety and effectiveness of the therapeutic approach [17]. While there is any sort of imbalance in the genesis and elimination of the RONS, they may start to accumulate and cause fatal effects on proteins, DNA, RNA and lipids causing them to die of genetic toxicity [18]. Where, this type of imbalance may instigate a chain reaction type of situation that will result in acute inflammation and immunogenicity [19]. The ROS generation is the most followed and supported mechanism that a nanoparticle follows for killing a cell. Other than that it is also reported that various metal nanoparticles can catalyse the generation of reactive oxygen and hydroxyl radicals driven metabolites that engender lipid peroxidation [20], This process can result in the malfunction of multiple cellular organelles, including the mitochondria, plasma membrane, and endoplasmic reticulum (ER) [21].

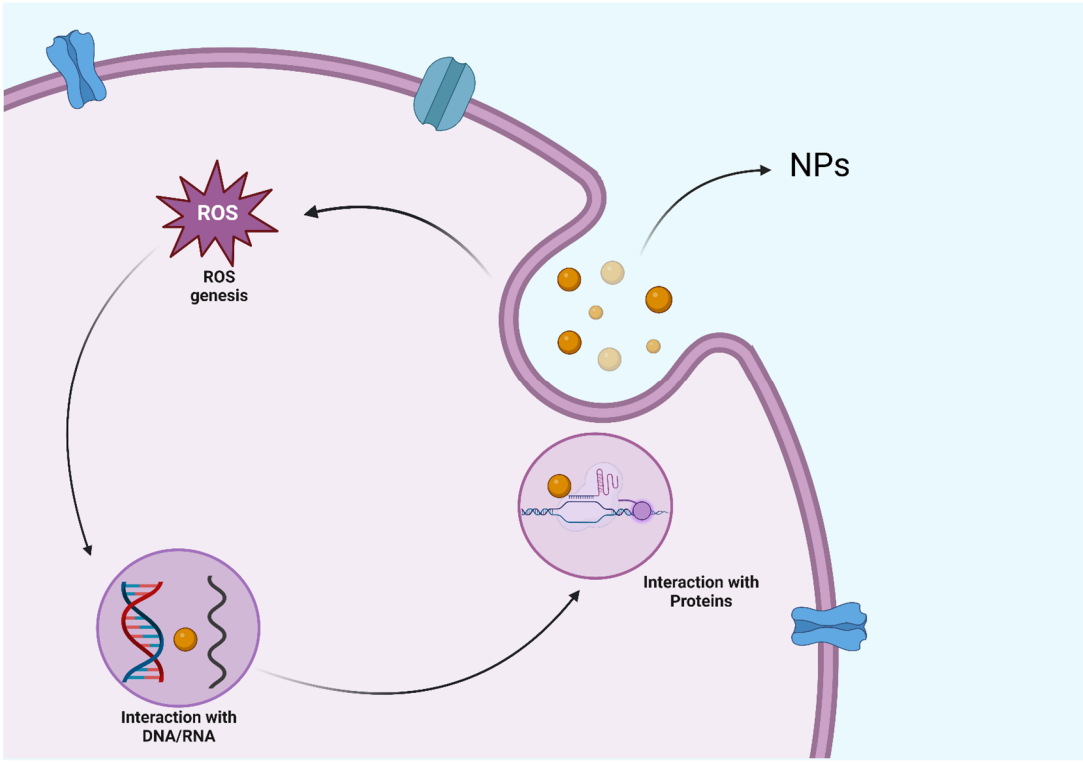

FIGURE 7.3
The Three Prime Mechanism a Nanoparticle May Follow to Cause Cellular Genotoxicity.

In normal cellular processes, cells generate superoxide dismutase (SOD), diminished glutathione (GSH), and α-lipoic acid as a defense mechanism against Reactive Oxygen and Nitrogen Species (RONS). These compounds work to neutralize RONS, preventing oxidative stress and safeguarding cells from the harmful effects of RONS accumulation, thereby averting potential genotoxicity [19]. Studies suggest that 50mg/kg concentration of synthesized ZnO nanoparticles may upset the SOD production, gravely, resulting is acute damages to studied rat's intestines [22]. The further studies by Sani A., et al., and Khalil A. T., et. al., also suggest that production of ROS may get to the DNA and cause genotoxicity resulting in cell death [10, 11, 23]. Furthermore, it has been observed that TiO2 NPs significantly disturb the balance between GSH and oxidized glutathione (GSSG) in hepatic (liver) cells. This disruption leads to an increased concentration of Reactive Oxygen Species (ROS), culminating in lipid peroxidation and a heightened frequency of micronuclei. The latter is recognized as a potential indicator of genotoxicity. [24]. On the other hand, concerning CuO nanoparticles, it has been discovered that they have a tendency to disrupt the transmembrane potential of mitochondria and decrease the levels of GSH in the blood. This disruption leads to an alteration in the normal functioning of macrophages. [25]. The literature asserts that nanoparticles can induce cellular genotoxicity through three primary mechanisms [1]. **Figure 7.3** shows the prime mechanisms followed by a nanoparticle to induce cellular genotoxicity.

- The direct approach/interaction by the NPs with the genetic material i.e., DNA (primary mechanism)
- The contact of nanoparticle with any biomolecule that may take part in the DNA replication of cell division (primary indirect mechanisms)
- The genesis of ROS that may cause damage to the DNA (secondary mechanisms).

The genotoxicity is mostly related to the rate and concentration of nanoparticle accumulation inside a cell, while rate of nanoparticle penetration/accumulation/uptake largely depends on the size, shape and morphology of nanoparticle [26]. Most of the time, metal and metal oxide nanoparticles are regarded to cause genotoxicity in a cell. Research on the cytotoxicity of Ag NPs has revealed that they impact metabolic activity and cause membrane damage by releasing LDH. This, in turn, triggers an inflammatory response and induces the generation of ROS [27]. The initial three studies reported that Ag NPs with a size of 15 nm led to a dose-dependent reduction in mitochondrial metabolic activity and an increase in LDH leakage in rat liver BRL 3A cells, C18-4 germline stem cells, and macrophages for doses up to 75 µg/ml. Additionally, they observed alterations in cell morphology, NP uptake, and low levels of apoptosis. Conversely, larger nanoparticles (55 nm) were found to induce a less pronounced cytotoxic response in macrophages, potentially due to the lower ease of internalization of larger agglomerates. Furthermore, Ag NPs demonstrated a significant impact on ROS generation and the release of inflammatory mediators such as TNF-α, MIP-2, and IL-1β in macrophages at doses starting from 5 µg/ml. However, Yen et al. reported a different finding, stating that pro-inflammatory cytokines TNF-α, IL-1, and IL-6 showed no response to Ag NP exposure, despite higher doses being administered [28]. As documented by El-Shorbagy, H., and colleagues, ZnO NPs obtained from Sigma Aldrich were utilized to investigate their impact on apoptotic induction in Ehrlich solid carcinoma (ESC) bearing mice models. The mice were administered with 50, 300, and 500 mg /kg of NPs of their weight continuously for a week, with each dose given every 24 hours. The outcomes demonstrated an increase in the expression of *Bax* and *P53* genes, accompanied by a decrease in *Bcl2* genes. Notably, there was a significant reduction in tumor size among mice treated

with ZnO NPs, which was 46% smaller compared to the control group. Additionally, histopathological analysis unveiled substantial infiltration of mononuclear inflammatory cells in the portal triad and vacuolar degeneration of hepatocytes. [29].
On the contrary research by Schulz et. al., the researchers, with the help of intratracheal installation, introduced Au NPs to the rats, over the time span of 3 days, suggested that there was no significant damage to the DNA od Au NPs exposed rat's cell compared to control rat's. but the question arises here is that if the time of exposure was enough or the size of nanoparticles was small enough to penetrate the cells or if the nanoparticles were active enough to produce ROS [30]. Whereas, a commentary published by Wang, Y., et. al., reported that Au NPs cause genotoxicity, and the group reached this conclusion after a careful study. The research group exposed varied sized Au nanoparticles, to be precise, 12nm against 3T3 cells (fibroblasts) of BALB mice and 14nm sized Au NPs with concentration of 6.2 up to 50µg/mL against human peripheral blood lymphocytes (PBLs). They group further investigated the genotoxicity cause by larger size AU NPs i.e., 10, 30, and 60nm against the HepG2 cell lines and found the nanoparticles cause significant damage to the cells that were exposed to Au nanoparticles and also corelated the concentration of NPs to the extent of damage and concluded that higher concentration was causing enhanced genotoxic effects. With that the group also studied the *in vivo* DNA damage on rats and fishes and studied the damage after exposing the animals for 28 days and found that the DNA damages were visible with the animals exposed with 80µg/L concentration of NPs [31].
Hence, despite the considerable benefits of nanocarriers and nanoparticles in the treatment of various life-threatening diseases, they are still regarded as potential hazards due to the incomplete understanding of their safety and toxicity. This limitation hinders their further progress and widespread adoption in medical applications.

Toxicity of Organs

Apart from other toxicities, nanoparticles have tendency to get accumulated inside the organs, and as discussed before, the higher accumulation of nanoparticles inside an organ may lead to toxicity also. The organs that nanoparticles have higher tendency to accumulate include Liver, Intestines, Bones, Kidneys, Eyes, Heart and brain, whereas the highest possibility of nanoparticles accumulating inside a single organ are in the liver. The liver is a major cleaner of our body, and every thins in the blood and every biomolecules passes through the liver, therefore, liver (specially macrophages) could accumulate the most concentration of nanoparticles in it and would be most affected [32, 33]. A significant challenge arises as over 30% of administered nanocarriers or nanomedicine into any animal body tend to accumulate in the liver. This accumulation not only enhances toxicity to the liver but also diminishes the effectiveness of targeted applications for the treatment of various diseases [34]. Carmo, T. L. L., and group studied the exposure of TiO2 NPs on the *P. lineatus* fish and found that TiO2 NPs were accumulating in the liver and caused damage due to ROS generation [16]. Furthermore, Jiang, X., led group reported the accumulation of GSH and Indocyanine Green (ICG) functionalized Au nanoparticles in the BALB/c mice model's liver. The group stated that more than 70% of introduced nanoparticle accumulated inside the liver, while the accumulation concentration decreased with passage of time, which may employ that the nanoparticles were accumulating in other organs also [35].
Aside from the liver, there are other organs where the nanoparticles may accumulate also. Nanomedicines are employed either for the cancer treatment, and neurodegenerative diseases.

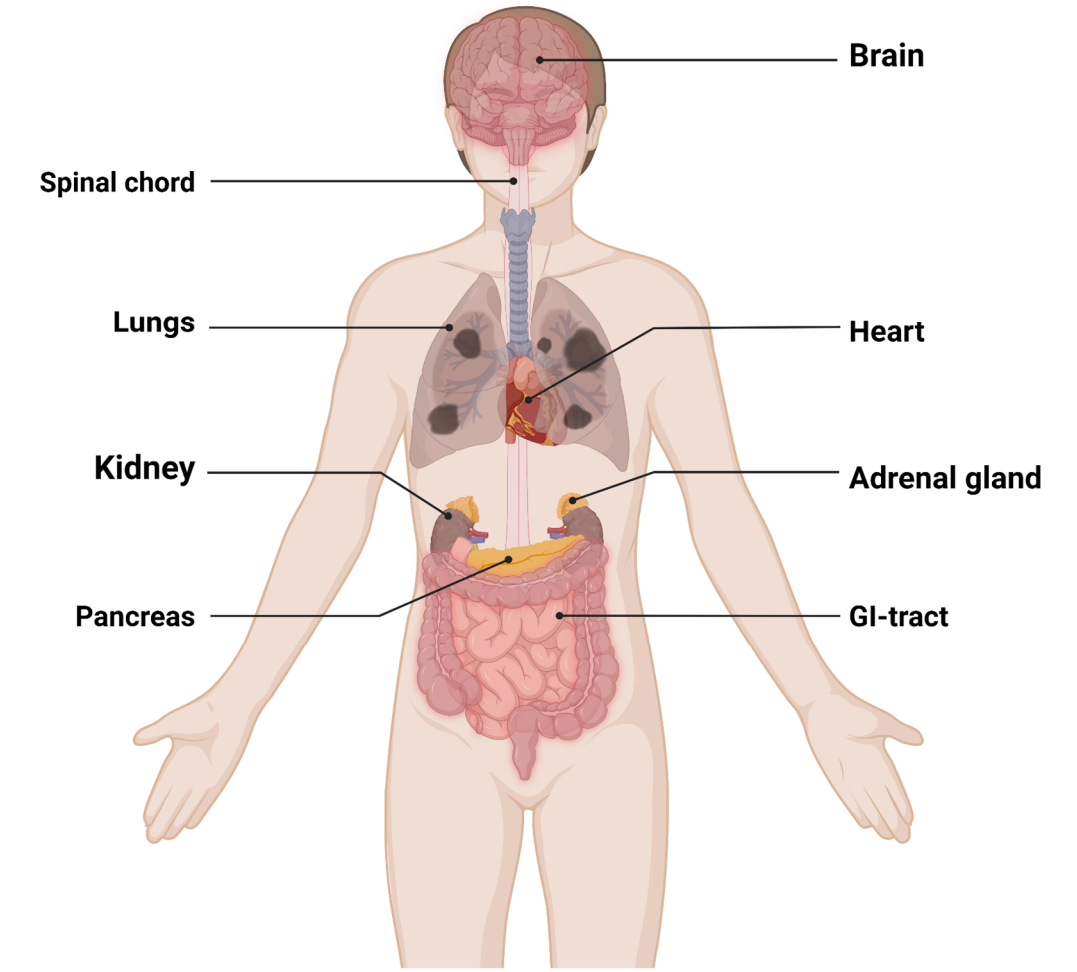

FIGURE 7.4
Organs inside the body where nanoparticles may accumulate and cause toxicity and damage.

Therefore, the accumulation can occur in the brain also. And due to accumulation, the nanoparticles may cause damage and toxicity to the brain cells and muscles. The group, Carmo, T. L. L., et. al., who earlier studies accumulation of nanoparticles and the damage brought by them in the muscles and liver and later they also evaluated the toxicity of TiO2 nanoparticles on the brain also. The group found that the TiO2 nanoparticles accumulated into the brain over the period of 14 days. At the end of 14 days the group found 1.92mg/mL of nanoparticles out of 50mg/mL initially introduced to fish [16]. The employment of nanoparticles for medicinal purposes is recommended due to their size, since due to their size they provide enhanced retention inside the body and increased bioavailability but on the other hand this may also cause enhanced toxicity inside the organs [1]. Due to small size the nanoparticles are also best candidate for crossing across the Blood Brain Barrier (BBB). Two potential mechanisms for nanoparticles to enter the brain through the olfactory nerve pathway have been recognized: paracellular transport and transcellular transport [36]. Quantitatively

differentiating between endothelium accumulation and transit into the parenchyma is challenging based on the available data on nanoparticle accumulation in the brain *in vivo* [37]. As discussed before, the morphology, shape and size of nanoparticles may impact their accumulation inside the organs and their penetration rates [38]. A study by Kolar et. al., evaluated the poly-styrene (PS) NPs functionalized with anti-TfR antibody having spherical and rod sized nanoparticles and compared their accumulation inside the brain. The group found that rod shaped PS NPs accumulated in higher concentration compared spherical PS NPs [39], while the study by Arnida, M., and group found that Au NPs functionalized with PEG with spherical shape accumulated in higher concentration in brain compared to rod shaped AU PEGylated NPs [40].

Other than liver and brain, kidneys are also the organs that are exposed to nanoparticles and may receive a load of nanoparticles, that could accumulate inside the kidneys and may cause damage to cells and organ. Bartucci, R., and group studied the accumulation of carboxylate loaded PS nanoparticles in lungs, kidneys and liver. C57BL/6J mice models were employed for this accumulation study and found that nanoparticles got accumulated inside the kidneys also and cause damage to the kidneys [41].

Other than these organs the nanoparticles may accumulate inside other organs like spleen, bones, skin, eyes and heart and others also[42] . Therefore, there is a grave need of studying the accumulation of nanoparticles inside the organs, before they could be put out for their potential use of medicinal purposes. **Figure 7.4** contains pictorial image of various organs where the nanoparticles may get accumulated.

Conclusion

There are various factors that could limit the use of nanomaterials for their potential medicinal uses. These factors may include but not limited to gene-toxicity, muscle toxicity, cell toxicity and accumulation in the body organs. Furthermore, it is to understand that the size of nanoparticle that is being employed matters a lot. Furthermore, there is still needed to analyse various factors in the coming studies that could possibly limit the use of nanomedicines. With that it must be considered that these studies are compulsory to determine if the use of newly developed nanomedicines will be safer to animal bodies and/or organs or will have any long-term effects or not.

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