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Increased Toxicity Risk from Nanoparticulate System in Food and Drug Applications

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***Abstract*— Nanotechnology involves manipulating matter at the atomic and molecular levels, leading to the creation of new materials for food and drug application with properties that are not always easily anticipated based on existing knowledge. Among the nearly infinite variety of these substances, some are toxic to biological systems, others are generally harmless, and yet others confer health advantages. The biocompatibility and distribution of these materials must be assessed prior to their use in biological and environmental. Consequently, it is essential to comprehend the toxicity of nanomaterials. Mechanisms of cellular uptake and nanoparticles dispersion in biological settings depend on their physicochemical properties. The pathways in which exposure to nanoparticles is harmful to health are through oxidative stress and inflammation. Recent research have indicated that nanoparticles may cause major health impacts when ingested, inhaled, or applied to the skin without precaution. The main sources of toxicity in nanomaterials include their size, shape, concentration, aspect ratio, crystallinity, surface charge, dissolution, and agglomeration. This review article aims to comprehensively summarize the toxicity aspects of nanoparticles for food and drug application, including physiochemical properties, mechanisms of nanoparticle toxicity, and the health risks, so as to provide an overview for future researchers to develop nanoparticulate in a safer way.**

***Keywords*— Nanoparticles; Toxicity; Physicochemical properties; Oxidative stress; Inflammation**

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I. INTRODUCTION

In the last few decades, nanomaterials have generated a considerable amount of interest due to their special properties, resulting from their relatively large surface area and high reactivity. In addition, the fast development of nanotechnology, nanomaterials are currently synthesized in an increasingly diverse variety of shapes and sizes are used in the production of a wide range of commercial and medicinal products. There are many methods for producing nanoparticles, including chemical, physical, and biological processes[1]. Nanomaterials may also be employed for specialized medical applications, such as the creation of novel drug delivery systems, for improvement of a performance of medical devices, and the manufacturing of diagnostic-imaging materials [2].

This article refers to several related terms—nanoparticles, nanomaterials, and nanocarriers—each differing in scope and application. Nanoparticles (NPs) are wide class of materials that include particulate substances, which have one dimension less than 100 nm at least [3]. Nanomaterials more broadly include substances with nanoscale internal or surface features, encompassing nanoparticles, nanofibers, nanowires, and nanosheets; these may be naturally occurring or engineered for both passive and active roles [4]. Nanocarriers are a subclass of nanoparticles specifically designed for drug or gene delivery, usually composed of biodegradable or biocompatible materials such as lipids, polymers, or proteins, aiming to enhance targeted delivery and minimize systemic toxicity [5]. While all nanocarriers are nanoparticles, not all nanoparticles function as nanocarriers, and nanoparticles themselves represent only one category within the broader class of nanomaterials. Despite the advantages of nanoparticles, several applications of nanotechnology have revealed the potentially harm to people and animals. People working in nanomaterials industries or research institutes are more probably to be exposed to with

these materials. Recent studies have shown that nanoparticles can readily infiltrate the human cells [3]. It's mostly because their nanoscale dimensions are so similar to those of common biological components. In addition, proteins NPs may circumvent natural mechanical barriers, which may result in unfavorable tissue reactions. When NPs are ingested, they can be absorbed across cell membranes, where they can then interact with nearby organs and potentially trigger or exacerbate organ defects [6]. In general, NPs with distinct physical and chemical features can enter cells through a variety of processes including uptake, endocytic, and "adhesive interaction" [7].

The physicochemical properties of nanoparticles determine their activities with cells and overall toxicity potential. Nanoparticle characteristics such as size, shape, composition, surface area, aspect ratio, and surface charge may affect their toxicity [8]. Small NPs have a large surface area per mass, which is frequently associated with an increased biological reactions. A large surface area may also facilitate the production of free radicals like superoxide anion and hydroxyl radical. It follows that oxidative stress can play a crucial role in the toxicity of NPs, especially metal-based NPs. For example, inflammation in response to NPs can be defined in terms of the free radicals formed during this process [9].

Several studies have demonstrated that chemically synthesized nanoparticles tend to exhibit higher cytotoxicity compared to biosynthesized nanoparticles, which often possess biocompatible surface functionalities derived from natural biomolecules, reducing their adverse interaction with human cells [10, 11, 12]. Howevesr, when nanoparticles interact with cells, degrade into less complex forms, or accumulate, some biosynthesized nanoparticles can be just as toxic [11, 12].

Biosynthesized nanoparticles are generally considered to have lower toxicity risks than chemically synthesized counterparts due to several key factors. First, biosynthesized NPs are often capped and stabilized with biological molecules (e.g., proteins, polysaccharides, polyphenols) that enhance their biocompatibility and reduce oxidative stress and inflammation upon cellular exposure [10]. Second, chemical synthesis methods frequently involve toxic reagents and stabilizers (e.g., $NaBH_4$, organic solvents) that may remain as residual contaminants, increasing the risk of cytotoxic and genotoxic effect [11].

Comparative studies have shown that chemically synthesized silver and copper nanoparticles, for instance, induce greater reactive oxygen species (ROS) formation, mitochondrial damage, and DNA fragmentation in human cell lines compared to their biosynthesized analogs [3, 12]. Moreover, biosynthesized NPs exhibit a more favorable immunological response profile and are less likely to disrupt metabolic or endocrine pathways, particularly in chronic exposure models [8].

According to the findings of animal experiments, nanoparticles in the environment ingested and accumulated in the respiratory system. The NPs can then enter the bloodstream and translocate to different organ [13]. Researchers discovered that exposure to droplets containing tiny and nanoparticles had decreased lung functions and respiratory disease. There isn't a lot of information about how ultrafine particles affect health compared to other fine airborne particles [9, 14, 15]. So, the widely use of nanomaterials has caused people to be concerned about the harmful effects of NPs on health, particularly on the sexual organs of both men and women [16, 17]. Due to NPs are small, biocompatible, and may be able to pass through the placenta. NPs are also associated with a range of diseases in animals, such as lung injury, heapatotoxicity, immuno-nanotoxicity, neurotoxicity, nephrotoxic, and irreversible testis injury [18 - 24].

Nanoparticles have a lot of great benefits, but we don't know much about their toxicity. This review article aim to present a comprehensive overview of nanoparticle toxicity, such as the physiochemical properties influence toxicity, the mechanism toxicity, and the health effects it causes.

II. DISCUSSION

A. Nanoparticles Toxicity

Nanomaterials are materials that have structures that are less than one micrometer in size along at least one dimension. This provides lots of advantages in a variety of aspects, such as enhanced specificity and therapeutic effect, causing nanotechnology to develop rapidly and be intensively explored by researchers. Nanomaterials are used effectively in medicine to deliver drugs, diagnose diseases, treat cardiovascular diseases, heal wounds, and make antimicrobial agents. Nanomaterials are being used in cells labeling, biological tagging, cancer therapy, DNA and protein detection, and biomedicine [25]. Because of their tiny size, catalytic characteristics, and high activity surfaces, NPs are able to rapidly traverse cell membranes and induce toxicity by altering intracellular biomolecules. This is possible because NPs have highly active surfaces. Despite the fact that nanocarrier systems are meant to reduce toxic side effects and improve biocompatibility [26], there may be some problems regarding their specific features. These nanocarriers have distinctive structures that allow them to exert their effects on the entire system while eliciting a variety of responses. These characteristics include their size, shape, and surface charge, as well as the manner in which their component parts are decomposed at the molecular level [27].

When nanoparticles enter the vascular system, they are linked to a variety of adverse health effects, including atherosclerosis, thrombosis, arrhythmias, respiratory problems, and eventually death from heart causes **(Figure 1)**. In addition, migration to other organs, such as the liver and spleen, might lead to diseases of these organs. Furthermore, an individual's genes complement that's what ultimately determines a nanoparticle's toxicity to that organism, which gives its metabolic inventory for adapting to and combating harmful compounds.

B. Types of Toxic Nanoparticles

This review primarily focuses on engineered nanoparticles (ENPs), particularly those that are intentionally synthesized for biomedical, pharmaceutical, and industrial applications, as reflected in the discussion of specific classes such as metal-based, carbon-based, lipid-based, protein-based, polymeric, and silica nanoparticles. The detailed mechanisms of toxicity, including reactive oxygen species (ROS) generation, mitochondrial damage, DNA fragmentation, and inflammation, are primarily derived from studies using engineered nanomaterials in controlled experimental settings—both in vitro and in vivo.

C. Metal-Based Nanoparticles

Fe_2O_3 NPs

Fe_2O_3 NPs have found use in a variety of biological, pharmaceutical delivery, and diagnostic applications. The liver and other organs that are part of the reticuloendothelial become a bioaccumulation site for these nanoparticles [28, 29]. In vivo tests have shown that Fe_2O_3 NPs, when entering the body, remain in cell organelles (such as endosomes and lysosomes), disintegrate into the cytoplasm, and promote to the build up of iron in cells. After inhalation, magnetic iron oxide nanoparticles may be found accumulating in the liver, spleen, lungs, and brain, indicating that they have the ability to cross the blood-brain barrier (BBB) [30]. There is evidence to suggest that the toxicity of these NPs is caused by the lysis of cells, inflammation, and disruption of the mechanism responsible for blood coagulation [31].

Additionally, in vitro studies have identified reduced cell viability as the primary common adverse effect of Fe_2O_3 nanoparticles. varies The cell survival of iron oxide nanoparticles treated with various compounds varies. Naqvi et al. found that Tween-coated supermagnetic iron oxide nanoparticles (30 nm) are harmful to mice macrophage cells [29]. Iron oxide nanoparticles in low concentrations (25-200 µg/mL for a 2-hour exposure) are thought to be more damaging to cells than iron oxide nanoparticles in high concentrations (300-500 µg/mL for a 6 hour exposure). After seven days of incubation, the presence of dextran-coated iron oxide nanoparticles (100-150 nm, 0.1 mg/mL) resulted in a 20% decrease in the viability of human macrophage cells [32]. In addition, a separate study on the mouse neuroblastoma (Neuro-2A) cell line found that Fe_2O_3 NPs (25 nm) had a less harmful impact on cell shape, cells permeability, cell damage, and mitochondria activity [33]. 10% of human hepatocellular carcinoma cells were active 12 hours after exposure to chitosan-coated iron oxide nanoparticles (13.8 nm) at a concentration of 123.52 µg/mL [34]. Nonetheless, rat mesenchymal stem cells maintained a viability rate of 70% after being exposed to 1-hydroxy-ethylidene-1,1-bisphosphonic acid-coated Fe_2O_3 NPs (20 nm, 0.1 mg/mL) over a period of two days. The MTS test was utilized in this investigation to determine the level of cell viability [35]. Excessive reactive oxygen species (ROS) formation has been hypothesized to be responsible for the adverse effects of Fe_2O_3 nanoparticles. These produced ROS exacerbate DNA damage and lipid peroxidation.

Al_2O_3 NPs

Chen et al. observed that aluminum oxide nanoparticles degrade cell growth, disrupt mitochondrial function, increase oxidative stress, and alter expression of tight junction proteins in the blood-brain barrier (BBB) [36]. Additional research, such as Radziun et al. have reportedt he presence of Al_2O_3 NPs in mammalian cells at concentration of 10, 50, 100, 200, and 400 µg/mL does not have a negative impact on the cells' ability to survive. Another research discovered that human mesenchymal cells are killed by doses of aluminum oxide nanoparticles ranging from 25-40 µg/mL, depending on the size of the particles (160 nm) [37]. In this work, cytotoxicity was also assessed using the MTT assay [38]. Balasubramanyam et al. showed that the amount of genotoxicity in 30-40 nm Al_2O_3 NPs depends on the dose [39]. Using rat blood cells, the comet assay and the micronucleus test were used to measure genotoxicity. The results of another investigation using a cell line derived from mouse lymphoma indicate that Al_2O_3 NPs with a size of fifty nanometers produce genotoxic effects in the shape of DNA damage but have no mutagenic effects [40].

TiO_2 NPs

Titanium dioxide (TiO_2) is chemically harmless, however research have shown that nanoparticles of titanium dioxide can cause Cell damage, genotoxicity, and lung inflammation in experimental animals [41, 42]. TiO_2 NPs cause oxidative stress and the formation of DNA adducts [43]. In animal experiments, TiO_2 NPs (5-200 nm) exhibit negative impacts on immunological function, liver, kidney, spleen, myocardial, hyperglycemia, and lipid homeostasis, in addition to genotoxicity [44, 45].

Ag NPs

Silver nanoparticles (Ag NPs) have been widely studied for their antimicrobial properties, as they exhibit distinct antibacterial activities against a broad spectrum of microorganisms, including bacteria, fungi, viruses, and microalgae [46] These effects are largely attributed to their ability to generate reactive oxygen species (ROS) and induce oxidative stress in microbial cells, which disrupts cellular function and viability. While bulk silver has limited antimicrobial activity, nanoscale silver exhibits significantly enhanced efficacy due to its increased surface area and reactivity [47]. Moreover, Ag NPs have demonstrated cytotoxicity toward cancer cells by inducing cancer-specific ROS generation [48].

The toxicity of Ag NPs varies depending on their surface coatings, size, and concentration. For example, Dang et al. reported dose-dependent cytotoxicity and DNA adduct formation in human lung cancer cell lines exposed to polyvinylpyrrolidone-coated Ag NPs (6–20 nm), confirming their ability to induce genotoxic effects [49]. Although these properties make Ag NPs promising for biomedical applications, growing evidence highlights their potential toxicity to the reproductive system. Roy et al. (2015) and Venugopal et al. (2017) demonstrated that Ag NPs induce oxidative stress in

germ cells, leading to DNA fragmentation, reduced sperm viability, and apoptosis [12, 48]. Kheirallah et al. (2021).further supported these findings by reporting ultrastructural damage in testicular tissues and impaired reproductive function following exposure to metal-based nanoparticles, including Ag NPs. Notably, Ag NPs have also been shown to cross the placental barrier[50]. Kovvuru et al. (2015) found that maternal ingestion of Ag NPs led to their accumulation in fetal tissues such as the liver, kidney, and brain, causing genomic instability and DNA damage. These findings collectively confirm the potential reproductive and developmental risks associated with silver nanoparticles and underscore the need for careful evaluation of their safety in medical and consumer products [51]

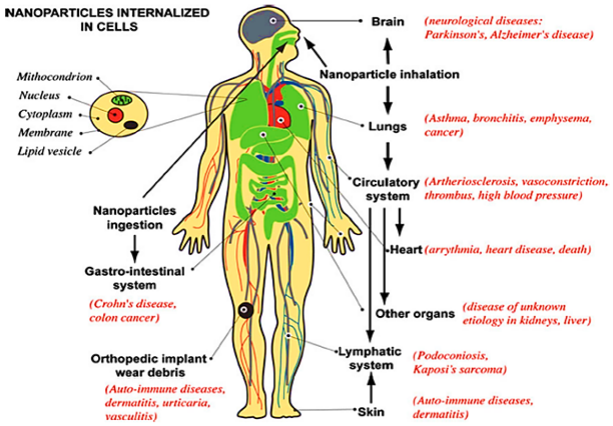

Fig 1. Diagrams of the human body showing the routes of nanoparticle exposure, the organs that are negatively impacted, and the diseases that have been linked to this exposure [27]

Au NPs

Au NPs inert, non-toxic core makes them harmless. In one experiment, the cytotoxicity of several Au NPs (4, 12,18 nm) with various chelating agents was examined against a leukaemia cell line [52]. The findings of this research suggest that spherical gold NPs are capable of penetrating cells and do not inhibit the function of cells in any way. The cytotoxicity of the sample was evaluated using the MTT test [52]. However, some results imply that the cytotoxicity of gold nanoparticles is dependent on dose, side chain (cationic), and stabilizer [53], [54]. Reportedly, the toxicity of human lung and liver cancer cell lines varies based on their cell lineage [55]. Recent research has shown a wide array of factors that, when combined, can result in cytotoxicity in human cell cultures. MC et al. investigated the size-dependent toxicity of gold nanoparticles on human embryonic stem cells and their neural derivatives [56]. In order to investigate its neuronal differentiation, viability, DNA methylation, and pluripotency, gold nanoparticles with particle sizes of 1.5, 4, and 14 nm were used. According to the findings of the study, gold nanoparticles with a size of less than 20 nm that were produced through chemical processes are especially dangerous to stem cells because they alter the methylation and hydromethylation patterns of cellular DNA [56].

Carbon-Based NPs

From the perspective of applications, carbon-based nanomaterials, which include carbon nanotubes, fullerenes, single-walled and multi-walled carbon nanotubes, represent the most desirable and widely used nanomaterials [57]. As cytotoxic agents, carbon-based nanomaterials have been documented in the scientific literature. Carbon nanotubes, also known as CNTs, have attracted a lot of interest in modern medical research, and they have been the subject of substantial investigation as potential drug delivery vehicles [58, 59]. Carbon nanotubes induce size-dependent toxicity. When injected into the peritoneal cavity of mice, multi-walled carbon nanotubes exhibited carcinogenic effects comparable to asbestos [60]. In contrast, macrophages were able to take in single-walled carbon nanotubes with relative ease. Long-term buildup of single-walled carbon nanotubes in the liver, on the other hand, has been shown to alter some biochemical parameters in experimental animals. These biochemical parameters include LDH, aspartate transaminases, alanine transaminases, glutathione, and malondialdehyde, in addition to organ indices [61]. When it comes to carbon nanoparticles, the amount of toxicity as well as the biological response of the cells is dictated by the size of the particles, the production process, and the concentration of trace metals [9, 62]. According to a multitude of studies, CNTs have been associated with a variety of harmful effects, including neurotoxicity, lung toxicity, immunological toxicity, embryotoxicity, genotoxicity, hepatotoxicity, and cardiovascular toxicity [63-69].

Lipid-Based NPs

Lipid-based nanoparticles (LNPs), including liposomes and solid lipid nanoparticles (SLNs), exhibit relatively low toxicity under physiological conditions due to their biocompatible phospholipid composition. However, adverse effects have been reported, particularly hypersensitivity reactions, complement activation-related pseudoallergy (CARPA), and cardiopulmonary distress, which are often mediated by opsonization and recognition by the reticuloendothelial system (RES) [70]. PEGylation is widely used to reduce immune

clearance, yet studies show that PEGylated liposomes can still provoke complement activation in up to 45% of patients [71].

Liposomes are micro structures that take the shape of spheres and are made up of layers of phospholipids to create an enclosed space. They are able to operate as amphipathic nanocarriers for a range of therapeutic medicines due to the cavity that is produced in the center as a result of the contact between the hydrophilic head and the hydrophobic tail [72-74]. This stimulates interest in gaining a better understanding of both the ways in which liposomes interact with the biological system as a whole and the toxicity of liposomes. When liposomes enter a biological system, they engage in a range of interactions with different biomolecules, such as LDL, HDL, and opsonins. When opsonins are present, the recognition system of the RES is triggered, and it then makes an effort to eliminate the opsonin from the system. It is well known that different lipoproteins in the blood can reduce the stability of liposomes within the biological system. This is accomplished by causing the surface lipids to rearrange. This is one of the key concerns about the utilization of liposomes as a drug delivery system [70]. PEGylated liposomes impede opsonization and promote escape from phagocytic cells to overcome this obstacle [75, 76]. It has been suggested that liposomes are a promising vehicle for the controlled release of cancer treatments such as antibiotics, transgene, and vaccination via antigen delivery [77-79]. The addition of cationic lipids to their formulation also makes them cationic, which confers the ability to transport DNA [80].

Protein-Based NPs

Protein-based nanoparticles (PNPs), such as albumin, gelatin, and casein-based carriers, are often considered safe due to their endogenous origin. Nonetheless, dose-dependent cytotoxicity has been documented. For instance, high concentrations of human serum albumin nanoparticles (HSA-NPs) have been shown to reduce cell viability by 30–50% in HEK 293T and HepG2 cells via disruption of mitochondrial membrane potential and induction of apoptosis [81, 82].

Protein-based nanoparticles have gotten a lot of interest recently because of their biocompatibility, amphiphilicity, ease of biodegradability, and low toxicity. Albumin, gelatin, ferritin, fibroin, and casein are among the most common protein-based nanoparticles utilized for medicine [83]. Albumin from human serum is a highly adaptable protein that is employed in the production of albumin-based nanocarriers (ANCs) for the distribution of cancer treatments [81, 84]. As a result of the presence of a large number of drugs binding sites inside the albumin molecule, serum albumin nanoparticles are capable of incorporating a significant quantity of medication into the particle matrix [85]. Mesken et al. published a report on the transmission of plasmids in HEK 293 T cells loaded with HSA nanoparticles coated with cell-penetrating peptide (CPP) [81]. They were produced using the desolvation method. When investigating nanomaterials transport at low plasmid concentrations, there was no cytotoxicity and no significant performance difference. Despite the fact that numerous studies and experiments have been conducted to examine the efficacy of HSA nanocarriers and their potentially hazardous effects at varying levels, substantial evidence of the nanocarrier's toxicity is still missing.

Polymeric NPs

Polymeric nanoparticles (PNPs) synthesized from PLA, PLGA, or chitosan show diverse toxicity profiles depending on their degradation rate, monomeric by-products, and surface charge. Positively charged chitosan nanoparticles have been reported to disrupt tight junctions in Caco-2 intestinal epithelial cells and induce oxidative stress, while PLGA nanoparticles generate mild ROS levels and DNA strand breaks at concentrations above 200 µg/mL [86, 87]. Quantitatively, cytotoxicity levels (measured via MTT assays) show 40–70% reduction in viability in A549 and HeLa cells after 24 hours exposure to 250 µg/mL chitosan NPs [88].

The most common application for polymeric nanoparticles is in the field of intelligent medicine administration, where they can be composed of either natural or synthetic polymers. They are the subject of much research and development in the pharmaceutical industry for use as carriers for regulated or sustained release in many types of medication delivery systems. The limited activity of these nanopharmaceuticals can be attributed to a number of factors, including concerns regarding safety and toxicity, concerns regarding biocompatibility, and physiological impediments [89]. The biocompatibility and biodegradability of nanoparticles made from synthetic polymers such as like polylactide (PLA), polyglycolide (PGA), poly (lactide-coglycolides) (PLGA), poly-ε-caprolactone (PCL), poly-alkyl-cyanoacrylates (PAC), and natural polymers like cellulose (CC), cellulose acetate butyrate (CAB), and cellulose acetate butyrate (CAB), Chitosan and gelatin make them usable for drug delivery [87]. Attaching surface ligands to these polymeric nanoparticles makes them "smart," so that the drug or biomolecule can be sent to the right place without hurting healthy cell [86]. For addition, chitosan NPS have been studied a lot as a way to deliver drugs. It has been shown that chitosan nanoparticles avoid RES clearance and tend to build up in tumors [88], [90-92].

Silica NPs

There are numerous advantages of using silica nanoparticles in medicine delivery systems. In addition to having use in medication delivery systems, 8% of all airborne NPs are composed of silicon dioxide nanoparticles [93]. In the earlier, nanosilica was thought to be a relatively friendly materials for use in drug delivery systems. However, recent findings suggest the NPs of silica cause the generation of reactive oxygen species (ROS) and oxidative stress [94]. Lin et al. found an increase in ROS, LDH, and malondialdehyde after treating human bronchoalveolar carcinoma cells with 15-46 nm silica nanoparticles at 10-100 µg/mL [95]. 2',7'-dichlorofluorescin diacetate, LDH, and a commercial kit detected ROS. Similarly, silica NPs have been shown to cause inflammatory indicators such as IL-1, IL-6, IL-8, TNF-(tumor necrosis factor), and

mitochondrial damage in other investigations [96-98]. Furthermore, in vitro research on liver cells revealed that silica-based nanoparticles (70 nm) at 30 mg/kg changed biochemical parameters and exhibited hepatotoxic effects [99].

To provide detail toxic effect information of nanoparticles types, this review lists (**Table 1**).

TABLE 1.
NANOPARTICLES TYPES, EXPERIMENTAL MODELS, AND TOXIC EFFECTS

Nanoparticles types	Experimental models	Toxic effect
• Fe_2O_3 NPs • Al_2O_3 NPs • TiO_2 NPs • ZnO NPs	• Liver, spleen, lungs, kidney, brain • Human hepatocellular carcinoma cells • Sperm, pulmonary organ • Human skin • Human hepatycytes • Human lymphocytes • Human bronchialepithelial cells • Mouse neuroblastoma • Mouse lymphoma cell • Rats	• Cell viability ↓ • Human macrophage ↓ • ROS (Reactive Oxygen Species) ↑ • DNA damage • Mitochondrial function ↓ • Dose-dependent genotoxicity • Apoptosis • Actin filament integrity • Blood brain barrier breakdown • Changes in gene expression
• Silver NPs • Gold NPs	• Human lung cancer cell • Human leukimia cells • Human hepatoma cells • Reproductive organs • Cancer cells • Bacteria, microalgae, fungi • Mammals	• ROS ↑ • Metabolic activity • Cell viability↓ • Mitochondrial damage • Mitochondrial integrity • Membran activity
Carbon–based NPs ; • SWNTs • MWCNTs • Fullerenes	• Liver, kidney, spleen, and bones • Human alveolar carcinoma epithelial cell line • Normal human bronchial epithelial cell line • Human keratinocytes cell line. • Mice	• Inflammation • ROS ↑ • Expression of genes that regulate cell proliferation • DNA strand breaking • Chromosomal damage
Lipid–based NPs ; • Liposomes • PEGylated liposome • Solid-lipid (SLNs)	• Drug delivery system	• Respiratory symptoms • Blood pressure • Hypersensitivity reactions (HSRs) • Cardiopulmonary distress, • Anaphylactoid reactions • Low solubility • Stability issues • Drug payload↑
Protein – based NPs; • Albumin • Geratin • Ferritin • Fibroin • Casein	• Drug delivery system • Rats	• Unstable • Damaging to healthy cells • Dose-dependent toxicity • Damage protein surface • Cell viability ↓
Polymeric NPs ; • Polylactide(PLA) • Polyglycolide(PGA)	• Drug delivery system • Mice	• Cytotoxicity • Genotoxicity • Oxidative stress ↑

Nanoparticles types	Experimental models	Toxic effect
• Poly (lactide-coglycolides) (PLGA) • Poly-ε-caprolactone(PCL) • Poly-alkyl-cyanoacrylates(PAC) • Chitosan • Gelatin		• DNA damage • Hydrolysis • Produce biocompatible metabolites
Silica NPs	• Drug delivery system • Human bronchoalveolar carcinoma cells • Rats	• ROS ↑ • Oxidative stress ↑ • LDH (Lactate Dehydrogenase) ↑ • DNA damage

Note : ↑=increased; ↓=decreased

D. Comparative Toxicity Across Nanoparticle Types and Knowledge Gaps

Numerous studies have reported distinct differences in toxicity among various classes of nanoparticles, depending on their composition, size, surface chemistry, and degradation behavior. Metal-based nanoparticles (e.g., Ag, CuO, TiO_2, ZnO) exhibit the highest toxicity, largely due to their propensity to generate reactive oxygen species (ROS), induce mitochondrial dysfunction, and cause DNA fragmentation. Silver nanoparticles (AgNPs), in particular, show strong antibacterial but also strong cytotoxic effects, even at low concentrations [49, 100].

Carbon-based nanoparticles, such as single-walled and multi-walled carbon nanotubes (SWCNTs, MWCNTs), are associated with chronic pulmonary inflammation, genotoxicity, and in some cases, asbestos-like pathogenicity [60]. However, their toxicity is highly dependent on surface functionalization and dispersion state.

Silica and polymeric nanoparticles generally show moderate toxicity, with toxicity increasing at smaller sizes or in agglomerated forms. Mesoporous silica NPs induce oxidative stress and inflammation, but their effects are dose-dependent [95].

Lipid- and protein-based NPs (e.g., liposomes, albumin NPs) are considered biocomspatible and show the lowest toxicity, especially when formulated using FDA-approved materials. However, hypersensitivity reactions and immune activation are still possible [80, 84].

Knowledge Gaps:

1. Lack of standardized comparative toxicity studies: Most available data are from isolated in vitro or in vivo studies under differing conditions, limiting cross-study comparisons.
2. Chronic toxicity data are limited, especially regarding low-dose, long-term environmental exposure and bioaccumulation in reproductive and nervous systems.
3. Few studies investigate combinatorial effects of different nanoparticles or their interaction with other environmental pollutants.
4. Biotransformation and degradation pathways of many NPs in vivo remain unclear, including how surface coatings evolve in biological environments ("protein corona").
5. Limited human epidemiological data, especially in occupational settings, hinder definitive risk assessment.

E. Physicochemical Properties of Nanomaterials Influence Toxicity

Size and Surface Area

According to the findings of research, the size of a substance can affect its toxic potential. Despite the fact that there is an inverse relationship between size and toxicity, there are some data that contradict this hypothesis. Because smaller particles have a greater surface-to-volume ratio than larger particles, the inverse relationship between size and toxicity can be explained by this fact [101]. The ratio of surface area to volume of nanoparticles develops exponentially as their size decreases, which leads to an increase in both the biological and chemical reactivity of the particles [102]. For instance, the number of surface molecules expressed increased from ten to fifty percent when the size of the NPs was decreased from thirty nanometers to three nanometers [6]. Some nanomaterials have a threshold size of 30 nm, and as they go smaller, the surface energy rises and the likelihood of surface interactions increases, which could render the molecule thermodynamically unstable and more toxic. One study's results show that nanoparticles with smaller sizes cause more reactive oxygen species to be made and cause more severe pulmonary inflammation than nanoparticles with larger sizes [103].

The size of nanomaterials plays a crucial influence in their physiological response, dispersion, and elimination [104]. Depending on their size, nanoparticles can migrate to different regions of the body. Following the intravenous injection of silica nanoparticles 200 nm in size and gold nanoparticles 100 nm in size into mice and rats, respectively, significant accumulations of these nanoparticles were seen in the macrophages of the spleen and liver [98, 105]. The smaller nanoparticles, on the other hand, were easily flushed away by the urine. In several cases, the level of toxicity rose along with the increasing size [106, 107]. Lopez et al. assessed the subcellular localization, toxic effects, and tissue distribution of three distinct sizes of AuNPs [108]. After administering particles with diameters of 10, 30, and 60 nm intraperitoneally to rats, the in vivo dispersion of these particles was evaluated. The 10 and 30nm gold nanoparticles traversed the nucleus'

membrane, hence encouraging DNA breaks. 10 and 30 nm gold nanoparticles appear to collect more in the liver, kidney, and gut than 60nm AuNPs. It was discovered that the spleen collected the most particles with a diameter of 60 nm. As a result, the accumulation of gold nanoparticles in the spleen rises along with the particle size of the gold. The body reacts differently to a comparable mass of billions of NPs as opposed to numerous microparticles. The increased surface reactivity and surface area of the nanomaterials could lead to the generation of more reactive oxygen species, which could cause cytotoxicity and genotoxicity [109, 110].

Shape

The structure and shape of nanoparticles affect their toxicity. Typically, nanomaterials can be shaped into a wide variety of shapes and structures, such as tubes, fibers, spheres, and planes. The cytotoxicity of multi-wall carbon nanotubes has been compared to that of single-wall carbon nanotubes and graphene in a lot of researches, and the results indicate a significant correlation between shape and toxicity [111, 112]. Several researchers have additionally compared the toxicity of nanocarbon compounds to that of NPs [113]. The shape of the nanoparticles plays a key factor in deciding how well it will be taken up by cells. In the epithelial tissues of the gut and gill, cube-shaped carbon nanoparticles were not taken up by cells, but spherical carbon nanoparticles and tubes of multi-graphitic sheets were [114]. The typical shape of nanomaterials are given in **Figure 2.**

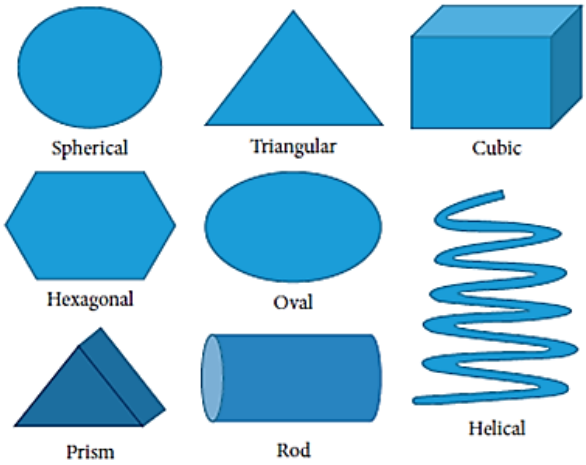

Fig 2. Typical shapes of nanomaterials

An alternative mechanism that has been hypothesized to explain the influence that shape has on toxicity is the link between form and toxicity. This relationship manifests itself as adverse effects on endocytosis or clearance of macrophages. For example, the shape of a particle can have an effect on the deformation of the membrane that occurs during endocytosis or phagocytosis [115]. It would appear that spherical nanoparticles are endocytosed more quickly and with less difficulty compared to rod or wire shaped nanomaterials [116]. Nanomaterials that are sheet-like, cylindrical, or otherwise nonspherical are absorbed by macrophages to a lesser degree. This is in comparison to nanoparticles that are spherical. As a result, particles that are not spherical have a greater chance of moving through the capillaries and adhering to the walls of blood vessels, which results in extra biological impacts [117].

One of the mechanisms associated with the form of nanoparticles is their capacity to cause direct physical harm. In concept, it is not difficult to imagine that some types of nanocrystals, such as those shaped like needles, could be harmful to cells and tissues. Uric acid crystals, for instance, are responsible for significant tissue damage as well as inflammation. In addition to this, graphene oxide nanosheets are toxic to human cells as a result of the direct damage that they produce when they engage with the cell membranes [118]. Titanium dioxide nanoparticles can have a variety of different crystal forms, each of which results in a unique set of toxic characteristics. Rutile nanoparticles, for instance, have been shown to be capable of causing oxidative DNA damage, as well as lipid peroxidation and the creation of micronuclei. Anatase titanium dioxide nanoparticles, on the other hand, have the potential to generate a greater number of reactive oxygen species as compared to rutile [119]. Embryos of zebrafish (Danio rerio) were reported to be more sensitive to silver nanoplates than silver nanospheres [120]. Compared to other shapes, cells take up a greater quantity of spherical nanoparticles [121]. Gold nanorods result in less autophagosome accumulation than gold nanospheres [122].

Concentration

The concentration of the NPs is important for their application. When it comes to determining the phototoxicity of NPs, one of the most important elements to consider is their concentration. In the vast majority of cases, there is a direct correlation between an increase in concentration and a rapid increase in the toxicity threshold. Sánchez-paradinas et al. to investigate the cytotoxicity of PtU2, a cisplatin derivative [123]. When coupled with 20 nm gold nanoparticles, this chemical exhibited low toxicity (AuNPs). Cisplatin is one of the most widely utilized anticancer medicines, and its conjugation with Au-NPs confers advantages due to Au's biocompatibility, inactivity, nontoxicity, and stability. In this approach, the chemical becomes an effective treatment for solid tumors. In this investigation, an osteosarcoma cell line (MG-63) was treated with varying amounts of AuNPs, PtU2, and a combination of the two, PtU2-AuNPs. One of the objectives was to identify the carrier activity. In order to accomplish this, the gold and platinum concentrations in the cells and supernatants were tested independently. The results demonstrated that the capacity of cells to absorb metal is identical for AuNPs and AuNPs connected to PtU2. The Annexin V-FITC assay was then evaluated using flow cytometry to determine how hazardous the cells were. When the two compounds were combined with MG-63, cells treated with PtU2-AuNPs exhibited more cytotoxicity 48 hours later. In short, PtU2-AuNPs are more hazardous to cells under identical culture circumstances [123].

Aspect Ratio

The ratio of a nanoparticle's width to its height is referred to as the particle's aspect ratio. The aspect ratio of a spherical particle is one, whereas the aspect ratio of a nanotube is very close to zero. The toxicity of the NPs increases in direct proportion to the aspect ratio of the NPs. Muller et al. injected carbon nanotubes with a high aspect ratio directly into the trachea of Sprague-Dawley rats in order to study the effects the nanotubes had on the lungs [124]. There was a significant amount of protein leakage as well as granulomas on the peritoneal side of the diaphragm, which is where the carbon nanotube samples were positioned. Li et al. investigated the effect that mesoporous silica nanoparticles with varying aspect ratios of 5, 1.75, and 1 had on the in vivo toxicity, excretion, and biodistribution of the particles [125]. When the aspect ratio was reduced, there was a concomitant increase in systematic absorption through organs such as the small intestine, while there was a concomitant decrease in urinary excretion. It has been shown that the toxicity of silica nanoparticles to the kidney might vary depending on their shape.

Crystallinity

The crystalline structure of nanoparticles may influence their toxicity. The chemical and physical properties of polymorphs, crystalline forms with the same chemical content but distinct crystal shapes varied. Lai et al. reported the cytotoxicity of 10-hydroxycamptothecin (HCPT) nanoparticle dispersions, which varies on the polymorph [126]. Three polymorphic nanoparticle dispersions of 10-hydroxycamptothecin, i.e., pancake, prismatic, and needle morphologies, were fabricated and studied. The cytotoxicity data demonstrated that the cellular toxicity of the various HCPT nanoparticles relied on their size and shape. Despite similar cellular absorption, the needle-shaped HCPT nanoparticles are more effective at inducing apoptosis in cancer cells than prismatic nanoparticles. This effect may explain the preference for polymorphs with unique thermodynamic features, such as lattice energy. Polymorph-dependent absorption and toxicity of titanium dioxide nanoparticles in A549 lung epithelial cells was also reported by Andersson et la. These results emphasize the importance of the precise characterisation of the polymorphic form (crystalline structure) of nanoparticles for the appropriate evaluation of their toxicity [127].

Solubility

Because the vast majority of nanomaterials are insoluble, they have a tendency to collect in the systems and cells of living organisms. It has been demonstrated that there is a considerable correlation between the solubility of nanomaterials and their toxicity [128]. While certain acute toxicity responses are caused by the high solubility of nanomaterials, low solubility can also result in a variety of long-term consequences, including cancer. During dissolution, crystal structures separate into smaller pieces and form distinct sheets of crystal. This, in turn, generates surface defects in nanomaterials, which leads to required for the production reactive oxygen species. Those reactions can be dangerous. Insoluble nanomaterials can stay in the respiratory system for years if they are not broken down. Keeping particles in the lungs for an extended period of time can potentially cause damage and biological reactions [[117].

Surface Charge

When particles have an oxidized surface, they usually produce a layer of negatively charged OH– groups at the surface. These groups attract positively charged protein side groups [129]. After proteins attach to particles, they either lose their ability to perform their function altogether or become less effective at doing that function. Due to the fact that this type of connection is irreversible, a more dramatic effect takes place when protein side groups attach covalently to the particles, such as cysteines to Au surfaces [13]. Unique manmade nanomaterials were found, through the use of a proteomic screen, to show some similar attraction to human proteins. Some of these proteins include peroxiredoxin 1, annexin A2, and ribosomal proteins [130]. The increasing application of nanomaterials in the medical field has resulted in a greater focus being placed on the effects of nanomaterial surface charge on cellular absorption, and numerous studies have been conducted to investigate the connection between nanomaterial surface charge and toxicity [131]. Positively charged polystyrene nanoparticles produce greater cytotoxicity in HeLa cells than negatively charged nanoparticles, according to the results of a study. Positively charged particles damage DNA and activate cell cycle checkpoints. However, negatively charged particles have no discernible impact on the cell cycle [132].

Protein Corona

Proteins have the ability to attach themselves to the surface of nanoparticles. Proteins that have a tendency for physical or chemical absorption at the nanoparticles' surface form a stiff corona when they come into contact with the nanoparticles. The formation of a gentle corona is caused by the weak contact that other proteins have with the surface of the nanoparticles. The physiochemical features of nanoparticles are what decide whether proteins will associate with them or dissociate from them. It is possible that the attachment of nanoparticles to proteins will make it easier for cellular receptors to absorb nanomaterials [133]. Consequently, the same nanoparticles can have significantly varying toxic effects depending on the proteins to which they bind.

Stabilizers

Since the hydrophobic nature of the vast majority of nanomaterials, the suspension of these particles in aquatic settings is unstable. Surfactants, polymer coatings, and functional groups are typically used in conjunction with one another in order to stabilize them. For example, citrate is utilized rather commonly in the process of functionalizing gold nanoparticles. According to the findings of a number of different research, the chemical properties of stabilizers and functional groups have the potential to provoke immunological and toxicological responses within the body [134].

Dissolution

The dissolvability of nanoparticles is a fundamental property that has a significant impact on the nanoparticles' capacity for safety, absorption, and toxic mechanism. The dissolution behavior of two identical NPs that are the same size and contain the same amount of compound can be dramatically different depending on the surface modification [135]. Typically, ion channels and ion transporters serve as the primary entry route for nanoparticles that undergo medium dissolution prior to being consumed by organisms.

Agglomeration

Due to their high free surface energy, nanomaterials tend to agglomerate together in solution [136]. In order to prevent agglomeration, nanomaterials are coated in barrier compounds that provide protection. The degree of toxicity of nanoparticles can be affected by whether or not agglomeration took place. Agglomeration of nanoparticles may be a contributory factor in the development of inflammatory lung illnesses in humans [137]. The agglomeration-dependent toxicity of carbon nanotubes (CNTs) and oxide nanoparticles is the type of nanotoxicity that is most commonly reported. It has been discovered that carbon nanotubes that are well-dispersed are significantly less toxic than carbon nanotubes that are agglomerated [138]. Zook et al. showed the importance of agglomeration controls by showing that large silver NPs aggregate noticeably less than small agglomerates, which leads to a reduction in hemolytic toxicity [139]. They presented their findings in their work.

F. Mechanism of Nanoparticles Toxicity

Role of Oxidative Stress

At the cellular level, exposure to nanoparticles can cause a number of different stressors, including oxidative stress, which is one of the most commonly recognized stressors. A general definition of oxidative stress can be stated as an equilibrium between both the activities of antioxidants and the production of oxidants in the environment [140]. An increase in reactive oxygen species (ROS) generation over antioxidants is what causes oxidative stress [141]. As a result, it is extremely important to explain the oxidant species that are produced either directly by nanoparticles or by the interaction of nanoparticles with biological systems (**Figure 3**). In this aspect, the vast majority of studies have supported the hypothesis that reactive oxygen species (ROS) are the most important oxidants produced by these systems. ROS encompasses a variety of chemical entities, including superoxide anion (O_2•−), hydrogen peroxide (H_2O_2), hydroxyl radical (HO•), peroxyl radical (ROO•), singlet oxygen ($^1O_2$), organic peroxides (ROOH), and peroxynitrite (ONOO−) [142]. Functions that are significantly different from those of other chemical and biological processes are exhibited by every single one of these compounds.

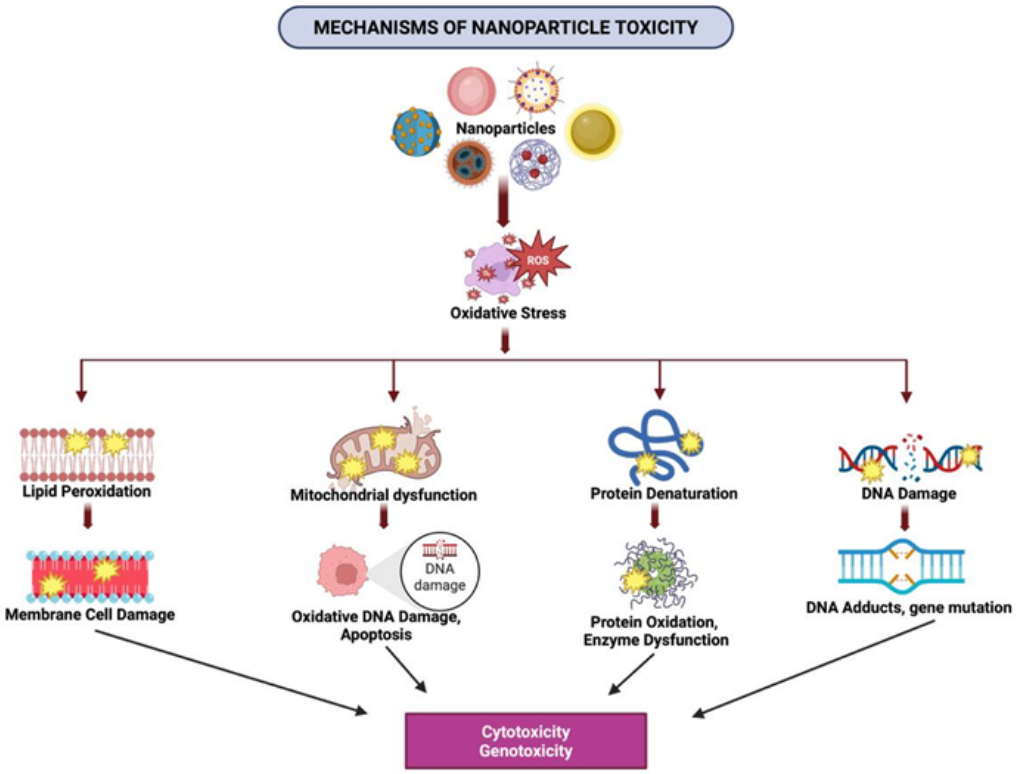

Fig 3. Mechanisms of nanoparticle toxicity

Oxidative stress is one of the worst possible outcomes of using nanoparticles. It can trigger the formation of oxidants and accelerate the generation of reactive oxygen species (ROS). This can happen due to the persistence of free radical intermediates on the reactive surfaces of the particles, or because NPs cause cells to react, or because functionalization of nanoparticles creates redox-active groups, especially if NPs can impede cellular uptake [143]. This imbalance, which may be produced directly or indirectly by nanoparticles, has the potential to have toxic effect and may lead to cytotoxicity [144]. ROS produced by NPs can cause DNA cross-linking, DNA strand breakage, and genetic mutations. NPs can also increase ROS production by stimulating inflammatory cells like neutrophils [50].

The main things that cause oxidants to be made by NPs are prooxidant functional groups on the reactive surface of NPs, active redox cycling on the surface of NPs because they are made of transition metals, and interactions between particles and cells. Most metal-based NPs are toxic because of free radicals. This is caused by the Haber–Weiss reaction, in which O_2• reacts with H_2O_2 Cu presence of transition metal such as Fe or Cu to make HO• (i) [145].

$$O_2•- + H_2O_2 \rightarrow O_2 + OH- + HO• \quad \text{(i)}$$

This is a reaction that takes place in 2 steps, and the first stage occurs when O2•− reduces an oxidized metal ($metal^{n+1}$) (ii)

$$metal^{n+1} + O_2•- \rightarrow metal^{n+} + O_2 \quad \text{(ii)}$$

In the next step of the process, which is known as the Fenton reaction, the reduced metal ($metal^{n+}$) combines with H_2O_2 to produce HO• (iii).

$$metal^{n+} + H_2O_2 \rightarrow metal^{n+1} + HO• + OH- \quad \text{(iii)}$$

For O_2-derived free radicals, the HO• radical has the shortest half-life (10^{-9}s) and highest standard reduction potential (E°=2.00V) [146, 147].

Certain nanoparticles based on metals are capable of having direct interactions with biological molecules, which can result in the production of free radicals or the activation of cell signaling pathways. The levels of efficiency at which various NPs are able to produce HO• vary greatly, despite the fact that they are all capable of doing so.

Silver NP (AgNp) was found in a variety of consumer products because it kills microbes very well. Several studies, on the other hand, have found that oxidative stress makes them cytotoxic. According to the results of one study, the presence of AgNPs led to the accumulation of intracellular ROS as well as other cell targets, which resulted in the programmed death of Candida albicans cells. The ultrastructure, cellular morphology, ergosterol concentration, membrane microenvironment, and membrane fluidity all changed as a result of this [148]. PVP-AgNPs are silver nanoparticles that have been coated with polyvinylpyrrolidone. In another study, mice who were given this treatment were shown to have lasting changes in their genes as well as DNA damage in several organs [51] . Finally, the oxidation of silver species in AgNPs after they are rapidly liberated from less silver-rich NPs after being consumed by lysosymes results in the production of reactive oxygen species (ROS), which alters the toxicity of AgNPs to cells [149]. It is the same with gold nanoparticles (AuNPs), which are frequently used in cancer treatments. However, it has been discovered that they have oxidative stress-induced cytotoxicity on multiple cell lines, such as HeLa, HepG2, and PMBC cells by producing reactive oxygen species. This is the case because these nanoparticles produce reactive oxygen species (ROS) [150, 151].

Recent studies have elucidated that oxidative stress induced by nanoparticles involves activation of intracellular signaling pathways, particularly the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), mitogen-activated protein kinases (MAPKs), and the Nrf2-Keap1 antioxidant response element pathway. Nanoparticles such as silver (AgNPs) and titanium dioxide (TiO_2NPs) stimulate mitochondrial ROS production, leading to cytochrome c release and activation of caspase-9, caspase-3, and ultimately apoptosis. Furthermore, the activation of NF-κB signaling upregulates pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, contributing to sustained inflammatory responses [144].

Some studies found that TiO_2 dose-dependently increased the formation of ROS within cells and the amount of MDA [113], [152]. TiO_2NPs have the potential to generate free radicals by inhibiting the activity of antioxidant enzymes within cells, such as SOD, CAT, GPx, and glutathione reductase (GR), or by decreasing the amounts of antioxidants within cells, such as GSH and ascorbic acid [153-156].

Nanoparticles that aren't made of metal can also be a source of oxidative stress, in addition to mestallic NPs. Oxidative stress, which can lead to cytotoxicity in the brain, heart, liver, and lungs, as well as cancer-causing and birth defect-causing qualities, has been demonstrated to be caused by ceramic NPs, which are frequently employed to transport medications. Ceramic nanoparticles have been shown to produce oxidative stress [157]. In addition, it has been demonstrated that silica nanoparticles (SiNP) elicit a time and dose-dependent NO/NOS imbalance and oxidative stress, resulting in inflammation and endothelial [158].

Role of Inflammation

The innate immune system is the first line of protection that the body has against foreign pathogens and substances that come into touch with it from the outside environment. Multiple cell types predominate in tissues that serve as an interface with their surroundings, such as the skin, respiratory mucosa, and the mucosa that lines the digestive tract and

intestines. These cell types are predominantly composed of phagocytic cells, such as polymorphonuclear leukocytes (PMN), tissue-resident macrophages, and monocytes that are derived from the systemic circulation. These cells not only have the potential to absorb particles, but they also release soluble mediators such as cytokines and chemokines, which play a role in the earliest stages of the inflammatory reaction [159]. Regardless of their density, nanoparticles between 10 and 100 nm in size can reach the alveolar space upon inhalation [160]. When there is a need to rid the alveolar area of foreign material, native macrophages will frequently take the nanoparticles into their bodies. However, if the NPs accumulation continues and the local phagocytic defense is overcome, epithelial injury will occur, which will lead to the recruitment of PMN cells and circulating monocytes. Because of NP's cytotoxicity, cells may be prevented from differentiating and from producing proteins. It is also possible that it activates genes that contribute to inflammation and produces inflammatory mediators. It is essential to take into consideration the fact that regular bodily defenses have no effect on NPs. PEGylated nanoparticles of a larger size are taken up by macrophages with more ease than those of a smaller size, which results in NPs accumulating in the body [161]. It has been proven that nanoparticles of superparamagnetic iron oxide disrupt or completely repress osteogenic differentiation of stem cells and stimulate the synthesis of signal molecules, tumor antigens, etc [162], [163]. In addition, the contact of nanoparticles with cells increases the expression of genes involved in the formation of lysosomes [164], disrupts their function [165], and inhibits protein synthesis [166, 167].

In summary, the following are the most common mechanisms of cytotoxicity exhibited by nanoparticles :

1. The generation of reactive oxygen species and other free radicals can be caused by nanoparticles, which can then lead to oxidation.
2. Nanoparticles have the potential to pierce cell membranes, which can cause damage to the membranes.
3. Nanoparticles damage cytoskeleton components, disrupting intracellular transport and cell division.
4. The acceleration of mutagenesis caused by NPs is due to their ability to interrupt transcription as well as cause DNA damage.
5. Nanoparticles cause damage to mitochondria and disturb their metabolism, which results in an imbalance of energy in the cell.
6. Nanoparticles inhibit the creation of lysosomes, which in turn stops autophagy and the breakdown of macromolecules and starts the process of apoptosis.
7. Nanoparticles change the structure of the proteins that are found in cell membranes, which in turn disrupts the passage of substances into and out of cells as well as the transfer between cells.
8. Nanoparticles cause disruptions in the normal metabolic processes of cells, tissues, and organs, which in turn stimulates the production of inflammatory mediators.

G. Distinction Between Acute and Chronic Toxicity

Acute exposure to nanoparticles may result in immediate cellular responses such as oxidative stress, cytoskeletal disruption, and cell death, observable within hours to days in in vitro or short-term in vivo models. For instance, AgNPs rapidly increase intracellular ROS and induce apoptosis in fungal and mammalian cells within 24 hours [148]. Chronic exposure, on the other hand, has been associated with long-term accumulation in tissues, persistent inflammation, fibrosis, and even carcinogenicity in animal models. Chronic toxicity is particularly relevant in occupational or environmental contexts where low-level exposure occurs over prolonged periods Accumulation of nanoparticles in organs like liver, spleen, or lungs is commonly observed in animal models[6], [168-173].

With the increasing use of nanoparticles (NPs) across various industries—including cosmetics, textiles, electronics, and pharmaceuticals—human exposure, both intentional and unintentional, has become a significant concern. Occupational exposure poses the highest risk to workers involved in the synthesis, processing, and handling of engineered nanomaterials, primarily through inhalation, dermal contact, or accidental ingestion, with studies linking chronic exposure to respiratory, cardiovascular, and systemic effects, although long-term cohort data remain limited [6], [15]. In parallel, environmental exposure arises from the release of NPs into air, water, and soil during production, use, or disposal, allowing persistence and potential bioaccumulation in ecosystems and food chains; for example, silver nanoparticles (AgNPs) from textiles have been detected in wastewater, raising concerns about ecotoxicity and contamination of drinking water sources [22]. Health implications are particularly alarming, as ultrafine and nanoscale particles may bypass protective barriers like the blood–brain and placental barriers, leading to accumulation in vital organs such as the liver, lungs, and reproductive tissues, with potential neurotoxic, developmental, and endocrine-disrupting effects, especially in vulnerable populations [21]. Despite these risks, regulatory and knowledge gaps persist, with no globally standardized exposure limits, limited occupational exposure guidelines from agencies like NIOSH (e.g., for TiO_2 and carbon nanotubes), and underreported environmental release data.

H. In Vitro vs. In Vivo Toxicity Models

In vitro models offer valuable insights into nanoparticle-induced cellular uptake, reactive oxygen species (ROS) generation, DNA damage, and mitochondrial dysfunction under well-controlled experimental conditions. However, they lack essential systemic components such as immune response, biodistribution, metabolism, and organ-level interactions. For instance, while gold nanoparticles exhibit

minimal cytotoxicity in vitro, they have been found to accumulate in the liver and spleen, causing adverse effects in vivo. In contrast, in vivo models provide a comprehensive understanding of nanoparticle behavior within the whole organism, encompassing organ-specific toxicity, systemic inflammation, and long-term retention. Notably, titanium dioxide (TiO_2) and silica nanoparticles (SiNPs) have been demonstrated to induce neurotoxicity, hepatotoxicity, and pulmonary inflammation in rodent models, even at low chronic exposure levels [52, 105].

I. Regulatory Approaches and Risk Assessment for Nanomaterial Safety

Given the growing commercialization of nanomaterials, various national and international agencies have started to develop safety frameworks and exposure guidelines to mitigate potential risks. The National Institute for Occupational Safety and Health (NIOSH) in the USA recommends a recommended exposure limit (REL) for TiO_2 nanoparticles at 0.3 mg/m³ for fine particles, but no safe level has been identified for ultrafine particles due to their carcinogenic potential via inhalation [174]. The European Union (EU), under the REACH regulation, mandates specific safety evaluations for nanomaterials, including toxicological and ecotoxicological profiling, while the European Chemicals Agency (ECHA) has issued guidance on reporting nanoforms with characteristics such as particle size, shape, and surface chemistry. The Organisation for Economic Co-operation and Development (OECD) encourages the testing and assessment of nanomaterials through its Working Party on Manufactured Nanomaterials (WPMN) and adapted testing guidelines that address nanospecific endpoints such as agglomeration and dissolution [175]. Additionally, the International Organization for Standardization (ISO) has released standards like ISO/TR 16197:2014 focusing on nanoparticle risk assessment, particularly in occupational settings, and these have been adopted in Indonesia for toxicity testing in environmental and pharmaceutical contexts [176]. While the FDA and EMA do not yet have nanoparticle-specific approval pathways, both agencies recommend detailed characterization, including size distribution and toxicity studies, for products involving nanotechnology [177]. Despite these efforts, challenges remain, including variability in test protocols, inconsistent nanoparticle definitions, and the lack of chronic human exposure data, highlighting the absence of a globally harmonized regulatory framework for nanoparticle safety.

III. CONCLUSION

Nanotechnology's growth has both positive and negative effects on the natural world and on the health of organisms. The application of NPs is growing across several industries. Because NPs are so widely used, it is impossible for humans to avoid coming into contact with them, whether on purpose or by accident.. Nanomaterials' harmful interactions with biological systems result for possible adverse effects. Nanoparticles potential toxicity is a significant concern that requires rapid consideration, such application in the medical field. However, both the positive effects of NP utilization, but also their possible unanticipated adverse consequences on the health, must be investigated. The toxic effects of NPs is inversely proportional to the extent to which they are dispersed in the respiratory and lymphatic system, as well as to their capacity to exert an effect on nearly all cells, to interact with a wide variety of molecules, and to cause changes in the structure of those macromolecules. This causes disruptions in the processes that occur inside of cells as well as in the operation of whole organ systems. There are several pathways for nanomaterial toxicity, including the generation of ROS, cytotoxicity to cells, genotoxicity, and neuro-toxicity. Size, surface area, shape, concentration, aspect ratio, surface charge, crystallinity, solubility, and agglomeration are all physicochemical properties that contribute to the toxicity of nanoparticles. Many types of NPs are unknown to the cell and body's defensive systems, which slows their destruction and can result in significant NP buildup in organs and tissues, even at extremely harmful and deadly amounts. For instance, acute toxicity tests on animals have shown that smaller nanoparticles are more toxic. Intensive study on nanomaterials characterization, molecular interactions, toxicology, and health risks is necessary. When producing nanoparticles, it is crucial to examine their harmful consequences at this stage. Size, shape, and other critical features should be modified for optimal performance. Nanoparticle toxicity can lead to friendlier, more productive nanoparticles. In conclusion, it is hoped that the increasing amount of research on nanotechnology will lead to a greater knowledge and compre-hension; therefore, more care and attention should be made to the usage, engineering production, and unintentional release of NPs, taking into all relevant factors. For a more exact risk assessment, nanoparticles and their complexes that underlie their interactions with biological materials must be considered.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

USE OF ARTIFICIAL INTELLIGENCE (AI) TOOLS STATEMENT

We used Grammarly (Grammarly Inc., 2025) to improve the clarity and grammar of the manuscript. The authors reviewed and approved all changes.

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