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Review Article

Review of Nanotoxicology and Safety Evaluation of Nanoparticles Formulation

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ABSTRACT:

Background: Nanomaterials have been widely used across medical and health sciences due to their unique physicochemical characteristics, versatile functionalisation, and remarkable tissue penetration abilities. As nanotechnology continues to evolve, concerns regarding the potential toxicological effects of these materials are growing. Despite their promising biomedical applications, comprehensive safety data remains limited. Purpose: This review details the physicochemical properties of the nanoparticles contributing to the development of potentially adverse effects on human health and the environment. It explores the cellular and molecular mechanisms through which nanoparticles induce toxicity. It assesses current nanotoxicity evaluation strategies, including In vitro, in vivo, and in silico models, along with supporting methodologies. The review also addresses the regulatory landscape of nanotoxicology, outlining the challenges in developing standardised protocols to ensure the safe and effective use of nanomaterials in the health sector. Key Observations: Factors such as particle size, dosage regimen, surface chemistry, and immunogenic potential of nanomaterials play a pivotal role in nanotoxicity. Nanoparticles may accumulate in diverse tissues, leading to oxidative stress, inflammation, and cellular and mitochondrial DNA damage. While regulatory agencies like the FDA, EMA, and CDE have issued guidelines for the safer use of nanomaterials, a globally harmonised framework is still absent

Keywords: Nanomaterials, Nanotoxicity, Formulations, cancer therapy, Bionanoscience

Introduction:

The development of nanotechnology in different industries, its modernity, and also the lack of information on its negative effects on human health and the environment originate from the novel mechanisms that are also related to nanotoxicology. Some researchers are fundamentally against using nanomaterials in human medicine and in the environment while others are in favor. The important point here is that because there are many nanomaterials with many different uses, it is difficult to test all of them and estimate their effects on human health. Therefore, some scientists believe that their side effects are acceptable.1,2 Considering all factors, testing the effects of nanomaterials on

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mammals and the environment is necessary. Only with more research, and using scientific evidence, microscopy tools, and modern analysis methods, can we discover the advantages or disadvantages of their applications. New features of nano-sized materials can be found, including electrical conductivity, reactivity, stability, colorability, and toxicity.2 Carbon in the form of graphite is soft and malleable, although at a nano-sized scale, it becomes a nanocarbon tube, which is tougher than steel. One gram of catalyst with a diameter of 10 nm is about 100 times more reactive than a similar particle with a diameter of 1 μm. However, toxicity occurs with nano- and micron-sized particles. The important fact about nanoparticles is their remarkable reactivity, a characteristic that may result in toxicity effects.1,2 In this review article, nanobiomaterials used in the field of medical sciences are discussed, along with their toxicity effects.

Nanotoxicology

Nanotoxicology is a branch of bionanoscience, which deals with the study and application of the toxicity of nanomaterials. Nanomaterials, even when made of inert elements such as gold, become highly active at nanometer dimensions. Nanotoxicological studies are used to determine whether and to what extent these properties may pose a threat to the environment and to human health.3

Nanoparticles play a remarkable role in toxicity, which is important for toxicologists, especially in respiratory diseases. Their size is an important factor in the occurrence of disease. Some studies on the different sizes of carbon and titanium oxide showed that reduction in nanoparticle size increases its toxicity in the lungs. Also notable is that combining some metals with each other causes complex toxicity, which does not occur with single metals. In 1975, a study showed the effect of oxidative stress caused by asbestos as the main factor in asbestosis and also in disturbing cell structure. In 1998, Zhang presented his findings on the effects of nanoparticles on respiratory toxicity and inflammation.4 Some of the particle features such as size, surface chemistry, and oxidative stress functions play important roles in nanotoxicity. Other features such as crystallinity, coating, and the longevity of particles have also been studied as important parameters.5 By gaining control over dangerous particles, we can increase the use of nanoparticles by reducing their harmful effects, and thus allowing them to be used in the curing of diseases.5–9

Important factors

a. Size

For particle toxicity, two factors are important: size and chemical compounds. A reduction in the size of nano-sized particles results in an increase in particle surface area. Therefore more chemical molecules may attach to this surface, which would enhance its reactivity and result in an increase in its toxic effects.8,9 Many studies on the absorption of nanoparticles from the mucus have examined these effects. After absorption, nanoparticles reach the blood stream and then spread through the tissue. In one study, 33% of 50 nm, 26% of 100 nm, and 10% of 500 nm particles were discovered in mucosal and lymphatic tissues of the intestine.9 Nanoparticles larger than 1 μm were weakly observed and nanoparticles larger than 3 μm were occasionally seen in lymphatic tissues. Researchers have concluded that:

1. Nanoparticles smaller than 100 nm are absorbed by the cells of the intestine but not the larger nanoparticles (300 nm).

2. The absorption of smaller nanoparticles (100 nm) in the lymphatic tissue is greater than in intestinal cells.

3. Intestinal cells cannot absorb nanoparticles larger than 400 nm.

4. •Only nanoparticles smaller than 500 nm can enter the circulatory system.

Scientists are discussing the relationship between particle sizes and their penetration into mesenteric lymphatic glands, but so far have reached no agreement.9

In addition to being able to cross cell membranes, and reach the blood and various organs because of their small size, nanoparticles have a bigger surface to volume ratio than larger particles. Therefore more molecules of the chemical are present on the surface, which may be one of the reasons why nanoparticles are generally more toxic than larger particles of the same composition.9

Particle surface

In vitro studies have shown that very small particles have more pathological and destructive power on the lungs rather than the same particles of smaller size due to their larger surface area, greater tendency to conjugate, and energy sustainability.6–8,10–12

Surface chemistry

Geiser et al13 studied the interaction between particle surface chemistry and the lung's surface-lining layer. They found that, regardless of the nature of the surface, the particles will be submersed into the lining layer after deposition in the small airways and alveoli. This displacement is promoted by the surfactant film itself as its surface tension falls temporarily to relatively low values.13,14 On the other hand, the reactive groups on a particle surface will certainly modify the biological effects. For silica, it has been shown that surface modification of the quartz affects its cytotoxicity, inflammogenicity, and fibrogenicity. These differences are mainly due to particle surface characteristics.15 The specific cytotoxicity of silica is strongly correlated with the appearance of surface radicals and reactive oxygen species (ROS), which is considered a key event in the development of fibrosis and lung cancer caused by this compound.16 Although the type of particle does not seem to play an important role in whether it is embedded in the surfactant lining of the alveoli, the embedding process itself is crucial. Particle–cell interaction is possible only after the immersion of the particulates in the lining fluid, and research is needed to study this phenomenon in detail in relation to the inhaled nanoparticles. Logically, as described in a report on silica,16 the reactive groups on nanoparticles influence their interaction with the lungs (or more generally with biological material). In some instances, it may be possible to predict the reactivity of the nanoparticle surface. The scarcity of data, however, suggests that verifying these predictions by laboratory testing would be sensible. The degree of hydrophobicity and hydrophilicity of a surface is the major feature used to estimate the toxicity. As well as size, it seems that the particle surface is critical in their absorption in the intestinal mucus. The absorption of nanoparticles produced by hydrophobic polymers is greater than that of nanoparticles produced by hydrophilic polymers.10–12

Chemical components

Chemical components of the particle surface have important effects on nanoparticles as they can react with metals. Iron can be affected by nanoparticles, which increases the induction of ROS in the free

cell system. The surface modification of nanoparticles can reduce toxicity. Researchers have also shown that the toxicity of super paramagnetic iron oxide nanoparticles could be reduced by coating them with pullulan.10–12

Dosage

Toxicity and other responses depend on the prescribed dosage and substances used. Research has shown that a high dose of nanoparticles in small or big particles could be harmful to health.10–12

Free radical production

Most or all pathogenic particles produce free radicals in the free cell system and this ability causes oxidative stress, which gives rise to inflammation, cell destruction, and genotoxicity. The particle surface of free radicals can activate the redox cycle and cause particle toxicity.10,17

Passage of nanomaterials through tissues

In body engineering and design, there are three important sites in relation to the environment: skin, lungs, and the digestive tract. These organs protect the body from harmful environmental components. In other words, they are important organs in the transmission of nutrients, water, and oxygen. The skin acts as a barrier against the substances (apart from special elements such as oxygen for the retina and UV rays for vitamin D synthesis).18–21

Nano-sized particles can enter and penetrate some organs such as the lungs, intestine, and skin. Some can penetrate into the deepest layers of the skin (dermis). Their penetration depends on their size and nanoparticle surface features. It must be noted that in vitro tests must be carried out on nanoparticle toxicity before in vivo tests are performed. Figure 1 shows the areas of the body that should not be penetrated by nanoparticles.

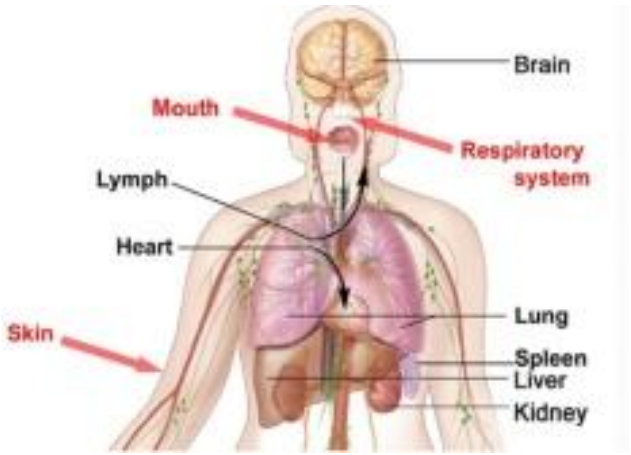

Figure 1 Parts of the body that should not be penetrated by nanoparticles

The digestive (gastrointestinal) tract has a close relationship with the environment. Materials come into the body through the mouth and all nutrition is exchanged there, apart from gas. The histology of these three organs in relation to other places is different. The skin surface area of the body, which has an area of about 2 m2 and a thickness of about 10 μm, is composed of keratin cells. These cells form a barrier against transmitting ions. The amount of penetration is related to organ, age, and other agents.20,21

Toxicity of nanoparticles

Knowledge of the toxicity effects of these small substances is limited, but is rapidly growing. Many studies have shown that some nanoparticles demonstrate toxicity in biological systems. Thus research in the internal and external environment is needed; external studies can direct the internal studies. Some researchers have shown that most of the nanoparticles can release active oxygen and cause oxidative stress and inflammation by the RES (reticoendothelial system). Acute toxicity resulting from nanoparticles has been investigated in the mouths of rats. The results indicate that toxicity depends on the size, coating, and chemical component of the nanoparticles. Also, the systemic effects of nanoparticles have been shown in different organs and tissues. The effects on inflammatory and immunological systems may include oxidative stress or pre-inflammatory cytotoxin activity in the lungs, liver, heart, and brain. The effects on the circulatory system can include prethrombosis effects and paradox effects on heart function. Genotoxicity, carcinogenicity, and teratogenicity may occur as a result of the effects of nanoparticles. Some nanoparticles could pass the blood–brain barrier and cause brain toxicity (Figure 2); of course more studies are required.22–24

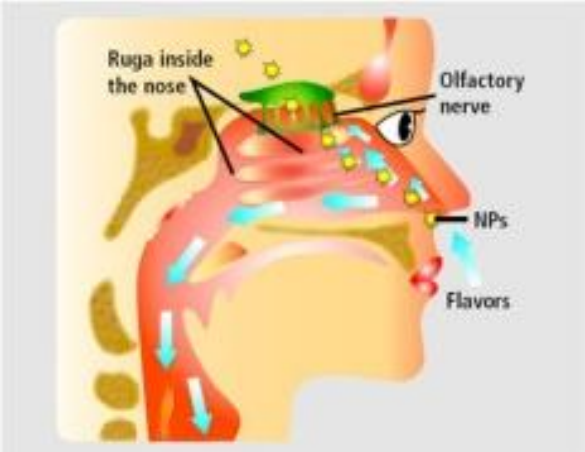

Figure 2 the passage of nanoparticles (NPs) from the nose to the cerebral system via the cibriform plate, which separates the nasal sinus from the brain and protects the nasal nerves and nervous receptors

Due to the high loading of nanoparticles, macromolecule absorption will increase, so that they can cross through the digestive tract. Because, for example, lectin is such an immunologic material for coating, it can be toxigenic and also cause inflammatory responses or digestive stimulation.25,26

Nanotoxicity- human health and environmental impacts

Despite the significant advantages of nanoparticles, critical concerns regarding their potential effects on human health and the environment remain unresolved. It is imperative to address these issues comprehen sively before the widespread production and application of nano materials. Engineered nanoparticles, increasingly used in the industry and consumer field, are emerging contaminants with potential ecolog ical and toxicological effects on populations, communities, and ecosys tems. The field of nanotoxicology analyzes the threats that nanomaterials pose to people and the environment. Engineered nano material toxicity is often determined by many factors, including (1) their plasmonic capabilities, (2) coating assessment, (3) particle shape and size, (4) surface charge, and (5) their phase stability27, 28. The use of engineered nanomaterials (ENMs) in human life is continuously increasing, which can be an alarming concern related to their safety. Such NMs may cause acute, chronic, and

systemic toxicities in humans after interacting with the biological microenvironment. As a result, it can lead to various life-threatening diseases and harm the environment Further analysing the toxicity of such ENMs before their use is essential to reduce their possible adverse effects. Nevertheless, studies conducted in the 20th century proved that materials with micrometre- scale dimensions demonstrated less inherent hazardous characteristics, whereas those at nanoscale dimensions exhibited plausible toxicity29, 30. A comprehensive understanding of nanomaterial toxicity requires an in-depth analysis of their physicochemical characteristics. Key factors such as particle size, shape, composition, concentration, structural di mensions, and stability play a crucial role. Additionally, surface-related attributes, including morphology, roughness, surface energy, charge, and the presence of functional groups, significantly influence nanoparticle behaviour31. These properties collectively determine how nanoparticles interact with biological systems, including their cellular uptake, internal distribution, and interactions with intracellular components. Such interactions can trigger a cascade of biological re sponses, including the release of reactive ions from the nanoparticle surface, generation of reactive oxygen species (ROS), lipid peroxidation, protein denaturation, inflammation, mitochondrial dysfunction, disruption of endothelial function, impaired phagocytosis, and distur bances in cell cycle regulation31, 32. Among cellular organelles, mitochondria are especially vulnerable to damage from nanomaterials. They play a central role in mediating nanotoxic effects. Research has demonstrated that exposure to nanomaterials can lead to significant changes in mitochondrial structure, disrupt membrane potential, trigger the opening of the mitochondrial permeability transition pore (MPTP), and impair respiratory function. Mitochondria are often involved in cell death pathways activated by nanomaterial exposure as the core regu lators of intrinsic apoptosis33, 34. Environmental stressors, such as increased reactive oxygen species (ROS), can prompt cells to adjust mitochondrial behaviour by modulating mitochondrial dynamics and initiating mitophagy processes essential for maintaining mitochondrial integrity and overall cellular homeostasis. Notably, nanomaterials can influence these dynamics: at lower concentrations, they promote mito chondrial fusion, while at higher concentrations, they are associated with excessive fragmentation. Fusion may serve as a cellular defence mechanism to sustain energy production and limit damage, whereas controlled fission can isolate damaged regions of mitochondria, pre serving healthy segments33, 34.

Nanomaterial toxicity in drug delivery systems

Nanostructures can be used to transmit drug targets (as a drug or transmitter) or increase drug effectiveness.35 Nanotechnology is important, especially for detecting and treating cancer, although many problems have yet to be solved. Essentially, nanostructures are studied for gene transmission and their usage in vaccination and cancer treatment. Gene transmitting has been done both in vitro and in vivo with different types of nanoparticles. In nanoparticle distribution in the body, immunological, pathological, pharmacological, and pharmacodynamic factors (time-base level of absorption, metabolism, and drug clearance) control the distribution of biological behaviors of nanoparticles in the body. In an in vivo environment, the fate of nanoparticles depends on these factors. After intravenous effusion of nanoparticles on rats, nanoparticles were rapidly removed from the blood. This action was done by the immune and reticuloendothelial system without considering the particle features. Autoradiographic studies have shown that nanoparticles are usually concentrated in the liver and bone marrow. Cyanoacrylic polymeric nanoballs decrease gradually in the liver and are cleared from the body in the feces and urine.36-38 Nanoball clearance was completed after 7 days. Briefly, we can say that the liver is an important organ for the clearance of nanoparticles. The highest concentration of nanoparticles is found in liver cells. On the other hand, the concentration of these particles on mononuclear phagocytes causes the drug to keep away from the target cells. We suggest

several methods for preventing this event. One is magnetic directional guidance of intravenous particles out of the body. It seems that this effect is intensified by increasing tumor angiogenesis. For example, albumin microballs with magnetic characteristics containing doxorubicin can penetrate into tumors more effectively than free drugs. On the other hand, by using nanoparticles to introduce the drugs into the body, drug distribution coincides with particle distribution. Changing the distribution model for nanoparticles is useful for some diseases such as cancer and also for reduction in drug toxicity. This advice has been used for anticarcinoma drugs and doxorubicin. The use of this drug has been limited because of chronic and acute cardiac failure. Doxorubicin nanoballs have lower toxicity than free drugs. Using this method, it is possible to transmit drugs into the MPS system with mononuclear phagocytes. Researchers have detected that ampicillin density in the liver is 20 times more than when free drugs are used. Intramuscular injection of labeled polymethyl methacrylate nanoparticles showed that these particles were fully absorbed after 70 days. After subcutaneous injection, the concentration of the nanoparticles decrease slowly in the tissues. Moreover, the injection of nanoparticles causes better performance in drug delivery systems, which results in increased bioavailability of peptic drugs after the drug injection has metabolized freely. As research has shown, nanoparticles are picked up on a large scale by mononuclear phagocytes after intravenous injection. On the other hand, it has been proved that distribution in the body can affect nanoparticle toxicity. Thus, in a new drug transmission system based on polymers, we must consider the possibility of activation or inhibition of mononuclear phagocytes. In this case, we must pay attention to changes in blood viscosity because the clot could be composed of nanoparticles and could cause red blood cell destruction. For other prescription methods, such as from the skin or through oral means, the stimulation of the local tissue is important.39-41

One of the problems of using nanoparticles in pharmacology is their uptake by the mononuclear phagocytosis system as they exist in the liver and spleen, although the targeting of the liver by nanoparticles may be suitable during treatment of liver diseases such as turmeric metastasis or hepatitis. Oligonucleotides can be used to control gene expression when they migrate to the liver when bonded with biodestructible polyalkyl cyanoacrylate nanoparticles. Besides the reduction in treatment effect, the uptake of nanoparticles in the liver may have a negative effect on liver function. The inflammatory responses by glycoprotein acid diffusion were caused by hepatocytes.42

Methods to determining the toxicity of nanomaterials

Dose considerations significantly influence toxicity assessments, with In vitro experiments being more prevalent than in vivo testing. In vitro, sedimentation diffusion, and dosimeter are models utilized in toxicity testing. This paradigm differentiates between the exposure (environmental attributes) of dosage aggregates at the surface of the cells and the cellular dose [52-54]. Considering the dosage rate as a predictor of response, a comprehensive understanding of the temporal dynamics of dose release is essential. Various methods like In vitro he molysis, genetic damage testing, and gene expression analysis [55] are used to identify toxicity alongside those that measure cell survival and proliferation [50,51,56]. SEM-EDX, TEM., AFM., VEDIC micro scopy, and photoluminescence are just a few of the microscopic and spectrometric techniques available for assessing cellular physio-chemical structure shown in Table 1 [57,58].

Table 1

Experimental methods of toxicity analysis of different nanomaterials.

Toxicity test	Purpose	Types of Nanomaterials	Ref.
Transmission electron microscopy	Determination of intracellular localization	TiO 2 , silver, fullerene	44
Light microscopy	Physicochemical properties	Singled-walled carbon nanotubes, silver	45
Comet assay test	DNA damage	Metal, metal oxide nanoparticles, metal- polymer nanocomposite	46
Lactate dehydrogenase	Cell viability	Carbon nanoparticles	47
Tetrazolium salts	Cell viability	Carbon nanoparticles	47
Alamar Blue	Cell viability	Quantum dots	48
ROSproduction	Oxidative stress	TiO 2, Polymer nanocomposites	46
Acridine orange/ ethidium bromide	Apoptosis	Silver nanoparticle	49

Regulatory framework for medical countermeasures against nanomaterial toxicity

The field of nanoscience has had significant advancements over the past two decades, leading to the widespread utilisation of various types of nanomaterials in clinical care [59]. This issue raises substantial concerns regarding the safe use of nanomaterials, necessitating more stringent nanotoxicity regulations. An obstacle hindering nano toxicology's progress is the absence of unified and defined methodolo gies for characterizing NPs and assessing their associated risks [60]. Standardising experimental design and data reporting is crucial for effectively training predicting computational models. Selecting appro priate doses for evaluating nanoparticle toxicity is especially important in data mining and machine learning [61]. The U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have established detailed regulatory frameworks and guidance documents to approve drugs and medical devices, offering a foundation that could be adapted to regulate biomedical nanomaterials [62]. Global regulatory bodies are actively addressing the multifaceted challenges posed by the unique properties of nanomaterials and nanomedicines, alongside the current lack of a harmonized international regulatory framework. The development of ISO standards typically begins with creating a pre liminary draft, which is first recognized as a national best practice following extensive consultation with relevant stakeholders. This draft is subsequently transformed into an ISO draft standard, which is circulated among all ISO member bodies for comments and expert evaluation [63]. The rigorous process may span two to four years before the final international standard is ratified. ISO Technical Committee 229 (Nanotechnologies), established in 2009, currently includes 33 partici pating countries and 10 observer nations. The committee has published 70 standards and guidance documents, with 40 under development. The committee's primary objectives include standardising terminology and nomenclature, advancing metrology and instrumentation protocols, and setting criteria for reference materials, testing methodologies, modelling and simulation. It also emphasizes science-driven approaches to health, safety, and environmental considerations in nanotechnology [64].

Nanomaterial toxicity mechanism for drug delivery in cancer therapy

As mentioned before, one of the advantages of using nanoparticles in drug formulations is their potential for crossing the blood–brain barrier, although this function could have harmful effects. The nanoparticles have a toxic effect on cerebral endothelium cells. Of course, this is not true when applied to all nanoparticles of the same size. The physical features of biological materials and their ability to adhere to nanoparticles are important. When nanoparticles with different surface features are tested, neutral nanoparticles and anionic nanoparticles do not have any effect on the blood cerebral system, while a high density of cationic nanoparticles has toxic effects on the blood–brain barrier system. The surface load of nanoparticles must be considered with regard to their toxicity effect and cerebral distribution profiles. The nanoparticles cause material transmission into the brain via the polysorbate surfactant Tween. The transmission mechanism by endocytosis of lipoprotein receptors at low density of endothelial cells after the absorption of blood plasma lipoprotein with nanoparticles is suggested.65, 66

The effect of paclitaxel entrapment and toxic drug effects in polysorbate/steel alcohol nanoparticles was studied in rats in a cerebral injection model. The results show that paclitaxel entrapment in nanoparticles clearly increases cerebral drug absorption and its toxicity in tumor cell P-glycoprotein expression.67

After oral injection, only 10% of 60 nm polystyrene particles were recovered again from the digestive tract. Most of these particles were found in the lymphatic tissue, such as Peyer's patches and the lymphatic tissues in the colon. The injection of nanoparticles in the dermis is optimal for cationic particles in the size range of 50 to 500 nm, and less effective with anionic and neutral particles of any size. Migration to the draining lymphatic nodes is important for detection and treatment. Polyisobutyl cyanoacrylate nanoparticle formulation and fluorescent quantum dots are found entrapped in the draining lymphatic nodes.68

The structures and features of nanogold particles make them useful for estimating their biological use. Although some of the effects were seen by using these systems at high density, using 2 nm gold cationic particles in microbiological estimates and in vitro hemolysis show slight toxicity. Since the anionic particles are comparatively nontoxic, these very small 2 nm gold nanoparticles are nontoxic when they are used in rats for tumor treatment.69-71 If we compare free TNF with PEG colloidal nanoparticles conjugated with TNF we observe increasingly antitumoral activity. While the best isomer inhibitors of topoisomerase are formulated in nanoparticle lipid, its antitumoral activity will increase in the in vivo model of human tumors grown on rat glands.72

Conclusion:

In this review article, nanobiomaterials, which are used in the field of medical science, have been discussed and their toxicity effects investigated. It is obvious that most nondegradable nanoparticles considered in this review are toxic and can influence the body's cells.

The swift evolution of nanotechnology has significantly impacted sectors such as healthcare, environmental management, and agriculture, offering unprecedented opportunities for precision medicine and reduced dependency on environmentally detrimental agrochemicals. However, the expanding integration of nanoparticles into biological systems has raised valid concerns regarding their potential toxicity and long-term biocompatibility. This review comprehensively analyses the current approaches to assessing nanotoxicity, highlighting recent ad vancements and persisting challenges. It delves into the underlying physiological and molecular mechanisms associated with nanoparticle- induced toxicity, including the

complex interplay of oxidative stress, inflammation, DNA damage, and neurotoxic effects. Developing advanced In vitro, in vivo and in silico testing platforms, high-throughput screening technologies, and alternative model organisms has enhanced nanoparticle toxicity evaluations' precision and ethical acceptability. Despite these improvements, the lack of standardized testing protocols and the need for more predictive, biologically relevant models remain significant barriers. The regulatory framework surrounding nano toxicology continues to evolve, with ongoing efforts to establish harmonized guidelines and robust risk assessment strategies.

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