ijlpr 2023; doi 10.22376/ijlpr.2023.13.5.P162-P190

International Journal of Life science and Pharma Research

ISSN 2250-0480

Review Article

Nanotechnology and Nanotoxicity

Deceiving The Myths of Nanotechnology in Relation to Nanotoxicity

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Abstract: The toxicity of nanoparticles (NPs) is a critical research topic in nanotechnology, as it is essential to understand the hazards posed by the wide spectrum of NPs that vary in shape, size, and composition. Previous reviews have yet to thoroughly explore the Biological Effective Doses of NPs, which drive toxicity and are influenced by factors such as solubility, charge, shape, contaminants, and the ability of NPs to translocate from the deposition site in the lungs. This review aims to fill the gap in the literature by providing an overview of the possible toxicity of nanoparticles in zebrafish during growth stages, with a focus on oxidative stress, and exploring the available modes of toxicity that are relevant to conventional pathogenic particles. This review also discusses the effects of nanomaterials on the reproductive system in animal models, providing insight into the potential toxicity of nanoparticles in humans. This review aims to provide a comprehensive overview of the toxicity of nanoparticles and to critically explore the challenges associated with implementing nanotechnology, particularly in the pharmaceutical development of novel therapeutic products and regulatory issues. The review also considers recent uses and projected nanotechnology advancements, providing a basis for future research in this field. In conclusion, this review rectifies the lacunae in previously published reviews by providing a comprehensive overview of the toxicity of nanoparticles and exploring the challenges associated with implementing nanotechnology. The aim and objective of this review are to provide a comprehensive understanding of the toxicity of nanoparticles and to guide future research in this field.

Keywords: Nanotechnology, Nanotoxicity, Biological Effective Dose, Zebra Fish, Nanomedicine, Regulatory Challenges

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Received On 19 December, 2022
Revised On 1 May, 2023
Accepted On 9 May, 2023
Published On 1 September, 2023

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Jeenatara Begum, Tamalika Chakraborty, Pramit Sahoo, Pritam Roy and Sebabrata Bhakta , Deceiving The Myths of Nanotechnology in Relation to Nanotoxicity.(2023).Int. J. Life Sci. Pharma Res.13(5), P162-P190 http://dx.doi.org/10.22376/ijlpr.2023.13.5.P162-P190

1. INTRODUCTION

Nanotechnology is the future in terms of enabling technological advances across a wide range of industries by offering potential[1]. The acceptance of nanotechnology and nano-enabled products are very reliable and have effective use on public cum consumer confidence in both human and environmental safety of this new technology. At the same time, we must ensure that it has been done effectively with the safety and regulation of new technology. The proactiveness of government regulators and international organizations such as the OECD, ISO, and BSI helps or tries to understand nanotechnology and how best to facilitate its safe development and use[2]. Abundant examples of other reports and opinions which have been more specific in their remit, like addressing nanoparticle terminology and definitions[3], inhalation toxicity testing for nanomaterials[4], management of the risk of carbon nanotubes[5] and specific regulatory frameworks such as REACH for nanoparticles with the effectiveness[6,7], and this all for improvement of the safe handling of nanotechnology. But despite the obvious hard work, funding, and good intention that is being focused on the safe development of nanotechnology, there still needs to be more certainty and Besides all the good side effects and therapeutic effects of nanoparticles, there is concern that nanomaterials may harbor an unknown mode of toxicity or 'nano-specific effects.' Much effort has been directed toward understanding these 'nano-specific effects' that lead to the various modes of action in identifying nanoparticle toxicity. All nano-sized particles have novel size-dependent properties, and indeed, it has been argued by Auffan and colleagues (2009) that the evidence for novel size-dependent properties, besides the particle size, should be the primary criterion in any definition of nanoparticles that have relation to health and safety[8]. Recently, a review by Fubini *et al*. and this argument was considered further. It was acknowledged that material at the nano-level should be 'new' by stating when and why it can be considered nano-material[9]. From the availability of the definitions of nanoparticles, an important consideration for a nanoparticle is based on a threshold dimension(s) of 100 nm[3,10]. That cannot be derived from toxicological evidence of a step-change in toxicity at 100nm nano-sized substances. Much of the evidence is far for 'nano' effects is acknowledged by Fubini et al., who noted that where the biological response is related to the surface area, which forms the interface of the particle, which is insoluble, or the biological interactions, as we know nanoparticles will, of course, show effects orders of magnitude greater than that of bulk particles at the same mass dose due to vast greater surface area[9]. By taking this, it becomes apparent that in the case of toxicity, at least, passing below this threshold into the nano-realm doesn't need to infer any new and specific properties; therefore, the arbitrary assumption of different and 'nano-specific' toxicity appears to be unfounded. Instead of all this, there is likely to be a more gradual magnification of the intrinsic hazard. This statement is echoed by Norppa *et al.,* that it cannot be generally assumed that nanoscale size would be increased the genotoxicity of nanomaterials, or we can say that nanoparticles are generically genotoxic[11]. Indeed, the view of 'nano-specific' toxicity could be intrinsically not helpful because it labels all the nanomaterials as hazardous or potentially like this, thereby prejudicing against their use. However, in most cases, as for conventional particles, in common use, nanoparticles show a range of inherent toxicities; the majority is low toxicity. In addition to that, the focus on the search for 'nano-specific' may lead to the effect of 're-inventing the wheel' of what is already known for the conventional particles and thereby delay the important issue of ensuring that the field of nanoparticle hazard can be adequately tested for, qualified and regulations put in place that can be facilitated this is an efficient and proportional manner. The main purpose of this article is to discuss this general basis of toxicity for nanoparticles because, as shown in recent research and studies, is to demonstrate that the mode of action is, in most, if it is not all, cases the same as that shown for the conventional bulk particles. Generally, we can say to understand the basis of toxicity is to understand the driving component, and this can be a variable entity between the materials of the same as well as differing the physicochemical characteristic, and this is described below about the Biologically Effective Dose (BED)[12].

2. NANOMATERIALS

2.1. Definition

According to the EC recommendation[13], nanomaterial refers to a natural, incidental[13], or manufactured material comprising particles[13], either in an unbound state or as an aggregate wherein one or more external dimensions are in size range of 1– 100nm for ≥50% of the particles[13], according to the number size distribution. In environmental, health, safety, or competitiveness concerns, the number size distribution threshold of 50% may be substituted by a threshold between 1 and 50%[13]. Structures with one or more external dimensions below 1 nm, such as fullerenes, graphene flakes, and single-wall carbon nanotubes, should be considered nanomaterials[13]. Materials with surface area by volume over 60 m^2/cm^3 are also included[14]. It defines a nanomaterial in terms of legislation and policy in the European Union[13]. Based on this definition, the regulatory bodies have released guidance to support drug product development[13]. For example, the EMA working group introduces nanomedicines as purposely designed systems for clinical applications[13], with at least one component at the nanoscale[13], resulting in reproducible properties and characteristics[13] related to the specific nanotechnology application and characteristics for the intended use (route of administration, dose)[13], associated with the expected clinical advantages of nano-engineering (e.g., preferential organ/tissue distribution[15])[13]. The Food and Drug Administration (FDA) has not established its definition for "nanotechnology," "nanomaterial," "nanoscale," or other related terms, instead of adopting the meanings commonly employed about the engineering of materials that have at least one dimension in size range of approximately 1 nanometer (nm) to 100nm[13]. Based on the current scientific and technical understanding of nanomaterials and their characteristics[13], FDA advises that evaluations of safety, effectiveness, public health impact, or regulatory status of nanotechnology products should consider any unique properties and behaviors that the application of nanotechnology may impart[12].

2.2. Size

The most important feature to consider is size. The conventional size ranges from 1 to 100 nm. However, the maximum size that a material can have to be considered nanomaterial is an arbitrary value because the psychochemical and biological characteristics of the material do not change abruptly at 100 nm[16].

2.3. Particle Size Distribution

The PSD is widely used in nanomaterial identification, reflecting the range of variation in sizes[17]. It is important to set the PSD because a nanomaterial is usually polydisperse, which means it is commonly composed of particles of different sizes[17].

2.4. Surface Area

Surface area determination by volume is a relational parameter. Therefore, the material is under the definition if the surface area by volume is larger than 60 m^2/cm^3 [10].

2.5. Composition

2.5.1. Metal Based

Metal-based NPs are an important class of NPs synthesized due to their functions as semiconductors, electroluminescent and thermoelectric materials[18]. Recently, interest and development in nanotechnology have been increasing, so many studies have been conducted to evaluate whether the original features of these NPs, such as the large surface area to volume ratio, negatively affect the environment[19].

2.5.2. Carbon Based

A typical carbon-based nanomaterial is carbon nanotubes. Carbon nanotubes were first discovered by Iijima and Ichihashi[20] and Bethune et al. [21]in 1993 [20]. Carbon nanotubes can show significant electrical conductivity[22]. Also, their tensile strength[23] and thermal conductivity[24] are outstanding due to their nanostructure and the strength of the bonds between carbon atoms; because of these properties of CNs can be utilized in many areas of technology, from biomedicine to nanoelectronics[25].

2.5.3. Metal Oxide

Metal-oxide NPs are used as industrial catalysts. TiO_2 nanoparticles may disrupt insulin response in Fao cells and cause pregnancy complications in some animal model studies[26,27]. Studies have shown that other metal-oxide nanoparticles adversely affect reproduction and neonatal development[28,29].

2.5.4. Quantum dots

Quantum dots are engineered nanoscale crystals that can transport electrons and convert a spectrum of light into different colors[25]. Quantum dots make it possible to study cellular processes and may notably improve the diagnosis and treatment of diseases such as cancers[30,31].

3. BIOLOGICAL EFFECTIVE DOSE

In conventional particle toxicology, the dose is defined by the mass or concentration of particles per unit tissue, number of cells, or surface area of cells in cell culture [32]. Particles are measured through Mass for risk management purposes; the exception is fibers are counted by number. Toxic effects are complicated and rely on the molecular effects at the cellular level and depend on various properties of the particle, basically the physicochemical properties. If the mass increases, it further increases the dose delivery that drives the toxic effect. Povey et al. define the BED as 'the active dose of the agent of interest' and that 'the nearer the dose specified can be to the active dose of the real agent of interest, the more likely it is that an association may exist between an agent and a disease'[33]. It has now defined the BED in particle toxicology as 'the entity within any mass dose of particles that drives a critical pathophysiologically relevant form of toxicity in tissue, such as inflammation, genotoxicity or cellular proliferation'[34]. The Biological Effective Dose of some pathogenic particles has been recognized; in the case of quartz, it is the unpassivated (active) surface area, and in the case of asbestos, it is the long, bio-persistent fibres[34]. BEDS are still measured by mass. However, no doubt in the future, the development of measuring instrumentation that directly measures the BED will allow the BED to be metric, improving epidemiological dose: responses and thereby improving risk management[32].

4. POTENTIAL HUMAN HEALTH EFFECTS OF NANOMATERIALS

4.1. Major Modes of Exposure

The population exposed, the amount of exposure, and the length of exposure, and these situations have very different types of material that people are exposed to [35]. During a new material's development, it is feasible to be produced under extremely controlled circumstances, usually in very small quantities. Exposures may happen during synthesis or downstream processes such as packaging, shipping, recovery, and storage once the substance enters commercial production[36]. Nanomaterials can be released intentionally in processes like contaminated land remediation or as waste or industrial pollutants into the air, soil, or water systems. As a result, nanomaterial contamination of the air, water, food supply, or commercial products containing nanomaterials could expose people to them[37].

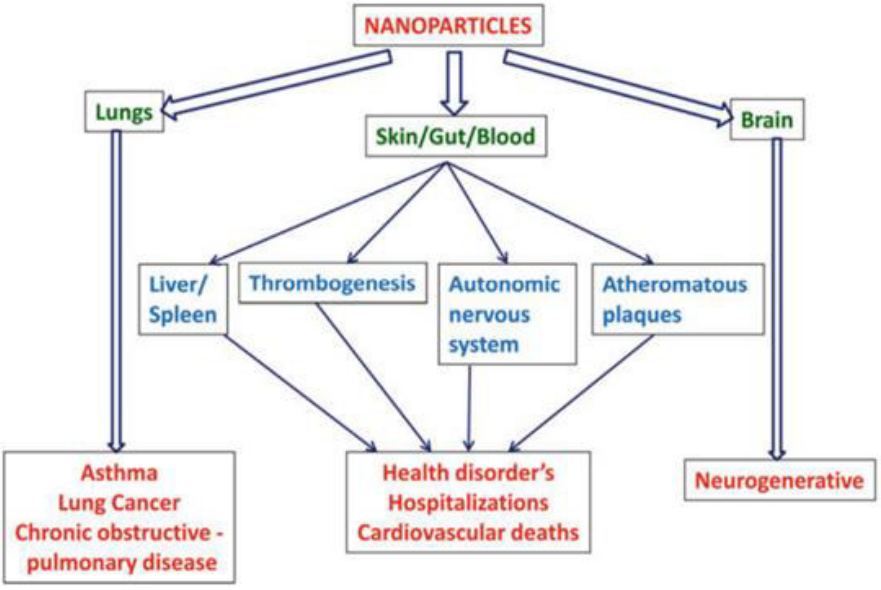

Fig 1: Systematic health effects of nanoparticles on the human body

Image adapted from Viswanath, B. & Kim, S. (2016). Influence of Nano toxicity on Human Health and Environment: The Alternative Strategies [Image]. doi: 10.1007/398_2016_12

4.2. Common Exposure Routes of Humans to Engineered Nanoparticles Present in Consumer Products

Table 1: Common Exposure Routes of Humans to Engineered Nanoparticles Present in Consumer Products

Route	Types of consumer products
Skin[37] (Dermal)	Sunscreen (lotion)
	Skincare (lotion)
	Paints and coatings
	Sealants
	Air fresheners (spray)
Lungs[37] (inhalation)	Paints and coatings
	Skincare (spray)
	Sunscreen (spray)
	Food additives and colorings
Gastrointestinal tract[37]	Food supplement
	Health supplements
	Food packaging

4.3. Effects of Inorganic Nanoparticles on Human Health

Among the most crucial nanomaterials employed in modern technologies are inorganic nanoparticles. Additionally, they are simpler to incorporate into applications [38]. Insoluble inorganic nanoparticles can be produced using pure metals or various inorganic materials or alloys. They can be distinguished from comparable products found on a wider scale by their nanometric size[39]. These inorganic nanoparticles lose their electrical, mechanical, and other properties as they become larger[40]. The study of nanomedicine has shown that drug sensitization employing various inorganic nanoparticles (NPs) is a workable and developing method[40]. For instance, when exposed to green light, the well-known photosensitizer Rose Bengal (RB) triggers the production of ROS, which results in cytotoxicity and cell death[41]. In addition, the substance releases ions and silver radicals that have an antibacterial effect when it comes into contact with moisture. Lam et al. (2004) identified the cytotoxicity of silver nanoparticles generated by ActicoatTM after finding a significant decline in cell viability in an in vitro investigation of cultured human keratinocytes[42]. Additionally, they showed that 100% anatase nanoparticles, regardless of size, cause membrane leakage and cell necrosis but do not produce ROS. On the other hand, rutile nanoparticles induce apoptosis by producing ROS. Therefore, the crystal structure and size interaction may be important in mediating nanoparticle toxicity. According to in vitro research by Lucarelli et al. (2004), cobalt (Co) and silica (SiO2) nanoparticles significantly increased the pro-inflammatory activity of human bone marrow monocytes. Gold nanoparticle (AuNP) particle size and concentration were examined by Yao et al. (2015) for their effects on uptake, accumulation, and cytotoxicity in model intestinal epithelial cells[43]. As the mean particle size of the AuNPs fell (from 100 to 50 to 15 nm), the rate of absorption by intestinal epithelial cells rose. Still, their cellular accumulation in the epithelial cells shrank. Additionally, mitochondrial membrane depolarization demonstrated that AuNP accumulation resulted in cytotoxicity in intestinal epithelial cells. The results offer crucial insight into the relationship between the dimensions of AuNPs and their absorption through the digestive tract and potential cytotoxicity[43]. Platinum medicines are given special consideration as anti-cancer treatments. However, no matter how effective they are, platinum medications have downsides. Examples include their dose-limited toxicities, ineffectiveness

against several common malignancies, and the patients' resistance to Pt-based therapy regimens[44]. The cell vacuoles contained PVC, TiO2, SiO2, and Co nanoparticles, according to Peters et al. (2004), who investigated the survival and behavior of human endothelial cells in vivo[45]. The synthesis, stability, and toxicity of engineered metal nanoparticles (ENPs) have been thoroughly studied over the past two decades because inorganic elements are an inescapable component of living beings. However, the study of naturally occurring nanoparticles (NNPs) and their creation, destiny, and ecological implications have recently attracted interest[40]. Solid organic nanoparticles, typically lipids or polymeric substances, make up organic nanoparticles (Lambert et al. 2014). This nanoparticle form has undergone extensive development and research over the past few decades due to its high potential in various industrial fields, including electronic and photonic, conducting materials and sensors, medicine and biotechnology, and others[46–48].

4.1. In vivo observed effects supported by in vitro evidence.

Table 2: In vivo observed effects induced by engineered nanoparticles supported by in vitro evidence.

In vitro evidence	In vivo observed evidence
Enhanced cytotoxicity in exposed cell culture samples	Chronic obstructive pulmonary disease (COPD)
Proliferative responses brought on by DEP component extracts	Hyperplasia
Gap Junction Intercellular Communication (GJIC) changes caused by macrophage-dendritic transepithelial cells	Particle translocation
Pneumocytes, macrophages, and other exposed cells in co-cultures that directly activate endothelial cells or indirectly trigger them. Tight junction-related changes to the TEER values	Systemic and endothelial dysfunction
When exposed to PM, lung epithelial cells' NADPH-oxidase produces more ROS.	Oxidative stress
IL-1b, IL-6, IL-8, TNFa, MCP-1, and other molecules are secreted by lung cells, macrophages, and cocultures.	Local and systemic inflammation

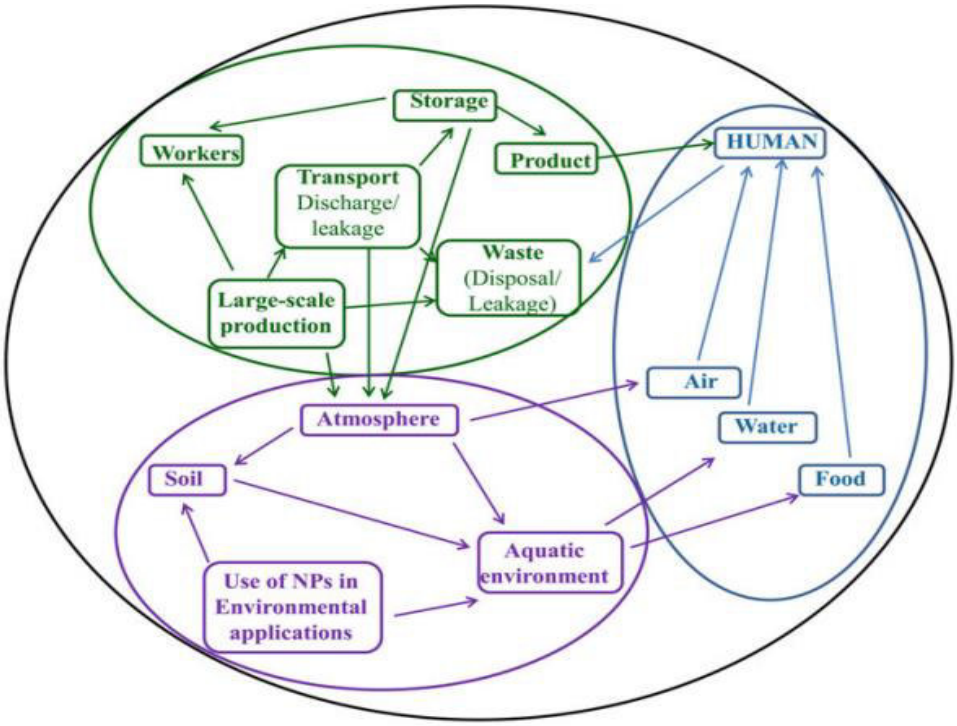

Fig 2: Multiple scenarios through which nanoparticles enter into the environment and humans

Image adapted from Viswanath, B. & Kim, S. (2016). Influence of Nanotoxicity on Human Health and Environment: The Alternative Strategies [Image]. doi: 10.1007/398_2016_12.

4.2. Environmental Issues

Energy, security, information technology, agriculture, environmental protection, and healthcare are just a few industries where nanotechnology is revolutionizing the landscape[82]. The development of nanomaterials has generated impressive scientific activity, with an exponential rise in the number of peer-reviewed papers on the subject during the past ten years. Currently, national nanotechnology projects exist in more than 60 nations. However, the success or failure of nanotechnology may depend on its capacity to address environmental challenges. Although there is limited advice for researchers on how to put such practices into practice, Responsible Research and Innovation provide a framework for assessing the ethical dimensions of innovation processes. Any research proposal should be anticipatory, looking ahead to potential technological effects; reflective, looking at the goals and purposes of technologies as well as the uncertainties in risk assessment; deliberative, looking at the idea that public and diverse stakeholders' perspectives are actively taken into account during design processes, and responsive, looking at

the actual alteration and shaping of technological trajectories in response to deliberation[83]. Many scientists are putting in a lot of effort to address several important environmental problems, such as the following: • To what extent might the manufacturing and usage of nanoproducts be expected to result in the release of hazardous elements into the environment? • What possible environmental problems can this nanotechnology cause? • Because nanoproducts bioaccumulate in living tissue, may nanotechnology contribute to environmental degradation? • What impact will laws have on this nanotechnology? Researchers looking at how nanoparticles affect ecology have found that some nanomaterials are hazardous to the environment. The precautionary principle should be used to reduce preventive risk, notwithstanding ongoing scientific uncertainties. Environmental inputs should be avoided as much as possible. The environmental relevance of materials and the complexity of natural systems should be increasingly the focus of ecotoxicological study. Due to their tiny size and increased specific surface areas, these products are expected to intensify chemical reactivities sensitive to exposed surface sites [84].

4.3. Environmental Fate of Nanomaterials in Air

The processes through which ultrafine particles in the air are lost have been clearly defined by numerous studies[75,85]. However, several pressing difficulties must be resolved to reveal the mechanisms that control their behavior, movement, and destiny[86]. In aerosol systems, nanoparticles will be very mobile and mix quickly. Unlike the other environmental compartments, engineered nanoparticles suspended in air will probably be exposed to sunlight, especially UV wavelengths, to a considerably larger amount[87]. It widens the range of photochemical changes. Additionally, the gravitational settling velocity, which is inversely proportional to particle diameter, influences the deposition of nanoparticles in the air. Smaller nanoparticles in the air deposit much more gradually than larger ones. Agglomeration, as a result, will greatly enhance the deposition of engineered nanomaterials. Other processes are considered significantly less significant or even inappropriate for nanomaterials in the air compared to photochemical reactions, aggregation, and deposition [84,88]. Understanding possible nanomaterial sources and their degradation, transformation, and existence is necessary to comprehend the fate and behavior of nanomaterials in the environment. Different outcomes for nanomaterials in the environment are anticipated depending on their physical and chemical characteristics, the medium in which they are contained, and interactions with other environmental pollutants. The three main sources of atmospheric nanomaterials are as follows: Specifically, there are three types of emissions: (1) primary emissions, which are defined as those that are outwardly released from industrial combustion and road traffic exhaust; (2) secondary emissions, which are defined as those that are produced in the atmosphere by the compression of low-volatility vapors from atmospheric gas oxidation; and (3) formation during diesel exhaust dilution[89]. Due to a lack of techniques that can separate manufactured nanomaterials from background concentrations from other sources, comparable to the situation in aquatic and terrestrial settings, there needs to be data on engineered nanomaterials in the atmosphere[89]. According to the literature, there are many processes that fine, ultrafine, and nanomaterials can go through in the atmosphere[90,91]. Some nanomaterials can be created by condensing low-volatility chemicals. They can be shrunk by evaporating adsorbed water or other volatiles, causing a departure in the particle size distribution but not the overall numerical concentration. Nanomaterials in the atmosphere can mix to produce larger particles while having a lower numerical concentration[92]. Dry and wet deposition, which may remove incredibly small particles of natural origin and presumably create nanomaterials, are other methods for removing nanoparticles from the atmosphere. As a result, particle number concentration falls, and the particle size distribution changes to bigger sizes[93].

4.4. Environmental Fate of Nanomaterials in Water

Aggregation and disaggregation, diffusion, the interaction of nanoparticles with natural water components, transformation, biotic and abiotic degradation, and photoreaction can all impact how nanomaterials behave in aquatic environments [64]. The destiny and behavior of manufactured nanomaterials released into the aquatic environment can be understood by referring to the existing literature on the fate and behavior of naturally occurring colloidal particles. Nanomaterials are currently highly suggested for wastewater treatment due to their outstanding features. Although certain studies have documented the numerous advantages of nanotechnology in wastewater cleanup, more research needs to be done on the fate and potential effects of the solid residues that these technologies produce[94]. The impact of particle size and coating material on these behaviors were examined in studies on the aggregation and sedimentation kinetics of citrate- and polyvinylpyrrolidone-coated silver nanoparticles (Cit-AgNPs) in calcium chloride ($CaCl_2$) solutions. Cit-AgNPs aggregated quickly and settled as the ionic strength increased[95], whereas PVP-AgNPs did not[95], due to the PVP coating's steric hindrance effects[95], even at an ionic strength of 10 mM $CaCl_2$[95]. It is interesting to note that PVP-AgNPs did not aggregate during the first week of sedimentation, and this propensity is influenced by particle size. These results suggest that the coating material type and particle size significantly impact how nanoparticles behave in water[95]. In addition, nanoparticles may interact with aquatic life and have detrimental consequences at different levels of biological organization. Despite a recent study of the ecotoxicological concerns that ENMs may pose to aquatic creatures[96–99], Their biological danger and mode of action are still unknown. Due to interactions with natural organic matter, natural colloids, and suspended particulate matter, nanoparticles in aquatic settings may aggregate and perhaps silt from the solution. Sedimentation and aggregation may aid in the movement of nanoparticles from the water column to benthic sediments. In addition, depositing and filter-feeding species in aquatic habitats bioaccumulate nanoparticles. Since there are no reliable and sensitive analytical techniques for identifying and characterizing nanoparticles in complex environmental matrices such as natural fluids and soils[100], although such interactions have not yet been well researched, they may have a considerable impact on the destiny and toxicity of nanoparticles.

4.5. Environmental Fate of Nanomaterials in Soil

A layered food web structure and a complex interface between gases, solids, water, organic and inorganic substances, and living things are matriculated by soil. Because they are so small, nanomaterials can pierce soil pores[101]. They can become immobilized because dirt particles adhere to their enormous surface area[102]. Sedimentation, filtration, or straining can be used to immobilize large aggregates of nanomaterials in smaller pores[101]. In the natural porous environment, there are

currently limited reports on the movement and destiny of nanomaterials. Reports state that the transfer speed depends on the kind of nanomaterials employed[103,104]. While most nanoparticle toxicity mechanisms are unknown, some probable causes include membrane rupture or membrane potential, protein oxidation, genotoxicity, interruption of energy transmission, creation of reactive oxygen species, and release of hazardous components[105]. High surface area to volume ratios, surface charges, hydrophobic and lipophilic groups enabling them to interact with proteins and membranes, complementary effects of nanostructures that inhibit enzyme activity, bioaccumulation, and increasing chemical composition their reactivity could all contribute to these toxicity mechanisms[106]. Polymers and surfactants improve the transport of nanoparticles. Numerous researchers are examining the part that natural organic matter plays in transport assisted by nanoparticles. The soil matrix's characteristics may influence the diffusion and mobility of nanoparticles. The physical-chemical features of the nanoparticles, the characteristics of the soil and environment, and the interaction of the nanoparticles with naturally occurring colloidal material all affect how mobile they are in soils. Table 3 lists some of the current ENPs along with their impacts on human health and the environment.

Table 3: List of some existing ENPs and their health and environmental effects

Nanoparticle	Environmental effects	Health effects
Carbon nanotubes	cause indirect impacts when in contact with environmental organisms' surfaces; harm the environment	Apoptosis, lowered cell viability, lung toxicity, oxidative stress, slowed cell growth, skin irritation, etc.
Fullerenes	Effects on aquatic ecosystems, soil organisms, enzymes, and chemical binding to fullerenes may impact the toxicity of other environmental pollutants.	Some examples are reduced cell viability, oxidative stress, apoptosis, and delayed cell growth.
Heterogeneous nanostructures	Numerous physical, chemical, and environmental factors, including ecosystem harm, affect toxicity.	Cellular growth arrest, and occasionally even cell death, chromatin condensation, and free radical production
Nanosilver	being released into the environment, it passes through various changes and manifests negative effects.	Non-specific immune system changes, altered cell signaling, apoptosis, cell necrosis, oxidative stress, etc.
Nanostructured flame retardants	persistent and have a propensity to build up in the environment, harmful to wildlife, flora, etc.	Cardiovascular effects, fibrosis, oxidative stress, cytotoxicity, carcinogenic, etc.
Polymeric nanoparticles	Environmental exposure risk factor potential	Oxidative stress, inflammation, changes in the shape and operation of cells, etc.
Silicon-based nanoparticles	Potentially dangerous environmental exposure factors, detrimental ecosystem impact, etc.	Heart problems, cytotoxicity, a rise in oxidative stress, etc.
TiO2 nanoparticles	Stress photosynthetic organisms and the carbon and nitrogen cycles in an aquatic habitat.	In humans, excessive exposure may lead to increased oxidative stress, slowed cell growth, minor lung abnormalities, etc.

5. NANO–BIO INTERFACE AND NANOTOXICOLOGY

5.1. Nano—bio interface

Research in numerous fields of nanotechnology has mostly centered on proteins and nucleic acids[107–111]. Compared to a 10 nm nanoparticle, a single cell, which is generally tens of microns, is immense (Fig 3). To research biological processes, including medication transport and cellular-level bioimaging, scientists worldwide have been using a variety of inorganic, organic, and composite nanoparticles[112–116]. Many publications have recently examined the relationship between a protein and a nanoparticle[117–119]. Compared to a 10 nm nanoparticle, the APP and a tiny therapeutic molecule (such as DHED) are incredibly small, making it challenging to probe biologically significant nanoparticle molecules. In truth, a nanoparticle put into a live system will interact with the environment endless times, regardless of size. Studies on the interface between biological systems and nanostructured materials, starting with proteins and moving up to the cell, will be a significant step forward in understanding bio-systems important to pharmacology, pharmacology, and medicine.

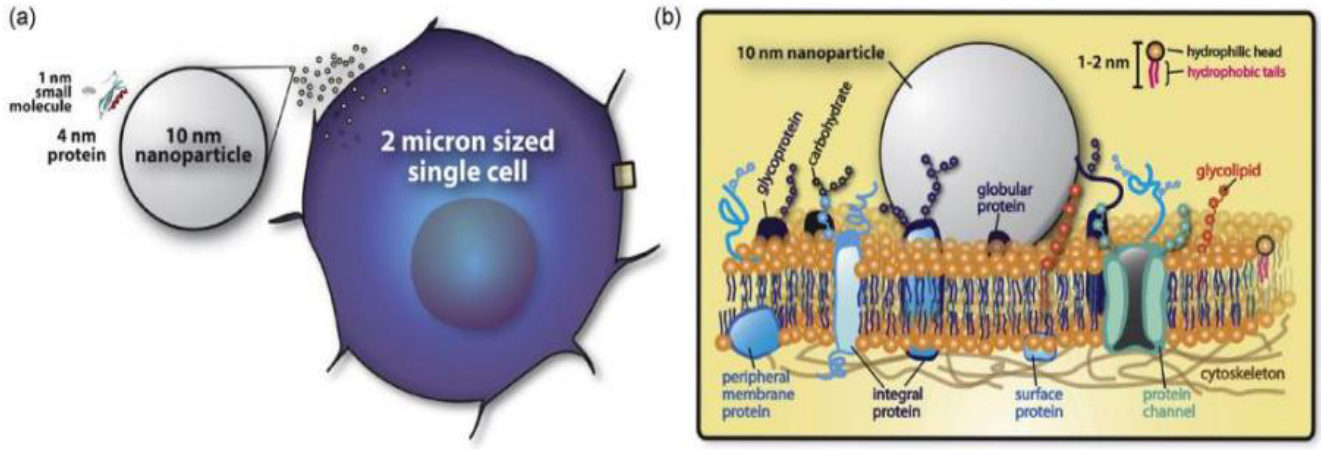

Fig 3: Compared to a 10 nm nanoparticle, proteins (e.g., APP; X-ray crystal structure obtained from www.pdb.org (Berman et al., 2000), protein ID 2FKL; visualization done by Accelrys Discovery Studio Visualization 1.7 software) and small molecules (e.g., DHED) are small in size and volume. A mammalian cell of proteins, nucleic acids, and other small to large molecules is a thousand times larger in volume and size compared to a 10 nm nanoparticle. (b) Cell membrane incorporating various proteins and a single 10 nm nanoparticle.

Image adapted from Viswanath, B. & Kim, S. (2016). Influence of Nanotoxicity on Human Health and Environment: The Alternative Strategies [Image]. doi: 10.1007/398_2016_12

Studying the bio-nano interface is a completely different undertaking because there are no straightforward tools for probing the interaction in real-time or in situ. On the other hand, nanotoxicology, which is the study of the bioeffects of nanomaterials, is a rapidly expanding discipline with some immediate use. Recent years have seen a significant increase in studies into the toxicity of nanomaterials on the environment and living systems. For instance, the University of California has a robust nanotoxicology program led by UCLA and UCSB as part of its UC Toxic Substances Research and Teaching Program (http://www.cnsi.ucla.edu/staticpages/education/nanotox-program; http://www.bren.ucsb.edu/news/press/nanotoxicology.htm). For the first time in US history, Berkeley (CA) has chosen to control nanotechnology through the law, with UC Berkeley and LBNL (Lawrence Berkeley National Laboratory) involved in various nanotech[120]. The International Council on Nanotechnology (ICON) and the Center for Biological and Environmental Nanotechnology (CBEN) at Rice University are both aiming to compile a database of materials based on nanotechnology (http://cben.rice.edu/; http://icon.rice.edu/). NCL (Nanotechnology Characterization Laboratory), run by a chemist specializing in nanomaterials with dimensions less than 100 nm, was recently established as a separate organization by the National Cancer Institute (NCI). Internationally, Singapore's IBN (Institute of Bioengineering and Nanotechnology), run by A*STAR, is a multidisciplinary research park that merges the study of biological systems at the nanoscale scale. The fact that a materials scientist serves as the organization's head suggests that IBN focuses more on the materials it creates, which will help the transition from nanotechnology to biotechnology. From the perspective of both the material and the biological system, a basic understanding of nanomaterial toxicity (nanotoxicology) is highly desired. Toxicology assessments of nanoscale materials should attract greater attention than ever from the general public, the government, or those involved in nanomaterial development, with the rising commercialization of goods ranging from tennis balls to cosmetics[121–124]. The knowledge gained from these studies on nanotoxicology should assist scientists in making better decisions on the kind of nanomaterial that can be utilized to investigate, for instance, the synaptic plasticity of a neuron. To do this, we will examine the literature on the development of nanotoxicology and offer a few tables to help with material selection. With the available data, however, it is usually challenging to determine the toxicity of particular nanomaterials since, like any tiny molecule (such as a medicine), toxicity is dose, exposure, and route dependent. Furthermore, it is impossible to predict the effects of nanotoxicology on humans just from investigations on cultured cells or animals.

5.2. Nanotoxicology

Different forms of artificial nanomaterials currently exist due to businesses' and academics' unprecedented and intensely focused efforts in recent years. Over 3200 papers were published exclusively on producing nanostructured materials between 2006 and 2007, an exponential rise (Figure 4). This enormous rise in publications has led to the release of hundreds of in vitro toxicology research[124–130], as well as countless evaluations and viewpoints[121–123,131–138]. Contrarily, in vivo, toxicology needs the test subject to internalize the test sample, whether a little mouse or a large creature like a dog or a monkey. This method examines toxicity (i.e., LD50, pathophysiology) through inhalation, injection, and oral digesting. However, given the extensive use of synthetic engineering, testing the toxicity of nanomaterials on whole animals is challenging[139–146] is carried out extremely specifically by various research groups, and access to proprietary information on synthesis—especially from the industry—can be challenging. Additionally, setting up, carrying out, and controlling an in vivo test is a difficult ethical and administrative task. Individual research initiatives must work with institutional approval organization(s) like IACUC (Institutional Animal Care and Use Committees).

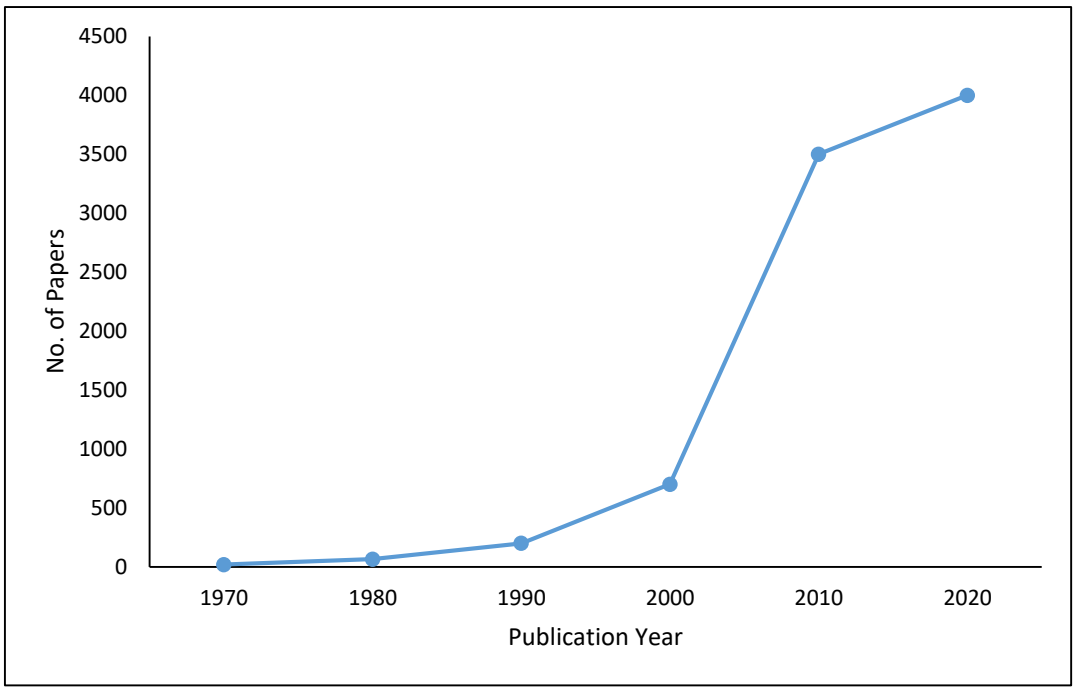

Fig 4: The number of papers published solely on the synthesis of nanostructured materials (According to Web of Science Search Results)

6. DYNAMIC BEHAVIOR OF NANOMATERIAL AND APPLICATION IN NANOMEDICINE

The application of nanomaterials in medical field purposes in the form of nanomedicine: which has three different areas in it is a diagnosis that is mainly known as nano-diagnosis, the second one is controlled drug delivery, also known as monotherapy, and the last one is regenerative medicine. A new area of the medical world that mainly combines diagnostics and therapy, termed theragnostic, is emerging and is a promising approach that holds in both systems, which are the same that are diagnosis/imaging agents and the other is medicine. The promise held by nanomedicine is the changes in clinical practice through the introduction of novel medicines for both diagnosis and treatment, which has enabled to address the of unmet medical needs (a)by integrating effective molecules that otherwise could not be used due to having the high toxicity (e.g., Mepact), (b) by exploiting multiple mechanisms of actions (e.g., Nanomag, multifunctional gels) (c) by maximizing efficacy (e.g., by increasing bioavailability) and also by reducing the dose and the toxicity, (d) by providing drug targeting, controlled and site-specific release, and by favoring a preferential distribution within the body (e.g., in areas with cancer lesions) and that is improved the transportation across biological barriers[147]. The result of the intrinsic properties of nanomaterials has brought so many advantages to the development of the pharmaceutical world. Because of the small size of the nanomaterials or nanoparticles, it has a high specific surface area about the volume. Therefore, the surface energy of the particle is increased by making the nanomaterials much more reactive. The absorbance characteristics of the nanomaterials towards the biomolecules, e.g., protein and lipids, have a large tendency when it is in contact with the biological fluid. Important interactions with living matter mainly rely on the plasma/serum biomolecule adsorption layer, known as "corona," which mainly forms on the surface of the colloidal nanoparticles[148]. Its composition mainly depends on the portal of entry into the body and on the particular fluid from which the nanoparticle comes, which may be blood, lung fluid, gastrointestinal fluid, etc. Changes in "corona" can be influenced by additional dynamic changes that constitute the nanoparticle crosses from one biological compartment to another one[149]. Besides that, the optical, electrical, and magnetic properties also can be changed and harmonic by the electron confinement in the nanomaterials. In addition, nanomaterials can also be engineered to have different sizes, shapes, chemical compositions, and surfaces, and they can interact with specific biological targets[138]. By restoring careful particle design, we will get a successful biological outcome. For these reasons, comprehensive knowledge of the interactions between nanomaterials and biological systems is required. Among of two, the first one is related to the physiopathological nature of the diseases. The main biological processes behind the diseases occur at the nanoscale and can rely on, e.g., mutated genes, misfolded proteins, viral infection, or bacterial infection. Understanding of the molecular processes will be provided with the rational design of engineered nanomaterials to target the specific action site that is mainly desired site of action in the body[150]. Another concern is the interaction between the environment of the biological fluids and the nanomaterial or nanoparticle surface. In the context of characterization of the biomolecules, the corona is of the uttermost importance for understanding the mutual interaction between nanoparticle and cell called nanoparticle-cell affects the biological responses. This intersection mainly comprises dynamic mechanisms involving the exchange between biological components' surfaces, e.g., proteins, membranes, phospholipids, vesicles, organelles, and the nanomaterial or nanoparticle surfaces. The interaction stems from the composition of the suspending media and the nanomaterial. The size, shape, surface area, surface charge, chemistry, energy, roughness, porosity, valence, conductance states, the presence of ligands, or the hydrophobic/hydrophilic character are some characteristics of the nanomaterials that influence the respective surface properties. In addition, the presence of water molecules, acids and bases, salts, and multivalent ions will influence the interaction. All these aspects will govern the characteristics of the interface between the biological components and nanomaterial and promote different cellular

fates[150]. A piece of deeper knowledge of how the physicochemical properties of the bio interface influence the cellular signaling pathway and kinetics and transport will thus provide critical rules that design the nanomaterials[151].

7. CONSIDERATION OF DOSE IN PERSPECTIVE OF NANOMEDICINE

The COVID-19 pandemic reminds us that we need high-value flexible solutions to urgent clinical needs, including simplified diagnostic technologies suitable for use in the field and for delivering targeted therapeutics[152]. Nanotechnology is an important resource for this, as a generic platform of technical solutions to tackle complex medical challenges[153]. Even though there are more than 50 formulations currently on the market, and the recent approval of 3 key nanomedicine products (e.g., Onpattro, Hensify, and Vyxeos), has revealed that the nanomedicine field is concretely able to model products that overcome critical barriers in conventional medicine in a special unique manner[154], and also to deliver within the cells new drug-free therapeutic effects by using pure physical modes of action and therefore make a difference in patients' lives[154]. One major advantage of nanomedicines as designed objects over other medicinal products is their high level of uncoupling between their functional requirements and their design parameters (nanoparticle & drug, for instance), described by the general theory of axiomatic design by P Suh in the 1990s[154]. However, it is often claimed that nanomedicine failed to meet the initial expectations in drug delivery since less than 2% of the active pharmacological ingredient (API) is locally released, e.g., in cancer treatment in the tumoral tissues[152]. On the other hand, Abraxane demonstrates a significantly higher response rate, longer time to tumor progression, and absence of hypersensitivity reactions[155]. Nanotechnology also expurgates transdermal delivery, a safe, noninvasive method of administering drugs[156]. Applied directly onto the skin, transporting large-molecular weight proteins like vaccines across the skin is relatively inefficient. Recent evidence has shown that this barrier can be covered by properly structured nanosized particles[156]. Nanoparticles can also provide an efficient delivery tool for drugs bypassing the blood-brain barrier, such as chemotherapeutic agents for brain malignancies, antiepileptics, and anesthetics (e.g., Dalargin)[157]. For example, Polysorbate 80-coated nanoparticles loaded with doxorubicin (5 mg/kg) achieved high brain levels of 6 μg/g brain tissue. In contrast, all the controls[157], including uncoated nanoparticles and doxorubicin solutions mixed with polysorbate, did not reach the analytical detection[157].

8. NANOTOXICOLOGICAL CLASSIFICATION SYSTEM

Hitherto, different risk assessment approaches have been reported. The DF4nanoGrouping framework concerns a functionality-driven scheme for grouping nanomaterials based on their intrinsic properties, system-dependent properties, and toxicological effects[158]. Accordingly, nanomaterials are categorized into four groups, including possible subgroups[13]. The four main groups encompass (1) soluble, (2) persistent high aspect ratio, (3) passive, that is, nanomaterials without obvious biological effects, and (4) active nanomaterials[13], that is, those demonstrating surface-related specific toxic properties. The DF4nanoGrouping foresees a stepwise evaluation of nanomaterial properties and effects with increasing biological complexity[13]. In case studies that include carbonaceous nanomaterials, metal oxide, metal sulfate nanomaterials, amorphous silica, and organic pigments (all nanomaterials with primary particle sizes smaller than 100nm), the usefulness of the DF4nanoGrouping for nanomaterial hazard assessment has already been established[13]. It facilitates the grouping and targeted testing of nanomaterials. It also ensures that enough data for the risk assessment of a nanomaterial are available and fosters the use of non-animal methods[159]. More recently, DF4nanoGrouping developed three structure-activity relationship classification decision tree models by identifying structural features of nanomaterials mainly responsible for the surface activity based on a reduced number of descriptors: one for intrinsic oxidative potential, two for protein carbonylation, and three for no observed adverse effect concentration[160]. Keck and Müller also proposed a nanotoxicological classification system (NCS) (Figure 5) that ranks the nanomaterials into four classes according to the respective size and biodegradability[161]. Due to the size effects, this parameter is assumed as truly necessary because when nanomaterials are getting smaller and smaller, there is an increase in solubility[13], which is more evident in poorly soluble nanomaterials than in soluble ones[13]. The adherence to the surface of membranes increases with the decrease in size, and another important aspect related to the size that must be considered is the phagocytosis by macrophages[13]. Above 100 nm, nanomaterials can only be internalized by macrophages, a specific cell population, while nanomaterials below 100nm can be internalized by any cell due to endocytosis[13]. Thus, nanomaterials below 100nm are associated with higher toxicity risks than nanomaterials above 100 nm[161]. Biodegradability was considered a required parameter in almost all pharmaceutical formulations[13]. The term biodegradability applies to the biodegradable nature of the nanomaterial in the human body[13]. Biodegradable nanomaterials will be eliminated from the human body[13]. Even if they cause inflammation or irritation, the immune system will return to its regular function after elimination[13]. Conversely, non-biodegradable nanomaterials will stay forever in the body and change the normal function of the immune system[161]. Two more factors must be considered besides the NCS: the route of administration and the biocompatibility surface[13]. When the NCS13 classifies a particle, toxicity depends on the route of administration. For example, the same nanomaterials applied dermally or intravenously can pose different risks to the immune system[13]. In turn, a non-biocompatibility surface (NB) can activate the immune system by adsorption to proteins like opsonins[13], even if the particle belongs to class I of the NCS (Figure 5)[13]. The biocompatibility (B) is dictated by the physicochemical surface properties, irrespective of the size and biodegradability[13]. It can lead to a further subdivision into eight classes I-B, I-NB, IV-B, and IV-NB[161]. NCS is a simple guide to evaluating the risk of nanoparticles, but many other parameters play a relevant role in nanotoxicity determination[161]. Other suggestions encompass more general approaches, combining elements of toxicology, risk assessment modeling, and tools developed in multicriteria decision analysis[162].

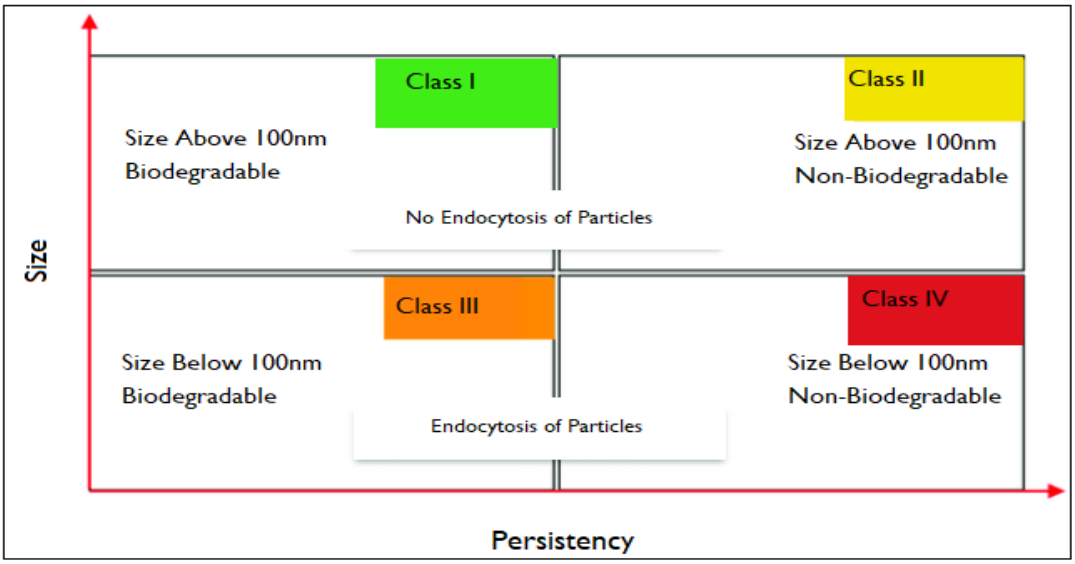

Fig 5. Nanotoxicological Classification

9. TOXIC EFFECTS OF NANOPARTICLES ON SYSTEMS

Experimental studies have demonstrated that nanoparticles harm numerous systems by entering the body in three ways. This section largely uses animal experiments to describe the harmful effects of nanomaterials on systems.

9.1. Circulatory system:

Nemmar et al. found that intravenous administering iron oxide nanoparticles to mice caused DNA damage and myocardial oxidative stress[163]. Magaye et al. discovered cardiac toxicity-arrhythmia and toxic effects in organs such as the liver, spleen, and lung in a study of rats receiving intravenous Ni nanoparticles[164].

9.2. Digestive system

Zirconia oxide nanoparticles at 100 ppm induce liver injury in rats, claim Arefian et al. [165]. Mice's liver is likewise harmful to iron oxide nanoparticles.[164].

9.3. Endocrine system

Oral iron oxide nanoparticles have been linked to abnormal thyroid hormone levels in rats, according to Yousefi et al. [164].

9.4. Immune system

According to Xu et al., Ti02 nanoparticles significantly increased the number of white blood cells in mice[166]. Additionally, white blood cell production is increased by iron oxide nanoparticles, with the liver and spleen being the most immunologically impacted organs[167].

9.5. Respiratory system

According to Cai et al., the lungs become hazardous when metal nanoparticles (cobalt oxide, nickel oxide, and titanium oxide) are delivered via oropharyngeal aspiration.[168] Iron oxide nanoparticles have also been linked to pulmonary damage in rats, according to Sadeghi et al. [169].

9.6. Urinary system

According to Saranya et al., kidney cells in monkeys, pigs, and cattle are toxic to zinc oxide, iron oxide, and copper nanoparticles[170]. Furthermore, TiO2 nanoparticles administered intraperitoneally to rats result in kidney deterioration, according to Fartkhooni et al. [171].

9.7. Nervous system

When vision and hearing toxicity in animal ears and eyes were investigated, very little or no harm was discovered overall[172,173].

9.8. Reproductive system

Zinc oxide nanoparticles were administered intraperitoneally to mice, and Mozaffari et al. found that this resulted in a loss and reduction of seminiferous tubule cells[174]. According to Kong et al., nickel nanoparticles affect rat sperm motility and FSH and LH hormone levels[175].

10. TOXICITY MECHANISMS OF NANOPARTICLES

The mechanical impacts brought on by the physicochemical characteristics of nanoparticles are what induce toxicity. Reactive oxygen species (ROS) are produced directly or indirectly, which is the fundamental process of creating hazardous effects. In vitro, ROS production is harmful via a variety of cell pathways[176,177]. In mitochondria, ATP is produced due to the conversion of molecular oxygen to water. During this process, superoxide anions and radicals with various oxygens are generated. Hydroxyl radicals, single oxygen radicals, hydrogen peroxide radicals, and superoxide anion radicals are some ROS generated[177]. Overproduction of free radicals, which interfere with cellular signaling and the mitogenic response in cells, causes damage to their physiological activities[178,179], resulting in cell disruption. Nanomaterials affect cells in cytotoxic and genotoxic ways (Figure 6). Nanomaterials have modest dimensions, but because of their high surface reactivity and specific surface area, they emit more ROS[180].

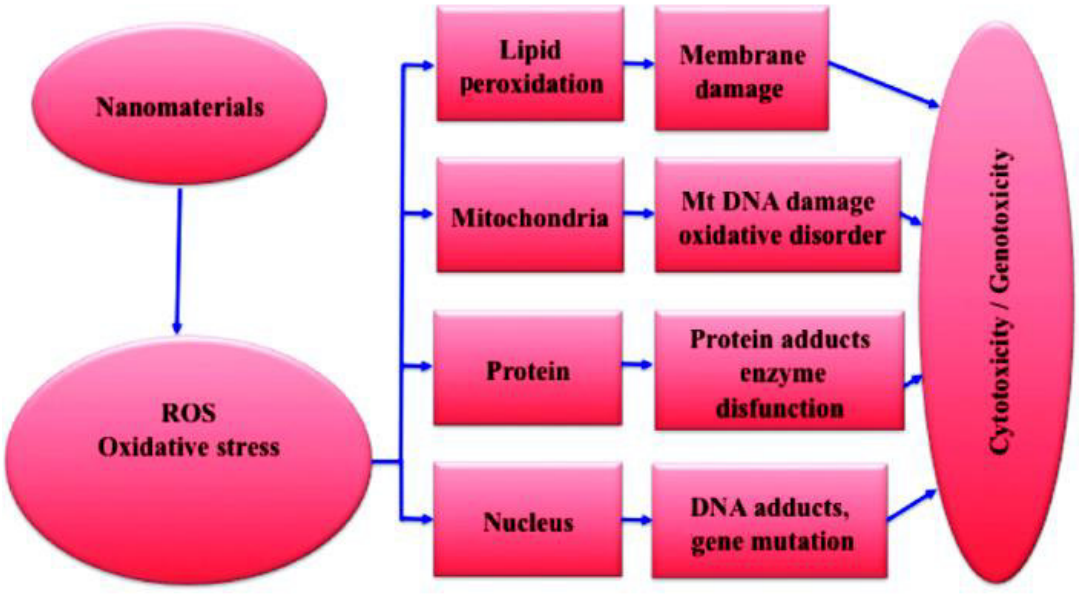

Fig 6: ROS and nanomaterial toxicity

Image adapted from Viswanath, B. & Kim, S. (2016). Influence of Nanotoxicity on Human Health and Environment: The Alternative Strategies [Image]. doi: 10.1007/398_2016_12

Studies in living tissues, including human erythrocytes and skin fibroblasts, have shown that different nanomaterials can be hazardous by activating ROS[124]. Kim et al. claim that nano-Ag produces genotoxicity and oxidative stress in cultured live tissue. Nano-Ag causes mutations in mice by boosting the generation of ROS, according to Mei et al. [181,182]. Hsin et al. claim that nano-Ag activates ROS in the mitochondrial pathway to induce cytotoxicity[183]. According to Akhtar et al., nano-CuO lipid peroxidation and ROS generation from silica nanoparticles cause cytotoxicity in cell membranes and mouse embryonic fibroblasts[184,185]. According to Girgis et al., nano-Au toxicity in mice was brought about by increased oxidative stress[186]. Shvedova et al. claims that keratinocytes and bronchial epithelial cells are cytotoxic to single-walled carbon nanotubes, forming ROS and mitochondrial dysfunction[187]. According to Winnik and Maysinger, quantum dots cause cytotoxicity by boosting ROS production[188]. According to reports, nano-ZnO damages human bronchial epithelial cells by causing them to produce more ROS[189]. When the cytotoxic effects of nano-Ti02, Co3O4, ZnO, and CuO in hepatocyte cells were evaluated, it was observed that nano-CuO had the highest cytotoxic effect. Nano-FeO was shown to have a cytotoxic effect via enhancing ROS production and apoptosis[190,191]. Nanomaterial toxicity is affected by various parameters, including surface area, surface coating, molecular size and shape, oxidation status, solubility, and the degree of aggregation and agglomeration[192]. It has been found that the size of the nanoparticles directly affects how dangerous they are. Amorphous nano-silica is hazardous to human cells, according to Yoshida et al., since it increases the production of ROS and damages DNA[38,193]. Additionally, based only on size, nanoparticles are more harmful to organs the smaller they are[194]. According to studies, the formation of ROS by wire-shaped nanoparticles damages DNA and has harmful effects[195]. Studies on the relationship between nanomaterial shape and toxicity have found that the shape does not significantly affect the toxicity of nano Au in human skin keratinocyte cells[196]. In contrast, hexagonal crystals are more hazardous than rod-shaped crystals, according to a study on nano-ZnO crystals[197]. Biocompatibility and nanoparticle contact area are closely proportional. In a study of zebrafish embryos, Ispas et al. found that dendritic zebrafish embryos were more hazardous than spherical ones[198]. A typical nanomaterial utilized in medication delivery systems is silica. Nano-silica has various harmful effects at different pore volumes[198]. The cationic-charged nano silica-titanium particles are extremely poisonous, according to Oh et al. [188,199]—studies on the dimensions, form, and association of the surface components of quantum dots with nanotoxicity[188,199]. In investigations on the toxicity of fullerene, the groups attached to the surfaces of these nanoparticles are crucial. Given that fullerenes are thought to produce free oxygen radicals, which are thought to cause cytotoxicity, there are fullerenes with antioxidant activity by adding malonyl groups to their surface[192]. The impact of a nanomaterial's solubility on toxicity has been studied. ZnO nanoparticles are less hazardous than soluble copper metal, claim Studer et al. [199]. Shen et al. found that dissolving nano-ZnO cells is useful for bringing about the cytotoxic impact[200]. According to Mahto et al.[201], When quantum dots are dissolved in water, more ROS are produced, which results in cytotoxicity. Nano-TiO2 and nano-ZnO materials are negatively impacted by UV and visible light. It is how toxicity is caused by photoexcitation using electrons[202]. Studies on the toxicity of graphene and aggregation have been carried out in various biological sectors, including drug delivery systems, biosensors, and labelling[203]. In addition, Kim et al.[181] highlighted the significance of aggregation and accumulation in the toxicity caused by nano-Ag. It is still being researched in toxicity tests on various organisms, including plants, rodents, and people. In engineering, metallic and carbon nanomaterials are frequently used in various applications. Additionally, metal nanoparticles are frequently applied in food, medicine, and cosmetics[204]. Depending on how often they are used, sun creams and lotions containing nano titanium and nano zinc can harm the skin and the environment[205]. Researchers have shown that carbon nanotubes harm cells and that nano copper oxide is effective in cytotoxicity and DNA damage[206,207].

10.1. Effect of Metal Oxide Nanoparticles in Zebrafish

Table 4: Properties and applications of mostly used metal oxide nanoparticles

Metal oxide nanoparticles	Physical and chemical properties	Potential applications in medicine (tested *in vitro*/*in vivo*)	Biomedical and applications in life science (in use and commercial products)
Aluminium oxide[208] (Al_2O_3)	high melting point, strong corrosion resistance, high melting stability, and high thermal and mechanical stability.	Drug delivery.	—
Copper oxide[209,210] (CuO)	Catalyst and high-temperature superconductorsClick or tap here to enter text.	Anticancer treatment.	Antimicrobial coating agents.
Iron oxide [209,211](α-Fe_2O_3, γ-Fe_2O_3, and Fe_3O_4)	Superparamagnetic and magnetic hyperthermia properties, catalyst.	Antibacterial agent, drug delivery, anticancer treatment (photothermal therapy, chemotherapy, and magnetic hyperthermia therapy), theragnostic (near-infrared imaging and positron emission tomography, single-photon emission computed tomography, and ultrasound imaging).	Iron-deficient anemia treatment (Venofer®, Feraheme®, and Rienso®). Solid tumor treatment (NanoTherm®). Magnetic resonance imaging (in liver: Feridex I.V.®, Endorem®, and Resovist®; in gastrointestinal: Gastromark™ and Lumirem®; and in blood pooling: Supravist®).
Magnesium oxide[208,209] (MgO)	High ionic character, catalyst, and semiconductor.	Antibacterial agent and anticancer treatment (hyperthermia therapy) and tissue engineering.	Antimicrobial agents (in the food industry).
Nickel oxide[210] (NiO)	Catalyst, magnetic properties, and high electrochemical stability.	Anticancer treatment (cytotoxic properties).	—
Silica dioxide [212](SiO_2)	Low density.	Antibacterial agent, drug and gene delivery, anticancer treatment, and biosensor.	Additive in drugs and cosmetics.
Titanium oxide[213] (TiO_2)	Semiconductor, photocatalyst, and high chemical stability.	Anticancer treatment (photodynamic, photothermal, so no dynamic therapy, chemodynamic therapy, and radiotherapy), theragnostic (bioimaging), drug delivery, and tissue engineering.	UV-A and UV-B radiation filters (in sunscreens, cosmetics). Antimicrobial agents (in food packaging and biomedical devices and dentistry & orthopedic implants).
Zinc oxide[214](ZnO)	In semiconductor, photocatalyst has high chemical stability, large exciton binding energy, and high isoelectric point.	Anticancer treatment (photodynamic, photothermal, and sonodynamic therapy), theragnostic (bioimaging), drug delivery, and tissue engineering.	UV-A and UV-B radiation filters (in sunscreens, cosmetics). Antimicrobial agents (in toothpaste, dental implants, food packaging, and as a food additive).

Table 5: Impact of IO NPs on zebrafish

Stage	NP diameter	Treatment time	Tested concentrations	General toxicity response	Specific ROS responses
Embryos[215]	22 nm	144 h	0.3; 0.6; 1.25; 2.5; 5; and 10 mg/L	High mortality rate and cardiotoxicity (reduction of heartbeat rate) and morphological alterations.	—
Embryos[216]	6-12 nm	120 hpf	SP IONs, S PION-DX, SP ION-CS,	SP ION-CS: reduced survival rate, SPI ON-	—

			SP ION-T, SPI ON-T-PEG, SP ION@SiO_2: 0.125 mM, 0.5 mM, 2.0 mM, and 8.0 mM	CS, and SP ION@SiO_2 delay in hatching rate; SP ION-DX, SP ION-T-PEG, and SP ION-T: slightly premature hatching; SP ION-CS and SPI ON@SiO_2: reduction in locomotor activity; and SP ION-CS, SP ION-T-PEG SP ION@SiO_2 reduction in escape behavior.	
Embryos[217]		168 hpf	0.1, 0.5, 1 and 5, 10, 50, and 100 mg/L	Mortality concentration and exposure time-dependent; LC50 = 53.35 mg/L; delay in hatching rate, LC50 = 36.06 mg/L; and different malformations (pericardial edema and tissue ulceration and body arcuation).	—
Embryos[218]	40 nm	96 h	Fe_3O_4 NPs: 100-800 μg/mL bare Cr@Fe_3O_4: 5, 150, 300, and 600 mg/mL	Fe_3O_4 NPs: dose- and time-dependent delay in hatching rate; slight decrease in embryo viability; Cr@Fe_3O_4: NPs high mortality in 2-week-old larvae; dose-dependent accumulation in the digestive tract.	—
Embryos[219]	100-250 nm	168 hpf	1, 5, 10, 50, and 100 mg/L	LC50 = 10 mg/L; delay in the hatching rate.	—
Embryos[215]	22-45 nm	96 hpf	10, 20, 40, 60, 80, 110, 120,140 ppm	LC50 =60.17 ppm; delay in hatching rate; reduction in heartbeat rate; and increased teratogenicity.	Dose-dependent decrease of $Na+K^+$-ATPase activity; the dose-dependent increase of AChE; increased levels of lipid peroxidation ROS, PC, and NO; an increase of apoptotic bodies; and a decrease of antioxidant enzymes, CAT, SOD, and Gpx.
Embryos/adults[220]	15 nm	Embryos: 96 hpf Adults: 2 weeks	Embryos: 1, 10, 100, and 1000 ppm Adults: 1, 10 ppm	Embryos: no adverse effect observed Adults: reduced locomotor and exploration activity, increased anxiety, reduced social interaction, tightened shoaling behavior, dysregulation of circadian rhythm locomotor activity and reduction of short-term memory retention, and reduction of serotonin and dopamine.	Increased CAT, cortisol level in the brain; reduction of AChE activity.
Adults[221]	21 nm	7 days	100 mg/L	Bare IO NPs accumulate mainly in	Altered expression of genes involved in inflammation,

				the gills, and coated IO NPs in the liver.	immune response, oxidative stress, antioxidant response, and mitochondria in the gills of Fe_3O_4-treated fish. Upregulation in the liver of genes involved in immune and inflammation responses and downregulation of genes involved in DNA damage and repair in both exposures and different expression of genes involved in DNA damage/repair and apoptosis (*tp53*) for starch-coated NPs and upregulation of *cyp1a*; and dysregulation of genes involved in the mitochondrial dysfunction pathway.
Adults[216]	Fe_2O_3: 80-90 nm Fe_3O_4: 140-160 nm	28 days	4 and 10 mg/L	Shift in coloration, extravasated blood, and chronic toxicity in the gut.	—
Adults[222]	23 nm	48 h	20, 50, 100. 140 and 200 mg/kg	Reduction of AChE activity; impaired swimming.	Increased expression of transcriptional *jun*, *caspase-8*, *caspase-9*, *gclc*, *Gpx1a*, *CAT*, *gstp1*, and *sod2*.

Table 6: Impact of ZnO NPs on zebrafish.

Stage	NP diameter	Treatment time	Tested concentrations	General toxicity response	Specific ROS responses
Embryos[223]	20 nm	96 h	0.1, 0.5, 1, 5, 10, and 50 mg/L	Significant decrease of survival rate and delay in hatching rate dose-dependent; 96 h LC50 = 1.793 mg/L; and several abnormalities (body accusation and pericardial edema).	—
Embryos[224]	20 nm	96 hpf	0.1, 0.5, 1, 5, 10 and 50 and 100 mg/L	Decrease of survival rate and delay in hatching rate and incidence of pericardial edema dose-dependent.	Increase in ROS production, low levels of *Gstp2* and *Nqo1* expressions, and a downfall in counteracting the ROS by oxidative stress responses.
Embryos[208]	<100 nm	144 hpf	1, 5, 10, 20, 50, and 100 mg/L	No effect on the survival rate, a significant decrease in the hatching rate, and different malformations (spinal curvature and hyperemia).	Important elevation in the SOD activity and MDA levels in a dose-dependent way; decrease in CAT activity; high levels of ROS; DNA damage only at the highest concentration tested; and important downregulation in *Bcl-2*, *Nqo1*, and *Gstp2* transcriptions and upregulation in *Ucp-2* level.
Embryos[225]	30 nm	96 hpf	1, 5, 10, 25, 50, and 100 mg/L	Decrease in survival rate and increase in hatching rate dose-dependent; severe decrease in body length.	—
Embryos[214]	<100 nm	96 hpf	1, 5, 10, 20, 50, and 100 mg/L	—	Increase in the lipid peroxidation and SOD activity; upregulation in the expression of

					the *ppaα* and *sod1*; downregulation of *cat*; altered expression of antiapoptotic genes (*bcl-2*) and proapoptotic (*Bax*, *puma*, and *apaf-1*; upregulation of *p53* gene, with overexpression of its protein; and increase in the activity of caspase-3 and caspase-9.
Embryos[226]	9.4 nm	96 hpf	0.2, 1, and 5 mg/L	Dramatic delay in hatching.	Upregulation of the cat and Cu/Zn-sod transcripts in embryos and downregulation in eleuthero; important upregulation of Mt2<; different expression of mRNA of *IL-1β*, *TNFα*, and proinflammatory cytokines in eleuthero-embryos in comparison to embryos; alteration in the jun proto-oncogene (*c-jun*) embryos treated with high concentration; and perturbation in antiviral and immune-related gene Myxovirus resistance A.
Embryos[227]	50–70 nm	144 hpf	0.1, 0.5, 1, 5, and 10 mg/L	Significant delay in hatching for ZnO NPs and Zn ions; no significant difference in cotreatment with ZnO NPs and NAC; and increased rates of delay in hatching in cotreatment with BSO.	ROS generation; cotreatment with BSO: lower production of GSH.
Embryos[228]	Nanospheres: 27 nm; nano sticks: 32×81 nmM; and SMPs: 202 nm	120 hpf	2, 4, 8, 16, and 32 mg Zn/L	LC50 for Zn^{2+} =7.9 mg Zn/L, LC50 ZnO SMPs =10.0 mg Zn/L LC50 nano sticks =7.1 Zn/L LC50 nanospheres =11.9 mg Zn/L, respectively; higher toxicity of Zn ions compared to the different shaped NPs; and decrease of hatching rate dose-dependent in the embryos treated with all the different kinds of nanoparticles and sulfate, strongest delay in samples exposed to nano sticks. Decrease dose-dependent of swimming activity; nano sticks are more toxic than the other NPs.	—
Embryos[229]	5, 10, 15, 26, 34, 62, and 70 nm	120 hpf	0.016 to 250 mg/L	Significant mortality at 24 hpf for all the coated NPs; no alteration in mortality with bare nanoparticles.	—

Embryos[225]	20-30 nm	96 hpf	0.01, 0.1, 1, and 10 mg/L	Higher mortality rate by ZnO NPs than $ZnSO_4$; LC25 for ZnO NPs =2.64 mg/L; LC25 for $ZnSO_4$ = 7.75 mg/L; and significant embryonic malformations after both treatments (tail malformation, pericardial edema, and yolk sac edema).	Downregulation of *ogfrl2* and *intl2* transcripts; upregulation of *cyb5d1*.

Table 7: Some of the main physicochemical properties of nanoparticles, as well as the exposure routes and main findings on various animal models

Animal Model	Administration route and exposure time	Nanoparticle	Surface Chemistry	Size/nm	Major observations
Mouse[230]	i.p. and i.v. injection, 1, 4, 24 h	Gold	Without surface modification	2,40	Macrophage uptake in the liver is less in the spleen, small intestine, and lymph nodes.
Rat[231]	i.v. injection, 24 h	Gold	Without surface modification	10-205	NPs of 10 nm entered the testis and brain.
Mouse[232]	i.v. Injection, 0.5,2, and 24 h.	MWCNTs	Carboxylated and aminated surface	20-30 × 0.5-2 mm	Accumulation in testis.
Mouse[233]	i.v. injection, 0.17, 1, and 24 h	SWCNTs	Without or coated by paclitaxel (PTX)-polyethylene glycol (PEG)	1-3 × 100 (diameter × length)	Accumulation in liver and spleen, less in the heart, lung, kidney, stomach, intestine, muscle.
Rat[136]	Whole body inhalation 12 days	MnO_2	Without surface modification	30	Accumulation in CNS via olfactory bulb.
Pig[234]	Intradermal injection <5 min	CdTe (CdSe) core (shell) type II QDs	Oligomeric, Phosphine	10 (naked); 18.8 (coated)	Accumulation in the sentinel lymph node.
Rat[235]	Gavage	Polystyrene microspheres	Without surface modification	50, 100, and, 300	Accumulation in the liver and spleen via lymph.
Mouse[236]	Intranasal instillation, 2, 10, 20, and 30 days	TiO_2	Without surface modification	10, 25, and 60	Accumulation in brain through the olfactory bulb.
Hairless Mouse[237]	Dorsal skin expos	TiO_2	Hydrophobic or hydrophilic surface	80, 155	Accumulation in the spleen, lung, kidney, and brain.

11. TOXICITY TESTING

Dosing concerns are crucial in determining toxicity, and in vitro, tests are more frequent than in vivo research. One of the models utilized in the toxicity test is the in vitro sedimentation diffusion and dosimeter. This model's core concept is the fundamental separation between exposure (concentration in the cell environment), dose accumulated on the cell surface, and cellular dosage. By being aware of how long it takes for a given dose to be released, we may assess the dose rate as a predictor of response[62]. Because in vitro techniques that assess cell viability and proliferation are widely used, gene expression analysis, genotoxicity detection, and in vitro hemolysis are also used to diagnose toxicity. Additional techniques for assessing the physicochemical structure of the cell include scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM-EDX), transmission electron microscopy (TEM), atomic force microscopy (AFM), video-enhanced differential interference contrast (VEDIC) microscopy, and fluorescence spectroscopy. The combination of these tests makes it simpler to identify nanotoxicity[238]. Current toxicity experiments, their intended use, and the tested nanomaterials are all summarised briefly in the table below.

Table 8: A summary of literature-related toxicity tests of nanomaterials.

Toxicity test	Purpose	Nanomaterials
Transmission electron microscopy	Determination of intracellular localization	TiO2, silver, fullerene[239–241]
Light microscopy	Physicochemical properties	Singled walled carbon nanotubes, silver[240,242]
Hemoglobin estimation	Homolysis	SiO2[243]
Micronucleus test	Genotoxicity	Different types of nanoparticles[244]
Commet assay test	DNA damage	Metal, metal oxide nanoparticles[245]
Lactate dehydrogenase	Cell viability	Carbon nanoparticles[246,247]
Tetrazolium salts		Carbon nanoparticles, fullerenes[248,249]
Alamar Blue		Quantum dots[71]
Propidium iodide		Carbon nanoparticles[58,250]
Neutral red assay test		Carbon nanotubes[248,251]
Caspase-3 activity	Apoptosis	Silver nanoparticles[240]
Acridine orange/ethidium bromide		Silver nanoparticles[252]
ROS production	Oxidative stress	TiO2[239]
Levels of glutathione peroxidase, catalase, superoxide dismutase		Polymeric nanoparticles[253]
Lipid peroxidation, vitamin		Singled walled carbon nanotubes[187]

Lung injury from nanoparticle exposure through the respiratory tract is common. Therefore, organ-on-a-chip research has become more significant in recent years, and many studies have been undertaken to establish the detection of lung toxicity. By more accurately simulating human reactions with the chip in a 3D human lung model that simulated in vivo settings, Zhang et al. explored nanotoxicity. Using accurate models, this study further illustrated the importance of organ-based toxicity[254]. According to studies, nanoparticles have a harmful effect after passing through the placenta of mice. In the 3D human placenta model, chip and TiO2 nanoparticle exposure studies may have similar harmful consequences, claim Yin et al. [255]. Additionally, research on nanotoxicity was conducted using a cell-on-a-chip (CoC) and a microfluidic system[256].

12. REGULATORY CHALLENGES

12.1. Importance of Nanomedicines in the Pharmaceutical Market

Over the last two to three decades means the last 20-30 years, the successful introduction of nanomedicine in both clinical practice and the continuous development in pharmaceutical research has created more sophisticated ones which are mainly entering clinical trials. The nanomedicine market in European Union is composed mainly of nanoparticles, liposomes, nanocrystals, nanoemulsions, polymeric-protein conjugates, and nano complexes[257]. There are currently available nanomedicines made and approved by the EU (European Union)[258].

12.2. Nanomedicines and Nanosimilars

In the approval process, nanomedicines were introduced under the traditional benefit or risk analysis framework. Another challenge related to the framework is developing a framework mainly for evaluating the follow-on nanomedicines at the time of reference medicine patent expiration[259]. Nanomedicine is comprised of both biological and non-biological medical products. Biological nanomedicines are obtained mainly from biological sources. At the same time, the non-biological products are mentioned as non-biological complex drugs (NBCD), where we can find that the active principle consists of different structures[260]. In introducing generic medicines in the pharmaceutical market, we must demonstrate several parameters, as described elsewhere. A more complete analysis is needed for biological and non-biological nanomedicines, which mainly go beyond the plasma concentration measurement. The therapeutic equivalence and, consequently, interchangeability can be requirable by a stepwise comparison of bioequivalence, safety, and efficacy and this relation to the related medicine[261]. The biological nanomedicines are under the regulatory framework set by the European Medicines Agency (EMA)[1]. This framework is an approach to the regulatory system for follow-on biological nanomedicines, which includes the recommendations for the comparative quality, clinical and non-clinical studies[262]. The regulatory approach for the follow-on "Non-Biological Complex Drugs (NCBD)" is still a process. The industry frequently asks for scientific advice, and the EMA analyzes a case-by-case analysis. Sometimes, the biological framework is the basis for the regulation of the "Non-Biological Complex Drugs (NCBDs)" because they have some common features: the structure cannot be fully characterized, and the *in-vivo* activity is dependent on the process of manufacturing, and consequently, the comparability needs to establish throughout the life cycle, as happens to the biological nanomedicines. Besides this, for some "Non-Biological Complex Drugs (NCBDs)" groups like glatiramer, liposomes, and iron carbohydrate complexes, there are draft regulatory approaches, which may help the regulatory authorities or regulatory bodies to create a final framework for the different "Non-Biological Complex Drugs (NCBDs)" families[263]. EMA has already released some papers regarding nanomedicines with a surface coating, block copolymer micelle, intravenous liposomal, and iron-based nano colloidal nanomedicines[264]. These papers released by the EMA are applied to new nanomedicines and nanosimilers, guiding developers in preparing marketing authorization applications. The principles outlined in these documents address general issues that are regarding the complexity of these nanosystems and provide basic information for the development of the pharmaceutical industry, both the non-clinical and early clinical studies of the

block-copolymer micelle, "liposome-like" and the nanoparticle iron (NPI) medicinal products mainly the drug products that have been created to affect the pharmacokinetics, distribution, and stability of incorporated or conjugated active substances *in vivo.* The important factors are mainly related to the exact nature of the characteristics of the particle, and that can influence the kinetic parameters and, consequently, the toxicity, such as the physicochemical nature of the coating, the stability, and respective uniformity (both in terms of susceptibility to degradation), the bio-distribution of the product and its intracellular fate are especially detailed.

12.3. Market Access and Pharmacokinetics

After obtaining nanomedicine by marketing authorization, there is a long way up to the introduction of nanomedicine in clinical practice or clinical trials in all the European Union countries. It occurs because of the reimbursement and pricing decisions for medicines taken at an individual level in each member state of the European Union (EU)[264]. In case to provide patients access to medicines, the multidisciplinary process provided by Health Technology Assessment (HDT) is being developed. The Health Technology Assessment HDT generates information about effectiveness, medicine safety, and cost-effectiveness to support the health and political decision-maker[264]. The study of pharmacoeconomics assumes a crucial role before the commercialization of nanomedicines at the current time. They mainly assess the economic and social importance through the added therapeutic value using indicators such as quality-adjusted life expectancy years and hospitalization[264]. To harmonize and enhance the entry of new medicines into the clinical trial, they have created the EUnetHTA to provide patients with novel medicines. The main goal of EUnetHTA is to develop decisive, appropriate transport information to help the HTAs in European Union countries.

13. ARGUMENTS FOR NANO-SPECIFIC TOXICITY

It is appropriate to mention that in contrast to the view taken in the published literature, nanoparticles do have nano-specific effects[265]. For example, Krug and Wick[32] refer to surface composition, size, and transport as the factors that contribute to the toxicity of any nanoparticle[265]. They suggest that for any specific nanoparticle, these three factors come together to form a unique combination forming[265] '. . . a basis for the description of specific reactions and interactions between nanomaterials/nanoobjects and biological systems . . .'[32]. These authors argue the obvious result of this contention, namely that each nanoparticle 'must be tested individually'[32]. We reject a 'counsel of despair' above[265], arguing that the lack of nano-specific toxicity forms a basis for benchmarking the large amount of available data on conventional particle-mediated pathogenicity[265]. We note that the final common pathways for pathological effects, oxidative stress[265], inflammation, and genotoxicity, are entirely shared by both nanoparticles and conventional particles, and no novel pathogenic pathways are anticipated[265]. Therefore while the proximate events such as the transport of nanoparticles into cells may be unusual or even novel[266], the final common pathways of oxidative stress inflammation and genotoxicity are impacted by all pathogenic particles[265]. Therefore, we can see no reason to invoke nano-specificity to the adverse effects, nor should we anticipate novel pathologies[265]. Kreyling has demonstrated that the translocation of NP from the lungs varies depending on the nanoparticle size[267], with a greater fractional translocation of the smaller nanoparticles[267]. However, the translocation fraction is extremely small and so of questionable significance[265]. That it is not significant is supported by the striking absence of reports of extra-pulmonary pathology in many chronic[268], high exposure, rat inhalation studies carried out with low solubility, low toxicity nanoparticles in the eighties and nineties, for example[268]. In the case of human epidemiology of ambient combustion-derived nanoparticles (air pollution/PM) exposure, the only clear extra-pulmonary effects — in cardiovascular disease — are now considered most likely to arise from oxidative stress or inflammatory signals from the lungs. However, translocation is not completely ruled out[269].

14. CONCLUSION

There is a huge amount of research and regulatory activity in nanoparticle health and safety. Toxicologists need to comprehensively understand this hazard in the context of varying composition, shape, and size for use in risk assessment. It is very important as the sheer degree of adaptability and variability of engineered nanoparticles against detailed testing of every form produced, so other judgments from other sources as to potential toxicity or mechanism of toxicity of nanomaterials are required. Current research shows that exposure to nanoparticles when administered in high concentrations, can cause severe adverse effects, as shown in zebrafish. TiO_2 NPs, IO NPs, and ZnO NPs are considered nontoxic and widely approved but can also show harmful effects. ZnO NPs cause an increase in the reactive O_2 in response to fluorescent light. ZnO NP increases ROS, which stimulates the apoptotic pathways regulated by caspases and mitochondria, which causes extensive cellular dysfunction even at a lower concentration. IO NPs are associated with oxidative stress and induction of redox-signal pathways(AP); NP size and coating seem to cause cellular dysfunction. Further research is needed to unravel the mechanism of nanotoxicity due to nanoparticles.

15. ACKNOWLEDGEMENT

We acknowledge Guru Nanak Institute of Pharmaceutical Science and Technology for providing us with the infrastructure and support system.

16. AUTHORS CONTRIBUTION STATEMENT

The authors of this review article, Pramit Sahoo, Pritam Roy, and Sebabrata Bhakta, contributed equally to the article's conception, research, and writing. In addition, Jeenatara Begum and Tamalika Chakraborty provided critical feedback and supervised the project. All authors have reviewed and approved the final version of the manuscript.

17. CONFLICT OF INTEREST

Conflict of interest declared none.

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