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Review on Nanoparticle Toxicity and their Methods of Assessment in Humans

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Abstract: *Nanoparticles, also known as zero-dimensional particles, are tiny, solid, colloidal forms of matter with diameters ranging from 1nm to 102 nm. Engineering nanoparticles (NPs) at the atomic scale (100 nm in diameter) has produced a number of unique and practical uses in a variety of fields, including electronics, chemistry, environmental protection, medical imaging, illness diagnosis, drug delivery, and cancer. This review aims to analyse potential toxicological portal routes connected to NPs exposures in order to better understand the effects of these exposures on health and how to create appropriate monitoring and control techniques. In actuality, the skin pores, weakened tissues, injection, olfactory, respiratory, and digestive tracts are all entry points for these ultrafine particles into the body. One of the mechanisms of NPs' toxicity is ROS production, which can result in oxidative stress, inflammation, and subsequent damage to proteins, cell membranes, and DNA. This review includes information on several types of nanoparticle toxicity, including neurotoxicity, genotoxicity, ocular toxicity, and dermal toxicity. This study aims to outline techniques for evaluating the toxicity of nanoparticles, including in-vitro techniques such size and surface charge evaluation, cellular interaction test, proliferation assay, apoptosis assay, necrosis assay, and DNA Assay, Endotoxin, Oxidative Stress, and Damage Assay and in vivo techniques including Hematology, serum chemistry, histopathology, and biodistribution and clearance. The review also discusses measures that can be implemented to reduce the toxicity of nanoparticles.*

Keywords: Nanoparticle (NPs), Exposures, Toxicity, Assessment, Preventions

I. INTRODUCTION

Nanoparticles are small solid colloidal objects of matter sizing between 1-10^2nm in diameter and they are also called zero-dimensional particles. Nanoparticles are not visible to human eyes. As the nanoparticles arenot size-independent, their physical and chemical properties are not the same as per the bulk form in which theyexist before. These properties changes as the concentration of atoms decrease in the molecules. The change in color of the Nanoparticle is also a unique optical property of it (ex. Gold nanoparticles of 100nm particle size are green in color whereas 25nm spherical-shaped gold NPs have an orange color). In contrast to size and composition classifications, NPs can be categorized as natural or artificial based on their origins[1].

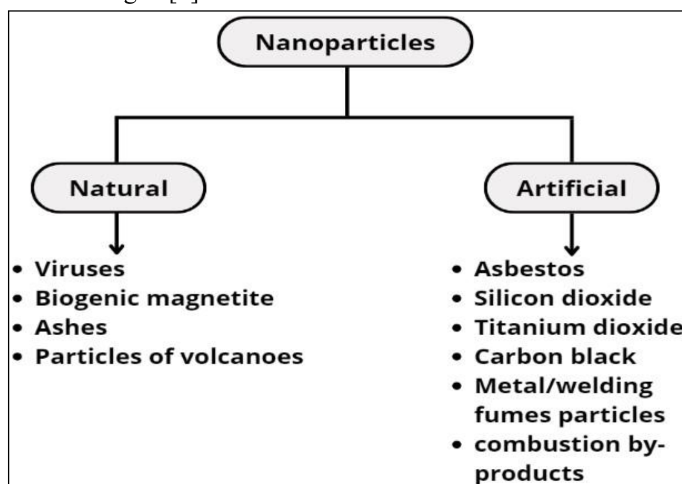

Figure 1.1: Classification of NPs based on their Origin

The use of nanoparticles has not only revolutionized medicine but has also aided in the accurate, precise treatment of diseases and drug delivery. Other applications of nanoparticles in medicine and cancer include fluorescent biological labels, drug and gene delivery, pathogen detection, protein detection, DNA structure probing, Tumour destruction through heating in tissue engineering, separation and purification of biological molecules and cells, MR imaging contrast enhancement, and phagokinetic studies[2].

1.1 Advantages

- NPs drug carriers have higher stabilities as well as capacity.
- NPs are biodegradable, Non-toxic and capable of storing for a long period.
- Drugs are kept apart from the surroundings they possess the ability to entrap both hydrophilic andhydrophobic substances.
- NPs are used in cosmetic products like Soaps, Sunscreen (UV filters), etc

The same novel properties making NPs attractive make them potentially toxic too, Nanotoxicology studies are intended to determine whether and to what extent these properties may possess a threat to the environment and human beings.

1.2 Disadvantages

- On repeated administration, metabolites that cause toxicity may be formed in between biotransformation of Polymeric carriers.
- The polymeric NPs relatively slowly degradable which might cause systemic toxicity
- Since these particles are very small, problems can rise from inhalation of these minute particles (ex. Asbestos particles)

Previous experiences, such as those involving asbestos and air pollution, have led to growing worries about the potential negative health effects of nanoparticles and nanostructures, latter these are proven to be the more problematic from a toxicological perspective. When introduced into the atmosphere, nanoparticles interactwith the air, water, and soil thereby changing the surface characteristics of the biological matter. This frequently alters the surface characteristics of the particles, which may cause them to aggregate or change their charge or

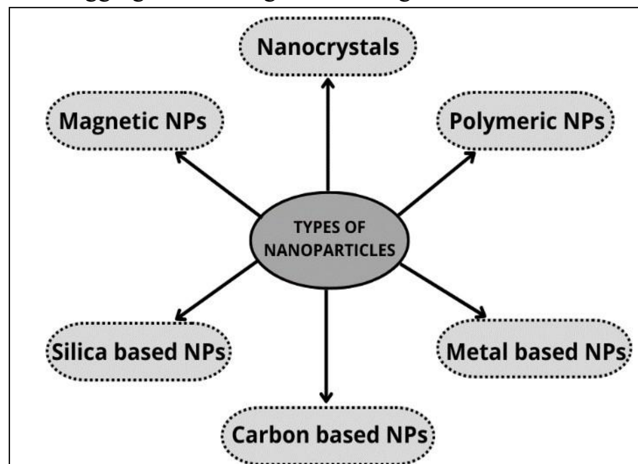

Figure 1.2: Types of Nanoparticles

other surface characteristics. These impacts, which have been researched in soil and water ecosystems, highlight the significance of recognizing nanoparticles and their surrounding en2vironment as a "complex" that must be examined in its completion to understand particle behavior within the environment. The question of whether nanoparticles may contaminate soil or water to generate toxicity is currently up for dispute. Although yet we don't know about all the toxic effects of NPs on the human body.

Nanomaterials, consisting of nanoparticles, nanotubes, nanofibers, and nanocomposites, in the forms of metals and alloys, ceramics, polymers, and composites are all produced by nanotechnology methods and are viewed to be the subsequent generation of substances for manufacturing quicker automobiles and planes, more effective computers and satellites, extra sensitive sensors, more suitable materials for structural applications, and better micro- and nanochips

and batteries. This is because nanomaterials have incredible mechanical, electrical, optical, magnetic, quantum mechanics, and thermal properties. Nanomaterials are already determinedin extra than a thousand one-of-a-kind products, such as bacteria-free cloth, concrete, filtration units, sunscreen, automobile bumpers, toothpaste, polymeric coatings, photo voltaic, lithium sensors. In the close to future, the use of nanomaterials will extensively increase worldwide. It is predicted that the international market growth ofnanotechnology is on the way to attaining one trillion dollars, and 50 percent of all new productions will be nanotechnology-oriented by the year 2015[3].

II. ROUTES OF EXPOSURE OF NPS

Most individuals are constantly exposed to particulates in the air, primarily from vehicle exhaust. Fuel combustion generates a large number of nanoparticles. The potential of nanoscale materials to enter the body, on the other hand, is one of the numerous criteria that scientists must investigate in order to determine if

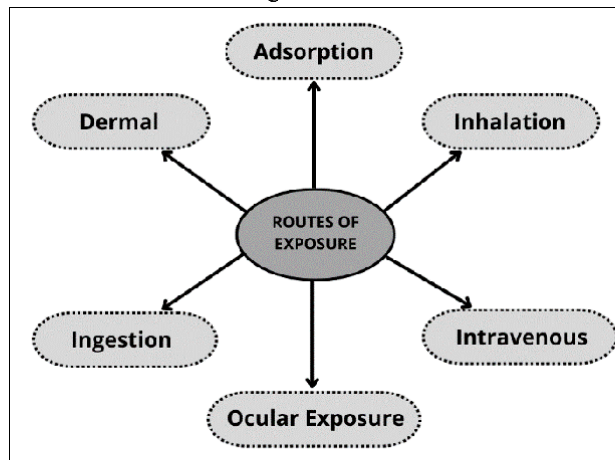

Fig. 2.1: Exposure routes of NPs to Human

such compounds constitute a health concern. If ultrafine materials become airborne or come into contact with the skin, they have the highest potential to enter the body as NPs, agglomerates of NPs, NPs aggregates, and particles from nanostructured materials[4]. Once in the body, NPs can traverse cells through perception, interactwith local tissues, and cause or trigger organ malfunctioning. Airborne nanoparticles can be inhaled and deposited in the respiratory system, according to animal research. NPs can then enter the circulation and reach other organs. There were several major routes of exposure are reviewed below.

2.1 Dermal Exposure

The epidermis and dermis, as well as sweat glands and hair follicles that run parallel to the skin, make up the skin's structure. Keratinocytes that migrate from the basal layer to the skin's surface create the external protective layer known as the stratum corneum. They provide a barrier that keeps out bacteria and other dangerous substances. This barrier is preferable for relatively tiny molecules (100 nm) that diffuse over the stratum corneum via cellular and/or intercellular channels since it is not entirely permeable. Most of the dermal exposure to the skin today comes from the usage of skin care products that include nanoparticles. Liposomes, poorly soluble solid materials like TiO2, ZnO2, and polymer particulates, and submicron emulsion particles like solid lipid nanoparticles are the three primary types of particulate materials employed in cutaneous formulations[41]. It seems doubtful that titanium dioxide nanoparticles used in sunscreens and other skin care products won't permeate through the dermis based on the scant information that is currently available. Light andelectron microscopy have not revealed the presence of micronized titanium dioxide in the deeper stratum corneum layers, the human epidermis, or the dermis. It is only deposited on the stratum corneum's outermost surface[5].

2.2 Inhalation Exposure

Engineered nanoparticles pose an entirely different toxicological challenge. In evolutionary terms, they are wholly unique; evidence suggests that they obtain entrance to the body, notably by inhalation, and then translocate inside the

body to distant places at low dosages. The lungs are components of the respiratory tract that exchange gases with the circulatory system, including oxygen and carbon dioxide (blood). The tubes become smaller and more numerously divided as they proceed down the respiratory tract beginning at the trachea. The total surface area is greater due to a large number of bronchioles, despite the fact that each bronchus or bronchiole has a smaller cross-sectional area[5]. This indicates that the terminal bronchioles experience less resistance. Humans are shown to be affected by nanomaterials mostly through cutaneous or inhalation toxicity during their manufacturing or use. The NPs breathed during the aforementionedprocedure are seen deposited in the alveolar areas of the deep lungs. Nanoparticles smaller than 100nm are frequently seen in the trachea, but those less than 10 nm are typically found in the bronchus, bronchiole, and alveoli. The majority of foreign particles are prevented from entering the airway epithelia by a barrier that is built up by the respiratory system. The bronchiolar epithelium, which is composed of ciliated cells and is kept together by tight connections, follows the surfactant film and mucus layer that make up the respiratory wall. Due to rapid urbanization, industrialization, vehicle emissions, extreme events (dust storms, volcanic eruptions,forest fires, etc.), and episodic events (such as fireworks and agricultural residue burning), the atmospheric concentration of nanoparticles is high in Asian countries. Most anthropogenic nanoparticles are composed of metal oxides, silicon, carbon, or silicon dioxide. More than 110,000 tonnes of particulate matter, 4.3 million tonnes of SO2, and 1.2 million tonnes of NOx are emitted annually by India's coal-fired power plants[6].

2.3 Oral Exposure

As nanoparticles (NPs) are utilized more frequently in food, it is important to carefully evaluate their toxicity when consumed. In fact, several investigations have demonstrated that oral exposure to NPs, particularly solid NPs, may cause toxicological reactions both in vivo and in vitro. However, the majorityof toxicological investigations exclusively employed NPs f or oral exposure, ignoring any potential interactions with food ingredients in actual use. There are numerous effects of exposure to nanoparticles through oral routesand can be given as follows

a) Food components directly affect nutrient absorption through physical-chemical modification; b) NPs directly affect food components absorption through disruption of microvilli or alteration in expression of nutrient transporter genes and c) The presence of food components affects oxidative stress caused by NPs. All these interactions could ultimately increase or decrease the toxicological reactions brought on by NPs after oral consumption[42]. Therefore, it is important to evaluate the synergistic effects of NPs in a complex system whenevaluating the safety of NPs used in food[7].

2.4 Ocular Routes

The eyes are utilized to detect light and transmit signals to the brain's visual regions via the optic nerve. The human eye can be separated into the anterior and posterior anatomical regions. There are veryfew papers that employ the eye as a point of entry for NPs into experimental animals. Topical application is used to deliver drugs. However, due to the quick precorneal elimination caused by solution drainage, lengthy diffusional channel length, induced lacrimation, and corneal epithelial in permeability, topical drug application for treating posterior eye problems is not very successful. The model that used mesoporous silica nanoparticlesto assess their toxicity in the eyes is one example of research on nanoparticle toxicity through the eye. The MSiNPs' porosity gives them a lot of surface area and the capacity to bind to nearby biological or chemical molecules, which increases their surface reactivity and hazardous effects therefore additional harmful compounds were added, such as silver ions (Ag+), to the system and then examined their synergistic nanotoxicity in order to better simulate MSiNP exposure in actual situations. As revealed by cell viability, apoptosis, reactive oxygen species (ROS) generation, and DNA damage, our findings demonstrated that exposure to MSiNPs-Ag+ and even Ag+ at a tolerable dose resulted in more toxicity than the MSiNPs alone[4].

2.5 Ingestion Exposure

In 1926, it was discovered that particles might move from the lumen of the digestive tract via aggregations of intestinal lymphatic tissue containing M cells. It is now discovered that inert particle absorption can occur not just through immune cells in Peyers' patches, but also through enterocytes and, to a lesser extent, through para-cellular routes. However, data on possible exposure through the GI tract are once again scarce in the literature. Cationic nanometre-sized latex particles were stuck in the negatively charged mucus, whereas repulsive carboxylated fluorescent latex

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nanoparticles were able to spread over this layer, according to Szenkuti the smaller the particle diameter, the faster it could pass through the mucus and reach the colonic enterocytes; 14 nm particles passed through in 2 minutes, 415 nm particles took 30 minutes, and 10^2 nm particles were unable to penetrate this barrier. After several days of oral gavage, researchers discovered a sparse build-up of charged latex particulates in the lamina propria as compared to uncharged latex nanoparticles in the same size range. The same researchers evaluated the body distribution of polystyrene particles ranging in size from 50 nm to 3000 nm following translocation. For 10 days, rats were fed by gavage at a rate of 1.25 mg/kg. It was discovered that up to 34% and 26% of the 50 nm and 100 nm particles, respectively, were absorbed. Those greater than 300 nm were not found in the blood. There were no particles found in the heart or lung tissue[9].

III. MECHANISM OF NPS TOXICITY

Nanoparticles enter the human body primarily by inhalation or skin contact, and subsequently through injection or ingestion (medicines or food). Oxidative stress is one of the possible mechanisms. Dysfunction of the immune system and autophagy NPs may cause phagocytic excess cells, protective fever, or a reduction in the body's immunological protection. NPs are not able to amass in the organs and progressively destroy because of their large surface area, NPs have an impact on enzymes as well as proteins[10].

3.1 Endo and Exocytosis

Endocytosis is classified into two types: phagocytosis (the uptake of big particles) and Pinocytosis (the uptake of fluids and solutes). Phagocytosis is a process that occurs in macrophages, neutrophils, monocytes, and dendritic cells. Pinocytosis, on the other hand, is found in all sorts of cells and comes in a variety of shapes depending on the cell's origin and purpose. Pinocytosis is a non-specific receptor-mediated passive uptake or adhesive interaction. The uptake of pinocytosis is triggered via electrostatic charges, Van der Waals forces, steric interactions, and interfacial tension effects, but it does not lead to the production of vesicles. Because of the direct access to cytoplasm organelles and proteins, this form of nanoparticle absorption and unfettered movement inside the cell makes them extremely dangerous. Following pinocytosis, NPs can be detected in a variety of sites, inside the cells, including the outer-cell membrane, cytoplasm, mitochondria, and lipid vesicles or the nuclear membrane within the nucleus depending on where they are in the cell, nanoparticles might harm organelles or DNA, or even lead to cell death. The size of the nanoparticle determines particle internalization. Environmental particles ranging in size from 2.5 to 10µm were discovered to accumulate in large cytoplasmic vacuoles, whilst tiny nanoparticles (100nm) were found to accumulate in organelles such as mitochondria, causing alteration of mitochondrial architecture. Very little with a diameter of 0.7 nm, NPs (C_{60} molecules) are capable of penetrating cells, most likely by ion channels or via cell membrane pores[11]. NPs tend to stick together in large quantities to a group, generating aggregates that are frequently bigger than 100 nm. Larger nanoparticles (100nm) can be quickly phagocytized via alveolar macrophages. In biological fluids, most NPs tend to agglomerate, increasing their total size. It was recently proposed that aggregates or agglomerates form when the van der Waals attractive forces between nanoparticles are greater than certain electrostatic repelling forces. Positively charged nanoparticles attracted more phagocytes than negatively or neutrally charged nanoparticles. Furthermore, surface functionalization with PEG, poloxamer and poloxamine was performed. Polymers shield NPs from ionic strength, improve particle dispersion and decrease protein absorption in blood on their surface as a result, it inhibits phagocytosis. The presence of an electric charge may have a function in the activation of Scavenger-type receptors for a certain kind of nanoparticles (such as titanium dioxide, iron oxide, quartz)[12].

3.2 Oxidative Stress

There could be several causes for the oxidative stress that nanoparticles cause. When both oxidants and free radicals are present, reactive oxygen species can be produced straight from the surface of the particles, the surface of the particle contains free radicals. Nanoparticles made of transition metals (such as iron, copper, chromium, vanadium, etc.) can produce catalysts such as reactive oxygen for reactions of the Fenton-type[30]. The function of mitochondria may be disrupted by nanoparticles after entering in it, resulting in the formation of ROS. Because of the absence of DNA repair enzymes, mitochondrial DNA appears to be more vulnerable to ROS-induced mutations. Protein oxidation can result in the aggregation of insoluble proteins, which is required as the molecular foundation for several illnesses, particularly

neurological disorders. TiO_2 NP- induced cellular alterations affect the redox state, diminish antioxidant enzymatic defence and mitocho-ndrial depolarization and activate apoptotic pathways. Furthermore, the activation of inflammatory cells such as alveolar macrophages and neutrophils can result in the formation of reactive oxygen and nitrogen species. There are various sources of oxidative stress in general: *Oxidant-generating characteristics of particles, as well as

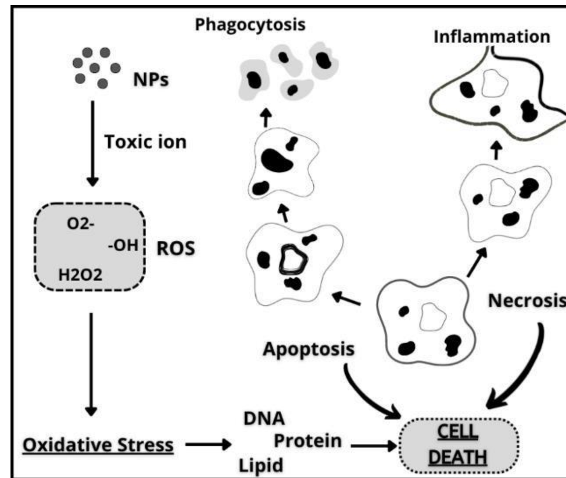

Fig. 3.1: ROS generation induced by Nanoparticle

their propensity to promote ROS production as part of the cellular response to nanoparticles. *Transition metal nanoparticles or transition metal contaminants are utilized as catalysts in the synthesis of non-metal nanoparticles. *Relatively stable free radical intermediates are found on reactive particle surfaces. *Redox- active groups formed because of nanoparticle functionalization[13].

3.3 Inflammation and Genotoxicity

NPs trigger microglia, causing the release of proinflammatory molecules that promote cell malfunction and cytotoxicity. NPs are thought to be a novel class of autophagy inducers. NPs cause autophagy in two different ways. ROS-dependent autophagy is mediated by NPs as well as NP-mediated lysosome-dependent autophagy. TiO_2 NPs, Si NPs, and polymeric NPs may all trigger autophagy in brain- derived human endothelial cells. The size and content of nanoparticles are the key elements in nanoparticle inflammation. NPs of varying sizes and chemical compositions can deposit in mitochondrial DNA, which results in mitochondrial electron disruption and $-O_2$ generation is increased because of the transduction chain. Following on from direct Oxygen synthase expression and nitric oxide production may be induced by hydroxyapatite NPs and TiO_2 microglial oxide release[14].

The genotoxic activity of nanoparticles is an oxidative stress effect. Increased oxidative species production leads to increased inflammation and antioxidant production. Finally, nanoparticles' interaction with cells may result in DNA changes, Cell damage and illnesses. Chromatin epigenetic effects (especially effects on histone acetylation and methylation), DNA damage and dysfunction that is premutagenic DNA healingmechanisms can be a component of the mechanism in charge of the acknowledged Ni(II) particle carcinogenicactivity[15].

IV. NANOPARTICLE TOXICITY

The primary causes for this are the manufacture, usage, disposal, and waste treatment of nano products. The relatively small size of NPs allows active chemical species to cross organismal barriers such as the skin, lungsS, body tissues, and organs. Thus, NPs can induce permanent oxidative stress, organelle damage, asthma, and cancer depending on their constitution[1]. The generation of ROS, such as superoxide anion radicals, hydroxyl radicals, and hydrogen peroxide is mainly responsible for nanoparticle toxicity. Excessive ROS levels can lead to lipid peroxidation, protein and DNA damage, cell death (apoptosis), genotoxicity and many other toxicities. The acute toxic effects of exposure to Nanoparticles and nanostructured materials include reactive oxygen species production, protein denaturation, mitochondrial dysfunction, and phagocytic function disruption. Because nanoparticles are used in so many industries,

scientists must investigate the effects of nanoparticles on human health and the environment. Nanotoxicology is concerned with the investigation and improved understanding of nanoparticle toxicity. Numerous in-vivo and in-vitro studies reveal that nanoparticles are highly toxic. With their size, and increased surface area interaction because of such properties nanoparticles have high reactivity. Nanotoxicity research assists in recognizing the characteristics of nanoparticles that cause toxicity via reactive oxidant species (ROS) and inducing biological macromolecule damage. Nanoparticles are also capable of crossing cell membranes and causing an impact on DNA. NPs can cause various types of toxicities some of which may even cause death[2].

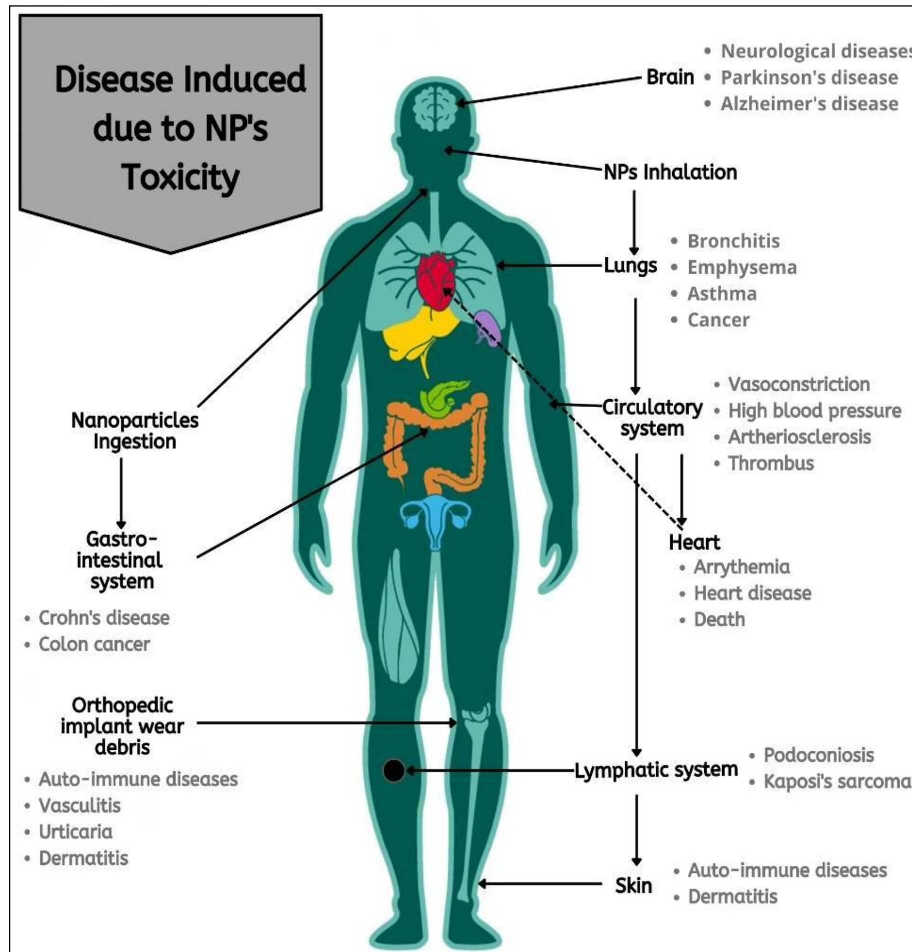

Fig. 4.1: Various diseases associated with NPs Toxicity

4.1 Neurotoxicity

Neurotoxicity is defined as any reversible or irreversible undesirable effect on the structure, function, or chemistry of the nervous system caused by physical or chemical aspects during development or at maturity. Neuronopathy, axonopathy, myelopathy, and gliopathy are the most common neurological deleterious consequences associated with morphological alterations. The principal mechanism involved in neurotoxicity comprises the overproduction of reactive oxygen species, which causes oxidative stress, the release of cytokines, neuroinflammation, and apoptotic deregulation, which causes neuronal death. As of now, there is a lack of sufficient knowledge about NPs induced Neurotoxicity so the development of an effective counteractionis very crucial.

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Table 4.1: Nanoparticles and their Neurotoxic effects

	Nanomaterial Type						
Neurotoxic Effects	**Liposomes**	**Dendrimer**	**Gold NPs**	**Silver NPs**	**Iron NPs**	**TiO2 NPs**	**Silica NPs**
	Necrosis	Cell Proliferation	Seizure Activity	Oxidative Stress	Neuro-Inflammation	Genotoxicity	Cognitive Dysfunction
	Neuro-Inflammation	Apoptosis	Cognition Defects	Necrosis	Apoptosis	Synaptic Plasticity	Neuro-Inflammation
	Haemorrhage	DNA Damage	Astrogliosis	Apoptosis	Macropha ge Infiltration	Apoptosis	Cognitive impairment
	Macrophage Infiltration	Oxidative Stress	-	Neuro-Inflammation	-	Oxidative Stress	-

A. Organic NPs

Liposomes: Liposomes are nano to micro-sized vesicles made of an aqueous solution core surrounded by oneor more amphiphilic lipid bilayers. They have the ability to encapsulate both hydrophilic and hydrophobic compounds. As a result, they have been widely employed in formulations as Nanocarriers for the efficient transport of medicines, vaccines, proteins, enzymes and nucleic acid. The in-vivo study of the anti-cancer therapeutic efficacy of cisplatin-containing liposomal formulations and the corresponding neurotoxic effects of drug-free liposomes showed that administration of commercially available drug-free Liposomal formulations or formulations having lower dosages of cisplatin causes mild to severe side effects such as Haemorrhage, necrosis,edema, and macrophage infiltration. As a result, the neurotoxicity was caused by the liposomes' inherent toxicitymixed with the neurotoxic effects of cisplatin

1. Dendrimers: Dendrimers are a group of artificial, extremely branched globular macromolecules with a tree-like topological structure and of nanoscale sizes. Dendrimers, also entrap both hydrophobic and hydrophilic molecules like liposomes. Dendrimers have been shown to produce a variety of neurotoxicological reactions depending on their physicochemical properties.
2. Polymeric NPs: It is one of the most extensively researched organic nanomaterials in nanomedicine. However, the drawbacks of polymeric nanoparticles include aggregation and probable toxicity caused by degrading processes and residues. Polysorbate 80-modified chitosan nanoparticles were tested for neurotoxicity in rats upon intravenous administration. Demonstrated that the nanoparticles accumulating in the frontal cortex and cerebellum, as well as neurodegeneration, a mild inflammatory response, body weight decrease and elevated oxidative stress. Although there are very few neurotoxicological studies that are able to prove the neurotoxicity due to polymeric NPs.

B. Inorganic NPs

The capacity of inorganic nanoparticles, such as gold, silver, iron oxide, titanium oxide, and silica nanoparticles, to cross BBB induces Neurotoxicity.

1. Gold NPs: Gold nanoparticles aggregate in the brain after crossing the BBB or migrating down the olfactory neurons, where they cause neurotoxicity. The primary neuropathological symptoms induced by gold nanoparticles are increased seizure activity and memory impairment and astrogliosis, which is characterized by a change in the morphology of astrocytes, resulting in the formation of astrocytes that are reactive.
2. Silver NPs: It has a wide range of applications, including the food, pharmaceutical, and environmental sectors,as well as medicine, cosmetics, and textile coatings. Recent research has suggested that the physical propertiesand release rates of silver ions, as well as their interactions with certain cells and proteins, may play a role in their neurotoxicity.
3. The mechanism of neurotoxicity entails the cellular uptake of silver nanoparticles, which causes the generation of intracellular reactive oxygen species and the promotion of cell death, decreased amounts of silver nanoparticles caspase activity and cytokine production from astrocytes increase, leading to apoptosis and respectively, neuroinflammation. Furthermore, the release of silver ions causes cell injury resulting in cell necrosis.

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4. Iron oxide NPs: Iron oxide NP's are extensively used in medicine for imaging, medication administration, cancer treatment, and cell separation due to their unique magnetic characteristics and biodegradability. Concerns about nanotechnology have been highlighted because of its fast development of neurotoxicity. Iron oxide nanoparticle exposure disruptions in synaptic transmission and nerve conduction, resulting in neuroinflammation, immune cell infiltration and apoptosis.
5. Titanium oxide: Titanium oxide nanoparticles are the most widely utilised nanomaterials in biomedicine, cosmetics, and food additives such as chewing gum, sweets, and toothpastes. The major causes of neurotoxicity caused by titanium oxide nanoparticle exposure include oxidative stress, neuroinflammation, apoptosis, genotoxicity, disruption of neurotransmission, synaptic plasticity, and disrupted signalling pathways.
6. Silica NPs: The nebulized route causes nanoparticle deposition in the brain, which results in memory impairment, synaptic disruptions, and diseases comparable to neurodegeneration. They can also induce an increase in oxidative stress and changes in microglial activity as a result of the striatum and dopaminergic neurons deterioration[2].

C. Genotoxicity

Genotoxic substances may bind directly to DNA or indirectly, causing DNA damage by altering enzymes involved in DNA replication, resulting in mutations that may or may not lead to cancer or birth abnormalities (inheritable damage). Nanoparticles can cross cell membranes and influence DNA. The genotoxicity mechanism is divided into two categories as primary and secondary mechanisms. At the single- cell level, the primary mechanism is the direct or indirect interaction between nanoparticles with DNA. In the case of direct contact, nanoparticles interact directly with the chromosomes during the interphase/mitotic phase, finally binding with the DNA and inhibiting the process of replication or transcription/chromosomal loss (aneugenic effect)/chromosomal breakdown (clastogenic effect)[16].

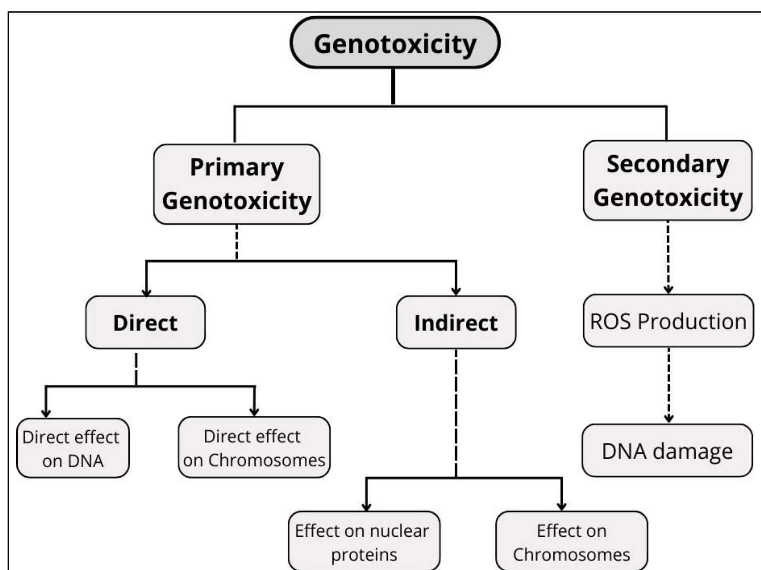

Figure 4.2: Mechanism of Genotoxicity induced by NPs

The intermediates of the nanoparticle process generate genotoxicity during the indirect contact by producing ROS and releasing toxic components that interfere with proteins required for DNA replication, transcription, or repair. The free radicals also produce oxidized base lesions in pyrimidine and purine bases, resulting in mispairing during the replication process and the possibility of fatal mutations. DNA content depletion was easily recognized in brain and liver cells, as mitochondrial activity reduction[17]

A secondary mechanism for genotoxicity occurs from the excessive production of ROS by activated phagocytes, including macrophages and neutrophils, because of a prolonged in-vivo inflammatory response. This inflammatory reaction increases oxidative stress, which affects the surrounding cells. These genotoxic pathways can result in DNA breakage, point mutations, chromosomal fragmentation, and changes in gene expression patterns, as well as

carcinogenesis and mutagenesis. Particle size, composition, shape,physicochemical parameters (temperature, pH, etc.), solubility, and surface coating are all critical factors in nanoparticle-induced genotoxicity. The chemical composition of Nanoparticles is the primary cause of genotoxicity. Smaller nanoparticles are more reactive and interact with biological entities, causing increased ROS generation and hence higher genotoxicity[18].

D. Ocular Toxicity

One of the most accessible sites for nanoparticle medication and gene delivery is the eye. The cornea and sclera are the principal barriers to the eye. The topical therapeutic's principal targets are tissues.Intravitreal injections are a popular and simple method of delivering nanoparticles to the eye.

Organic NPs:

- Polycaprolactone: The ARPE-19 cell line was used to investigate the toxicity of polycaprolactone (PCL),
- PLGA, and PEGylated PLGA (PEG-PLGA) models of human
- retinal vascular endothelial progenitor cells It was discovered that PEG-PLGA did not cause toxicity, but both PCL and PLGA were shown to be highly toxic.
- Quantum dots: When quantum dots are used, they can enter and accumulate in the cornea when the epithelial barrier was breached. The viability of corneal stromal cells after being exposed to lower concentrations, decreased by 50% concentrations (5-20nm) in-vitro for 24–48 hours. The study investigated that the ocular toxicity of formulations of fluconazole NE in situ gel.

4.2 Inorganic NPs

1. Gold NPs: Gold nanoparticles can be utilized to diagnose and treat visual problems. The research examined the toxicity of gold nanoparticles on the mouse retina. In this work, gold nanoparticles with diameters of 20 and
2. 80 nm are induced at modest levels of oxidative stress and apoptosis, and retinal cells are damaged concentrations (0.4 g/ml for 80 nm NPs and 0.0065 g/ml for 20 nm NPs). Furthermore, apparent neurotoxicity, including toxicity to Photoreceptors and glial cell activation was identified. This will result in vision damage or perhaps blindness. Furthermore, the harmful effects of NPs on the embryonic development of zebrafish were also spotted in exposition to 16.7 nm silver NPs At 100 g/ml, which were harmful to the rabbit's corneal cells.
3. Titanium dioxide NPs: Titanium dioxide nanoparticles are easily absorbed by the eyes. Titanium dioxide nanoparticles were discovered to trigger apoptosis in endothelial cells and to increase the fraction of cells in the G2/M phase after primary mouse corneal endothelium cells were exposed for 24 hours to 25 g/ml.
4. Silica NPs: In a toxicity study conducted on human corneal and conjunctival epithelial cells, 100 nm, 12.5 g/ml ZnO-NPs were shown to be cytotoxic. Following human corneal epithelial after 48 hours of exposure to silica NPs, the cells produced Intracellular ROS levels that were elevated in a dose-dependent manner.
5. Carbon NPs: Carbon nanotubes, fullerenes, graphene, graphene oxide, and reduced graphene oxide are the most common types of carbon nanoparticles.Carbon nanotubes are categorized as single-walled carbon nanotubes and multi-walled carbon nanotubes (MWCNTs). Only one form of multi-walled carbon nanotube was responsible for only a few reversible conjunctival congestions and the others were non-irritating to the eyes. Previous studies have revealed that graphene oxide NP exposure was harmful to human primary corneal epithelial cells and human conjunctival epithelial cells in a dose- and time-dependent manner[19].

4.3 Dermal Toxicity

Dermal toxicity testing assesses a chemical's local and systemic effects after dermal exposure. These tests identify chemicals that penetrate the skin and cause systemic toxicity. However, the overall amount of chemicalsabsorbed cannot be determined by dermal toxicity testing. Passive diffusion is a common route of dermal penetration. However, prior to systemic absorption, biotransformation of the test chemical might occur in the deeper viable parts of the skin. The capacity of the stratum corneum (SC) as the outermost layer, the degree of dermal penetration is controlled by the skin and its bi-lipid layers. Certain biological factors influencing the absorption process are body, SC integrity, and epidermis thickness.

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Penetration into the skin occurs via four routes: intercellular, transcellular, trans appendageal, and through sweat glands and hair follicles. However, because of there is inadequate information on nanoparticle dermal absorption and skin penetration. Further researches are crucial in this field. Nanoparticle penetration into healthy skin results in the formation of free radicals, oxidative stress, and collagen depletion. Even though such depletion causes keratinization, dermal atrophy, and skin wrinkling; when the epidermal barrier has been disrupted the penetration of NPs is enhanced.

1. Iron Oxide NPs: According to previous research, nanoparticles' percutaneous penetration is restricted to the upper regions of hair follicles or the surface layers of the SC. Iron nanoparticles accumulated in the skin's surface and epidermis.
2. Titanium dioxide NPs: $TiO2$ nanoparticles reached the SC but not the dermis; nevertheless, according to one study, Over 40 days of dermal exposure in hairless mice, nanoparticles were found to permeate the deeper portion of the skin, reaching other tissue which induced some pathological alterations in a number of vital organs. 3)Gold NPs: Hagar et al. investigated the penetration of gold nanoparticles and the metabolic effects of nanoparticles on human skin. They demonstrated that after 24 hours of exposure, 15-nm gold nanoparticles in an aqueous solution aggregated on the surface SC. 6nm gold nanoparticles in toluene, on the other hand, entered the SC and human skin's epidermal layers[20].

V. METHODS OF ASSESSMENT OF NPs TOXICITY

The growing usage of nanomaterials in consumer and industrial products has sparked global concern about their status in biological systems, prompting equivalent risk assessments. There has been a lot of researchpublished on the effects of nanoparticles in-vitro and in-vivo scenarios. However, because of the numerous experimental issues and challenges faced when measuring the toxicity of nanomaterials, there is still a need for additional research that unambiguously confirms their safety/toxicity. Many toxicity assessment approaches were developed and standardized with chemical toxicology in perspective. However, nanoparticles have variousdistinct physicochemical features that can interact with or complicate traditional toxicity assessments. Some novel approaches and updated versions of established methods have recently been developed for NPs toxicity assessment which we are highlighted below in brief[21].

The NPs toxicity assessment methods are differentiated into two types likewise In-Vitro and In-Vivo methods of analysis. Additionally, NPs characterization studies are also performed.

NPs Characterization:

Standardized guidelines for the physicochemical characterization of nanomaterials should be established to improve the quality and relevance of toxicological research. Size, surface area, chemicalcomposition, surface charge and reactivity, shape, solubility, and crystallography are all important physicochemical properties to analyse. NPs characterization is generally achieved through techniques that are mentioned in below table[22,23]

Table 5.1: Summary of Characterisation of NPs and their measurement techniques

Characteristics	Solid	Liquid	Gaseous
Size	Electron microscope and laser diffraction for bulk sample	Photon correlation Spectroscopy and centrifugation	SMPS and Optical particle counter
Surface area	BET Isotherm	Simple titration and NMR experiments	SMPS, DMA
Composition	XPS and Chemical digestion followed by wet chemical analysisfor bulk samples	Chemical digestion for mass spectrometry, atomic emission spectroscopy and ion chromatography	Particles are collected for analysis by spectrometric or wet chemical techniques
Surface Morphology	Image analysis of electron micrographs	Deposition onto a surface for electronmicroscopy	Capture particles electrostatically or by filtration for imaging using electron microscopy
Surface Charge	Zeta potential		DMA

Crystallography	Powder X-ray or neutron diffraction	-	-
Concentration	-	-	CPC

5.1 In-Vitro Methods:

A key method for nanotoxicology is in-vitro toxicological assessment. In vitro studies provide severaladvantages over in vivo investigations, including speed, reduced cost, more control, and a reduction in the number of lab animals needed for testing. However, because in vitro exposure circumstances often include significantly larger concentrations and exposure durations than those encountered in the cellular microenvironment in vivo, extrapolating results from this research for the prediction of in vivo toxicity is difficult. Proliferation, necrosis, and apoptosis are the three main viability-based test types listed below, while oxidative stress or DNA damage detection techniques are the two main toxicity mechanism studies. Given the substantial overlap across the tests, the category classification of these assays is somewhat imprecise. For instance, in addition to resulting directly from nanoparticle interaction in the nucleus, DNA damage can also belinked to apoptosis and oxidative stress. Detection methods for DNA damage or oxidative stress are the two categories under which toxicity mechanism analyses lie. Since there is a considerable degree of overlap across assays, the category division of these assays is somewhat arbitrary. For ex. in addition to emerging directly from nanoparticle interaction in the nucleus, DNA damage can also be associated with apoptosis and oxidative stress. In this review, we addressed the review of those techniques that are currently applied to nanotoxicology and alternative techniques available for assessment of NPs toxicity[24].

5.2 Size and Surface Charge Evaluation

There are several analytical approaches available to describe the toxicological features of nanoparticles, but two of them are frequently utilized to provide essential quantitative information: dynamic light scattering (DLS) and zeta potential (ZP) studies.

The apparent surface charge of nanoparticles is identified by zeta potential, which is frequently a supplementary capacity of DLS systems. ZP is calculated by matching the velocity of particles passing through the device's anode or cathode to the magnitude of an externally applied electric field. Although DLS and ZP are the principal nanoparticle characterization methods, Numerous additional strategies existed before use in in- vitro experiments. Provide comparable analysis or more useful information Typical techniques include electron microscopy (such as transmission electron microscopy (TEM) and scanning electron microscopy (SEM)) For size and analysis, scanning electron microscopy (SEM), laser diffraction, and atomic force microscopy (AFM)are used. X-ray diffraction, Fourier-transform infrared spectroscopy (FTIR), and geometry (XRD), SERS (surface-enhanced Raman spectroscopy) and solid-state nuclear magnetic resonance (SS-NMR) spectroscopy for composition investigation, and UV-Visible spectroscopy and fluorometry for photonic characteristics. Using are used. X-ray diffraction, Fourier-transform infrared spectroscopy (FTIR), and geometry (XRD), SERS(surface-enhanced Raman spectroscopy) and solid-state nuclear magnetic resonance (SS-NMR) spectroscopy for composition investigation, and UV-Visible spectroscopy and fluorometry for photonic characteristics. Usingmultiple techniques in conjunction with DLS and ZP analysis aids in identifying some of the most significant multiple techniques in conjunction with DLS and ZP analysis aids in identifying some of the most significant factors that comprise nanoparticle toxicity profiles, and their evaluation associated with in-vitro toxicityassessment can show the major pathways of toxicity that must be addressed prior to their application[25]

Table 5.2: Summary of In-Vitro assessment methods of Nanoparticle toxicity

Assay type	Category	Cellular Property	Assays
Viability	Proliferation	Metabolic activity DNA synthesis Colony formation	MTT, XTT, WST-1, Alamar Blue Thymidine incorporationCologenic
	Necrosis	Membrane integrity	LDH, Trypan Blue, Neutral Red, Propidium iodide
	Apoptosis	Membrane structure	Annexin-V
	LIVE-DEAD	Esterase activity/membrane integrity	Calcein acetoxymethyl/ethidium homodimer

Mechanistic	DNA damage	DNA fragmentation DNA double-strand breakage	Comet, CSETUNEL
	Oxidativestress	Presence of reactive oxygenSpecies(ROS) Lipid peroxidation Lipid peroxidation Presence of lipid hydroperoxides Antioxidant depletion Superoxide dismutase (SOD) activity SOD expression	DCFDA, Rhodamine C11-BODIPY TBA assay for malondialdehyde Amplex Red DTNB Nitro blue tetrazoliumImmunoblotting

Particle surface charge may influence particle cellular absorption as well as particle interactions with organelles and biomolecules. As a result, particle surface charge has an effect on cytotoxicity.

While using DLS, the Brownian motion causes random motions of dilute nanoparticles distributed in solution are identified by examining Rayleigh or Mie scattering from a monochromatic laser[26].

A. Cellular Interaction Assay

The capacity of a nanoparticle to transit through and interact with cellular barriers is an important test for nanoparticle toxicity. Cationic surface charges and coatings inviting active transport increase nanoparticle penetration through the cell membrane, facilitating the interaction of therapeutic or harmful substances with vulnerable organelles in the cytoplasm.

Cells are treated with nanoparticles for 24 hours before staining and microscopic inspection. Fluorescent approaches often employ anterior chemical alteration or immunostaining to pair fluorescein tags. Fluorescence-activated cell sorting utilizing isothiocyanate (FITC), cyanine, and Alexa dyes. For example, flow cytometry (FACS), confocal laser scanning microscopy (CLSM), and imaging flow cytometry (IFC) Internalization of nanoparticles is identified quantitatively and qualitatively in an independent manner after sample incubation[25].

B. Proliferation Assay

This technique is used to analyse metabolically active cells to determine cellular metabolism. The most often utilized tetrazolium salt for in vitro toxicity evaluation of nanoparticles is 3-(4,5- Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)[24]. The method is because of the rapid yields, reproducible outcomes, and minimal model cell manipulation.

1. Tetrazolium salts assay: This assesses a cell population's viability in comparison to untreated control cells.
2. Alamar Blue: It has indeed nowadays been used in nanotoxicological research by measuring cellular redox potential.
3. Incorporation of [3H] thymidine into the DNA: It is a sensitive measurement of cell proliferation.
4. Cologenic assays: The cologenic analysis enables researchers to investigate the effects of certain drugs on cell survival and proliferation[27].

C. Apoptosis Assay

Apoptosis is among the key markers observed in the in-vitro assessment of nanoparticle toxicity. Excessive free radical production is thought to be the cause of apoptosis and DNA damage. Oxidativestress has been observed in cell culture systems to produce apoptosis and DNA damage. Apoptosis caused by nanoparticles has been described in numerous investigations. Cell death metrics such as annexin-V and propidium iodide (PI) are commonly utilized in toxicity testing[21]. The assay is based on the theory that whenAnnexin-V binds to phosphatidylserine, it increases fluorescence, indicating that the plasma membrane has been externalized. The initiation of the caspase-dependent process causes the plasma membrane to externalize. PI isan impermeable pigment that only stains the nucleus when the integrity of the cell membrane is disrupted, which is associated with the late stage of apoptosis[24]. When human HepG2 hepatoma cells were exposed to silica nanoparticles, morphological alteration in the nucleus and induction of apoptosis were detected in the followingassays:

1. DNA laddering: By isolating and fluorescently and tagging DNA from cells exposed to a suspected toxicantin culture, the earliest DNA damage assay approach assesses this fragmentation. Gel electrophoresis is then usedto detect DNA damage

2. Caspase assay: The caspase assays are based on zymogen processing to the active enzyme and proteolytic activity measurements
3. Comet assay: The Comet Assay, also known as single cell gel electrophoresis, is a sensitive and quick technique for measuring and assessing DNA damage in single cells.
4. TUNEL assay: The TUNEL test, which draws its name from Terminal deoxynucleotidyl transferase dUTP(deoxyuridine triphosphate) nick end labelling, is based on double-strand breakage, identical to the damage involved in DNA fragmentation during apoptosis.

Annexin-V: This is commonly used to identify apoptotic cells, interact significantly with phosphatidylserine in a calcium-dependent form[28].

D. Necrosis Assay

This method of assessment has the following assays,

1. Neutral red uptake: The Neutral red uptake cytotoxicity assay technique is a cell viability assay based on viable cells' capacity to ingest and bind neutral red, a weak cationic supravital dye that rapidly penetrates cell membranes via non-ionic diffusion and mainly accumulates. intracellularly in lysosomes, with lysosomal fragility and other progressive alterations become irreversible Cytotoxicity is defined as a concentration-dependent decrease in the intake of neutral red upon chemical exposure, resulting in a sensitive, integrate Both cell integrity and growth inhibition are illustrated by this signal.
2. Trypan blue assay: Cells are treated with agents, trypsinized, and stained with trypan blue, a diazo dye that is taken up by dead cells but excluded by living cells, in the trypan blue experiment. The total number of live cells retrieved from a particular plate is shown by unstained cells. This technique is beneficial since it transmits the real amount of live cells and raises or reduces in contrast to untreated control cells.
3. LDH: LDH is a soluble cytosolic enzyme that serves as an indicator of lytic cell death. The colorimetric lactate dehydrogenase (LDH) assay which is based on the oxidation of the yellow tetrazolium salt, INT, to a red formazan, has a long tradition in the clinic to evaluate tissue or cell damage. As significant amounts of LDH are released from the cytosol upon cellular necrosis, LDH activity is measured in the cell culture supernatant[27].

E. DNA Damage Assay

Unrepaired single- and double-strand DNA breaks can develop from nanoparticle exposure via changes in the oxidative environment, apoptosis, or physical interactions between the DNA and nanoparticle, resulting in DNA damage that is known as genotoxicity. The oldest DNA technique is DNA laddering. This fragmentation is characterized by the damage assay procedure such as separating and fluorescently tagging DNA from cells that have been subjected to a possible toxin in culture. Gel electrophoresisis used to identify DNA damage. The Comet test, also known as the single-cell gel electrophoresis (SCGE) assay, is most typically used to assess DNA damage. Originally, in the Comet assay As explained by Singh et al., the cells are embedded in agarose gel, which lyses the cells and denatures the DNA before subjecting it to Ethidium bromide labeling (for quantification) and electrophoresis (for separation).

Comet assay: The quantity of tailing, or DNA fragments trailing after electrophoresis, indicates the level of DNA damage. The Comet assay has been used in several investigations to detect DNA damage, when exposed to nanoparticles. Barnes et al. work has demonstrated that the TiO_2 NPs concentrations (4 and 40 mg/mL) testedcomet test revealed no genotoxicity. While not concentrating on They were able to demonstrate the toxicity of TiO_2 nanoparticles. that in vitro testing may be done using a consistent procedure utilizing everlasting, accomplished quantitatively and reproducibly 3T3 cells and TiO_2 nanoparticles (both purchased and provided created in-house). While this study suggests that the Comet assay is repeatable, the authors also state that rigorous attention to sample preparation and handling was required to achieve harmony as a result, although it is conceivable to succeed. Handling discrepancies are likely due to reproducibility complicating the interpretation and comparison of the genotoxic NP's impacts[21].

1. TUNEL assay: TUNEL, which stands for 'TDT-mediated dUTP-biotin nick-end labeling, is another DNA damage test that depends on double-strand breaks such as the damage required for DNA fragmentation while in apoptosis. The most often used approach for this test is to repair isolated fragmented DNA, exogenous DNA polymerase-I was used. DNA from the nucleus to integrate the polymerase, 5-Bromo-2-deoxyuridine, a

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synthetic deoxythymidine analogue, is introduced. BrdU is incorporated into repaired double-strand breaks in apoptotic cells. Anti-BrdU antibody coupled with FITC is used to mark BrdU-containing DNA in order to measure the number of double-strand breaks in the nuclear DNA

F. Oxidative Stress

The engineered nanoparticles in a biological environment may disrupt the cell's oxidative balance. This is characterized as oxidative stress, and it results in excessively high amounts of and other intracellular reactive oxygen species (ROS) like Superoxide (O2), Hydroxyl radical (-OH), Peroxy radical (ROO), and other radicals hydrogen peroxide (H2O2) or reactive nitrogen species (RNS) Nitric oxide (NO), Peroxynitrite anion ($ONOOCO_2^-$), Peroxynitrous acid (ONOOH), and Nitrosoperoxycarbonate anion are among examples. The development of inappropriately high levels of ROS and RNS can have a variety of toxicologicaleffects via reactivity with proteins, lipids, or nucleic acids, resulting in improper cellular function via reaction with intracellular components such as proteins, lipids, or nucleic acids. An illustration of oxidative stress, as thecell, either respond to increased stress by increasing antioxidant generation or exhausts cellular resources of superoxide dismutase (SOD) or glutathione (GSH) because of high amounts of RNS or ROS[21].

1. 2, 7-dichlorofluorescein (DCFH) assay: The dye is produced as a diacetate precursor in the 2, 7-dichlorofluorescein (DCFH) test, which is cleaved by high pH to produce the non-fluorescent product DCFH. When ROS is present, DCFH is converted to a fluorescent product, 2, 7-dichlorofluorescein, which may be detected by fluorimetry[1].
2. Electro paramagnetic resonance (EPR): This is a technique frequently used to evaluate nanoparticles and particle-induced ROS production. Quantification can be accomplished by using certain spin traps or probes in conjunction with specific reagents that also identify the free radical species produced.
3. Lipid peroxidation assay: The lipid peroxidation assay has been widely utilized to demonstrate the potential of various nanomaterials to induce lipid peroxidation in a number of cell types, including fullerenes in human dermal fibroblasts (HDF) and human liver cancer cells (HepG2).

Plasmid assay: ROS generation has been measured using the plasmid assay. The unwinding and linearizationof a coiled bacterial DNA plasmid is employed in this experiment to quantify free radical and/or ROS exposure[27]

G. Viability Assay

Mitochondrial activity was detected. The colorimetric MTT test is a commonly used cell viability assay that relies on the reduction of the yellow tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) to purple water-insoluble formazan in cells with undamaged mitochondria. It has been developed and used in several cytotoxicity investigations, as well as to verify other techniques and to detect the toxicity of nanoparticles[25]. Lactate dehydrogenase (LDH) is a cellular enzyme that regulates pyruvate and lactate levels via nicotinamide adenine dinucleotide (NAD) oxidation.[1] LDH is released into theextracellular space when cells suffer haemolysis or necrosis while preserving enzymatic activity. The time- dependent change in spectroscopic absorbance of the reduced tetrazolium molecule assessed by an enzyme- linked immunosorbent assay (ELISA) reader or UV-Vis spectrophotometer is directly proportional to the sampleLDH content and by extension, the level of cellular damage[29].

H. Endotoxin Assay

Endotoxins generate acute inflammatory reactions in humans. Because many nanoparticles are made in non-sterile circumstances, they may be contaminated with bacteria or endotoxins, both of which can trigger inflammatory responses. Particles should be avoided, as a result, be identified and treated appropriately to eliminate any contaminants that interfere. The LAL technique gives rise to three major assays: the gel clot test, the coagulogen-based (turbidity) assay, and the chromogenic assay.[6] Endotoxin-activated enzymatic coagulogen is used in the gel clot technique. Cleavage is accomplished by mixing a portion of the LAL solution with the endotoxin sample solution and following appropriate incubation, checking for clotting. The method is somewhat subjective because of confirmation caused by a simple positive or negative tube inversion to view the clot Inference from successive dilutions is required for formation and quantification. The quantification of tests varies by analysing either change in turbidity or coagulogen decreased clotting time or peptide fragmentation. The alternative chromogenic method replaces chromogens for coagulogen that

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emit chromophores when cleaved[25].

5.2 In-Vivo Methods

Beyond in-vitro experiments, another field of research for nanotoxicity is to investigate the impact of nanoparticle exposure on whole live organisms. Typically, these in vivo experiments are carried out in mice 152-155 or rats 156,157 While in-vivo experiments show potential because of the usage and sacrifice of animals,there is an ethical consideration. These investigations allow for the investigation of long-term impacts including tissue damage. Localization, biodistribution, and retention/excretion are these various sorts of in-vivo investigations that can guide the selection of an appropriate model framework for future in-vitro experiments, as well as offer toxicity information In-vitro research, yielded no information in addition to the LD50 (lethal dosage for 50% of participating animals) experiment. Typically, in vivo nanoparticle toxicity studies focus on one or more of three primary areas: changes in blood serum chemistry and cell population, changes in tissue shape as measured by histology, or overall nanoparticle biodistribution[24].

A. Biodistribution and Clearance

Biodistribution and clearance investigations are typically conducted concurrently to study the entire process of tissue or organ localization and to track the transit of nanoparticles through the animal over time. Nanoparticles are detected in entire animals and fixed tissues that are alive or recently slaughtered using fluorescence (native or tagged) or scintillation (through radiolabels), as well as ICP-MS or ICP-AES (using homogenized samples of tissue) Long-term investigations of nanoparticle metabolism and excretion are used to conduct clearance studies at various points of time following exposure. This is mostly accomplished through the examination of blood samples or killed animals investigating the number of nanoparticles present 168–170[8]. The technique of quantitating nanoparticles using ICP-MS, radiolabelling, and fluorescence is depending on the type/properties of the particles. Nanoparticle biodistribution and clearance studies put a spotlight on nanoparticle retention and transit in mammals. However, because many of the frequently utilized quantification methods require the inclusion of a radiological or fluorescent tag, the presenceof which may indicate these nanoparticles' biodistribution and clearance are altered. There is still a significant demand for quick analytical techniques for the In-vivo detection and localization of nanomaterials[30].

B. Haematology and Serum Chemistry -

Assessing blood composition, both cell type and serum chemistry, forchanges that occur after nanoparticle exposure is one of the most frequent in vivo toxicity studies. In this case, blood homeostasis is used as a surrogate for toxicity, where any variation in blood components, either an increase or a reduction, is reflective of the usual (pre-exposure) situation toxicity. In haematology, cell population refersto either a broad category of cells such as red and white blood cells or a narrower category of cells such as T cells and macrophages. These studies take dispersion into account. Both typically, haematological and serum chemistry tests are conducted on computerized analysers. Protein levels are usually quantified during automated investigations of serum chemistry using either turbidity or nephelometric techniques, in which antibodies for the proteins of interest are introduced to the sample resulting in aggregation and changed light transmission/scattering. (For ex. Wistar rats were utilized as models to investigate the dose-dependent in vivo toxicity of silver nanoparticles, and haematological parameters such as WBC and RBC count, platelet count, haemoglobin levels were measured)[31].

C. Histopathology

Another common in-vivo test is the assessment of histological alteration in specificcells/tissues/organs post nanoparticle exposure. A histological investigation is carried out on tissues repaired following the sacrifice of the exposed animal when alterations in tissue and cell morphology are assessed via microscopy using light. The normal approach for histological preparation is to fix the tissue/organ before embedding it in a paraffin matrix, then thin pieces were cut with a microtome. After that, tiny portions can be dyed with dyes, most often the H & E stain or hematoxylin (blue nucleus/nucleic acids) and eosin (pink stain for the cytoplasm). Image contrast is provided via staining in order to highlight the visually detected changes incell or tissue shape. Histological/histopathological tests give actual evidence of

morphological alterations, showing that toxicity is associated with changes in tissue and cell morphology on a scale that can be seen with a light microscope[21].

VI. PREVENTIONS

Earlier there were around 100-150 foods, food additives, and material that comes in contact with food like aluminium foil paper, and kitchen products were reported to contain hazardous NPs in the international market but nowadays the range of NPs is becoming vast that around 400-550 Nano foods were reported worldwide. Although the health concerns of nanoparticles are not clearly illustrated, work practices and engineering control measures for avoiding exposure are widely recognized[2]. Most measurements utilized arecomparable to normal laboratory techniques regarding the use of harmful chemicals and gases. So there is an urgent need for unified terminology and nomenclature for characterizing the physical properties of nanoparticles[18].

The aspects that must be considered to improve risk assessment of Nanoparticle Toxicity werementioned below;

- Protocols must be devised to assess the release of nanoparticles from a wide range of manufacturing processes, product composition, and use.
- Proper disposal of Nanoparticle containing products should be performed via proper authorities.
- Safety guidelines must be employed while the manufacturing, processing, and handling of nanoparticles.
- If alternatives with less toxic elements have similar properties to nanoparticles, then the use ofnanoparticles should be avoided.

As NPs show interaction with cell surface so NPs morphology must be designed as they can show lessbinding with the cell surface and use ligands polyethylene glycol.

VII. CONCLUSION

The physicochemical characteristics of a material determine how likely it is to interact with the different proteins and cells that make up the biological milieu, which leads to nanomaterial toxicity. Surface area, shape, agglomeration, aggregation solubility, and size with protein (opsonisation) interactions inside the host are determined by NPs routes of entry and their possible bio-distribution safety problems. In addition to physicochemical characteristics, NPs such as carbon nanotubes, titanium dioxide NPs, quantum dots, gold NPs, and silver NPs cause cytotoxicity by the formation of toxic ions, fibrous structure, high surface charge, and generation of radical species. To evaluate NP toxicity, in vivo and in vitro experiments are also performed. The harmful species can be replaced with less toxic ones to decrease the dissolution of the nanoparticles to poisonous ions. The toxic species can be substituted with less toxic elements with comparable properties, the nanoparticle can be capped with a shell material, the morphology of the nanoparticle can be chosen to minimise surface area and thus minimise dissolution, or a chelating agent can be co-introduced or functionalized onto the surface of the nanomaterial.

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