Environmental Nanotoxicology

Ayush Bisoyi
Department of Chemistry, Private International English School
Mr. Manzar Alam & Mrs. Deepa Dinesh

Abstract

Nanotechnology is among the most important domains of leading science and technology and is a highly promising field for future research. It deals with theoretical and practical structures at the nanolevel (10^{-9} m). Nanotechnology is being used in various fields and has led to the evolution of many new groundbreaking inventions such as nanodrugs, nanotubes, nanorobots, nanocrystals, nanosensors, nanoactuators and nanomotors. Therefore, it has been an integral part of pharmaceutical, surgery, mechanics, electronics, material science, and many more fields. These nanoparticles have had huge success in finding scientific breakthroughs. However, they give rise to harmful conditions as well. The behavior of substances at the micro and nano levels differs from its macro level. Due to this, these substances interact with their surroundings distinctly. Hence, these nanoparticles are mostly non-biodegradable, cause contamination and are highly toxic for the environment. That brings us to the issue of environmental nanotoxicology. With the growth of nanotechnology, it becomes very important to address the issues of nanotoxicology. The corrosive nature of some nanoparticles is clinical in causing certain diseases in humans and animals whereas they also pollute the water sources and lead to land degradation since they are of chemical origin and are mostly inorganic substances. It is also vital to have a detailed analysis of the harmful effects of certain nanoparticles on the human body as well as the environment.

Introduction

Nanotechnology has been the most explored and extensively studied area in recent times since the nanoscale stands out as perhaps the most exciting field in which different sciences and disciplines converge such as physics, chemistry, engineering, mathematics, health sciences and much more. It's an essential element in multiple areas of science and technology. The distinctive behavior of matter at the nanoscale has drawn significant scientific interest. At such small scales, matter behaves differently, allowing us to witness quantum effects. These effects are crucial for the understanding of matter and the working of quantum processes and phenomena. Therefore, nanotechnology is the creation and utilization of materials, devices and systems through the control of the properties and structure of matter at nanometric scale. The various branches of nanotechnology that have emerged with the development of it are: nanobiotechnology, nanotoxicology, nanophysics, nanoengineering, nanoelectronics, nanoplasmonics, and nanomechanics.

The application of nanotechnology is as diverse as communications, medicines, energy production, water treatment, agriculture, textiles and cosmetics. Most of these applications are nothing short of revolutionary, including improved disease treatment and sustainability in food production. The rise of nanotechnology has also seen the development of products such as carbon-based molecules including nanotubes, fullerenes, graphene as well as multi element materials such as quantum dots.

In 1974, Norio Taniguchi, working at Tokyo University of Science, coined the term nanotechnology as the process of manufacturing materials from single atoms and molecules.

Nanotechnology And Its Applications

A brief look into the fundamentals and application of this field of science illustrates the increasing demand of nanotechnological materials and may help us to find ways to use nanoparticles in the right ways.

Nanotechnology is defined as the manipulation of matter at nanoscale. It is a field of science and engineering that deals with design and manufacture of extremely small devices and structures. In the nanoscale, quantum effects such as quantum hall or the Casimir quantum effect become visible.

Nanotechnology has developed into nanobiotechnology, nanoelectronics, nanoplasmonics, nanoengineering, nanomedicine and nanorobots. It is applied in food and agriculture, energy, environmental preservation, for diagnosis and treatment of diseases such as HIV, cancer, and Alzheimer's. In cosmetics, nanotechnology has been used in UV filters and in drug delivery agents. In the automotive industry, this technology is used in nanocoating or nanopaint technology, carbon black and nanofilters. Some sports equipment like tennis, badminton and golf use materials like carbon nanotubes, silica nanoparticles, fullerenes, nanofibers and nano-titanium.

Nanoelectronics

Quantum Dots are man-made semiconducting nanoscale crystals that are capable of transporting electrons. When semiconducting materials are small, quantum effects emerge which quantize the energy levels of electrons or holes in the particles. They can be useful for self-assembled nanodevices.

Graphene is an allotrope of carbon which consists of a single layer of atoms arranged in a honeycomb lattice. It is a zero-gap semiconductor. There have been many developments like graphene superlattices, nanoribbons, graphene quantum dots, graphene oxide among others.

The uniform and symmetrical structure of nanotubes and nanowires allows higher electron mobility, a higher dielectric constant and a symmetrical electron/hole characteristic. Nanofabrication can be used to construct ultradense parallel arrays of nanowires. Silicon nanowires are being increasingly studied for diverse applications in nanoelectronics, energy conversion and storage.

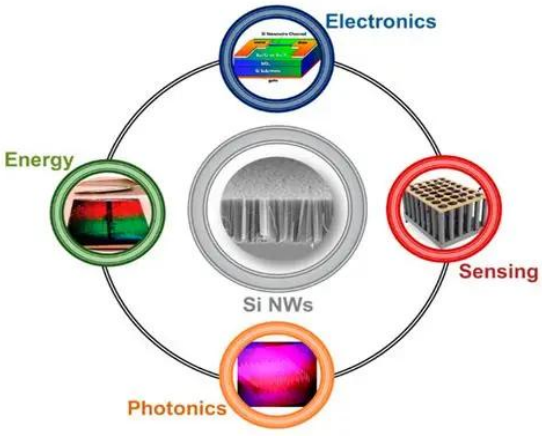

Figure 1: Uses of Silicon Nanowires

Nanorobots

Nanorobotics involves the development of nanoscale systems and procedures such as nanofabrication, nanomotors, nanoactuators, nanosensors, and modeling of materials and processes at the nanoscale. It consists of assembling nanometer-sized parts, and manipulation of biological cells or molecules. It is used in fields such as medicine and environmental science, particularly for the removal of pathogens and toxins from biological fluids and water sources. These are activated by UV light, DNA origin based nanorobots, light induced nanotransducers, magnetic nanolink nanoswimmers and other mechanisms and techniques.

Nanotechnology in Medicines

Nanotechnology is involved in intracellular targeting, treatment of chemotherapy, avoidance of multidrug resistance, treatment of leprosy, ocular drug delivery, brain drug delivery, DNA delivery and Lymph targeting. It is also used in detection of pathogens in humans, separation and purification of molecules and cells and detoxifying agents.

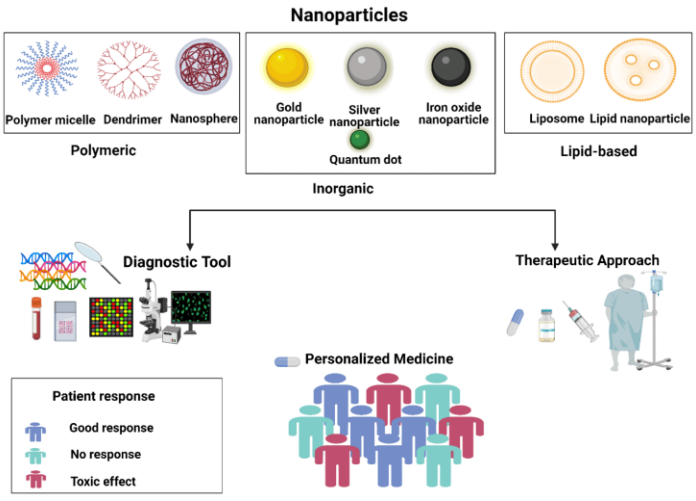

Figure 2: Nanoparticles in Healthcare

Pharmaceutical nanotechnology is used to construct a delivery system that combines targeting, imaging and therapeutic functionalities into nanoplatforms.

Nanotechnology in Agriculture and Livestock

It is used for creating nanofabricated gel-free systems and high throughput DNA sequencing, micro-chips and expression profiling, creation of DNA microarrays and protein microarrays. It is used to determine genomic sequences, scanning of genes for polymorphisms among others.

Nanovaccines are used in prevention of diseases. Nano-apoptosis can be used to detect tumors and cancer. Researchers at Rice University are using nanoshells injected into an animal's bloodstream with targeted agents applied to the nanoshells to seek out and attach to the surface receptors of cancer cells. Some research groups have been experimenting with ‘smart’ super-paramagnetic nanoparticles. These are injected in the bloodstream which target tumor receptor cells. These are made of iron oxides when subjected to a magnetic field to locate tumor cells and the site of tumor these nanoparticles emit a drug to kill the cancer cells. Quantum dots are injected into the bloodstream of animals to detect malfunctioning cells. When the quantum dots respond to light it may illuminate with light and stimulate the quantum dot to heat up enough to kill the cancerous cell.

For post-harvest management and food biotechnology, nano bar codes and identity preservation, enzymatic nano-bioengineering and for monitoring the quality of agricultural products are used.

Nanotoxicology

The advancements as noted above aren’t the only direct effect of the use of nanoparticles, these come along with the rising concerns of toxicity of such materials and the study of this is called nanotoxicology.

Nanotoxicology is an aspect of nanotechnology and nanoscience which deals with the adverse effects of engineered nanomaterials and nanoparticles on living organisms. Due to quantum size effects and large surface area to volume ratio, nanoparticles exhibit unique features leading to higher toxicity. Inhalation exposure is the biggest concern while various pulmonary effects like inflammation, fibrosis and carcinogenicity. Skin contact and ingestion exposure are other concerns as well.

Nanoparticles may cause toxicity in various ways. It can interact with blood, tissue fluid and can enter the central nervous system as well and affect cardiac and cerebral functions. Nanoparticles may bind with mediators which can activate inflammatory responses.

Several studies have shown that chemically synthesized nanoparticles have high toxicity towards human cells due to the presence of chemicals as surface functional and capping agents. Certain biosynthesized nanoparticles also exhibit toxicity upon reaction with cells, due to disintegration into simpler forms or accumulation.

Nanoparticles are used as nanomedicines and nanocarriers of drugs due to their small size and exclusive properties. Thus it becomes very important to manage the toxicity of nanomaterials. The scope of nanotoxicology is aimed at identifying potential hazards that are useful for the safety evaluation of nanomedicines.

Factors Affecting Toxicity

Size And Surface Area

Smaller nanoparticles have a significantly higher surface area-to-volume ratio, enhancing their biological and chemical reactivity. When the size of a nanoparticle reduces from 30 nm to 3 nm, the number of surface molecules expressed increases from 50 to 10%. The cytotoxicity of nanomaterials results from the interaction of surface molecules and cellular components. Thus, even nanoparticles with similar chemical composition show different levels of cytotoxicity depending on their sizes and surface areas.

Cho et al. reported silver nanoparticles' size-dependent acute toxicity on BALB/c mice after intraperitoneal administration of silver nanoparticles of diameters 10, 60 and 100 nm. Histopathological changes such as thymus cortex apoptosis, focal necrosis, single-cell necrosis, vacuolation, congestion in the liver and congestion in the spleen were only seen in nanoparticles of diameter 10 nm. Du et al. investigated cardiovascular toxicity of different sized amorphous silica nanoparticles (90, 60 and 30 nm) and 60 nm of the silica nanoparticles after intratracheal instillation in rats. Blood levels of inflammation-related proteins, cytokines and tumor necrosis factors were found higher in rats administered with fine silica nanoparticles. Braakhuis et al. showed size-dependent pulmonary inflammation after inhalation of 15 and 410 nm of silver nanoparticles. The larger nanoparticles were cleaned more easily than the finer ones from the lungs which can further cause lung cancer. Lopez-Chaves et al. evaluated subcellular location, toxic effects, and tissue distribution of three different gold NPs' sizes. They used particles of 10,

30, and 60 nm sizes and assessed *in vivo* distribution after intraperitoneal administration in the rat. The gold nanoparticles of 10 and 30 nm crossed the membrane of the nucleus, consequently favoring breaks in DNA. These 10 and 30 nm gold NPs seemingly accumulate more in liver, kidney, and intestine than 60 nm gold nanoparticles. The highest accumulation of 60 nm particle was observed in the spleen.

In summary, NPs have larger surface areas and higher particle numbers per unit mass in comparison with the bigger particles. The engineered nanoparticles possess high surface reactivity, as well as high surface area, which could result in producing higher reactive oxygen species level, thus leading to cytotoxicity and genotoxicity.

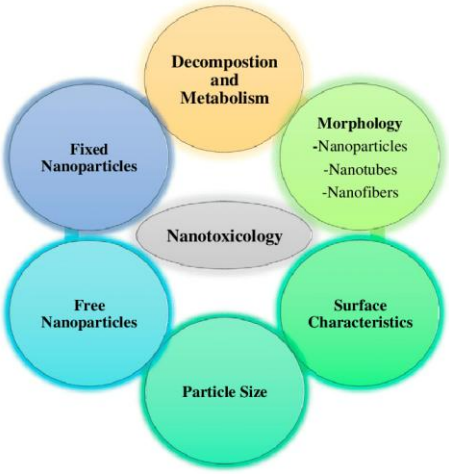

Figure 3: Factors affecting Nanotoxicology

Shape

Shape is an important factor of nanoparticles that play a vital role in determining their biological reactivity as well as toxicity. The typical shapes of nanoparticles are sphere, cylinder, cube, sheet, or rod. The shape of the nanoparticle is important in determining its cellular uptake.

The cellular uptake of carbon nanomaterial of spherical shape and tubes of multi-graphitic sheets was observed in epithelial tissues of both gut and gill. Silver nanoplates were found to be more harmful than silver nanospheres in zebrafish (*Danio rerio*) embryos. The spherical nanoparticles are taken up in greater numbers in cells compared to the other shapes. Gold nanorods cause less accumulation of autophagosomes than gold nanospheres. Steckiewicz et al. examined the cytotoxic properties of gold NP of stars, rods, as well as spheres against human fetal osteoblast, osteosarcoma, and pancreatic duct cell line. The star-shaped gold nanoparticles are the most cytotoxic against human cells. Both cytotoxicity and anticancer potentials of gold nanoparticles depend on shape. The needle-shaped nanoparticles exhibit more toxicity than those with spherical shape, because of their improved multiple endocytic mechanisms, internalization rates, and more efficient adhesiveness to the surface of the target cell.

Aspect Ratio

A nanoparticle aspect ratio is the width to height ratio. An aspect ratio of 1 represents a spherical particle, while a nanotube has an aspect ratio close to zero. The greater the NPs' aspect ratio, the higher the toxicity of the NPs. Aspect-ratio-dependent toxicity is generally observed in the lung. The nanofibers with about 150 nm thickness and 2, 5, and 10 μm length show asbestosis, mesothelioma, and lung cancer, respectively. Muller et al. studied the pulmonary toxicities of carbon nanotubes with a high aspect ratio in

Sprague-Dawley rats following administration directly into the trachea. Carbon nanotube samples caused significant protein exudation and granulomas on the peritoneal side of the diaphragm. Renal toxicity which depends on the shape of silica nanoparticles was reported.

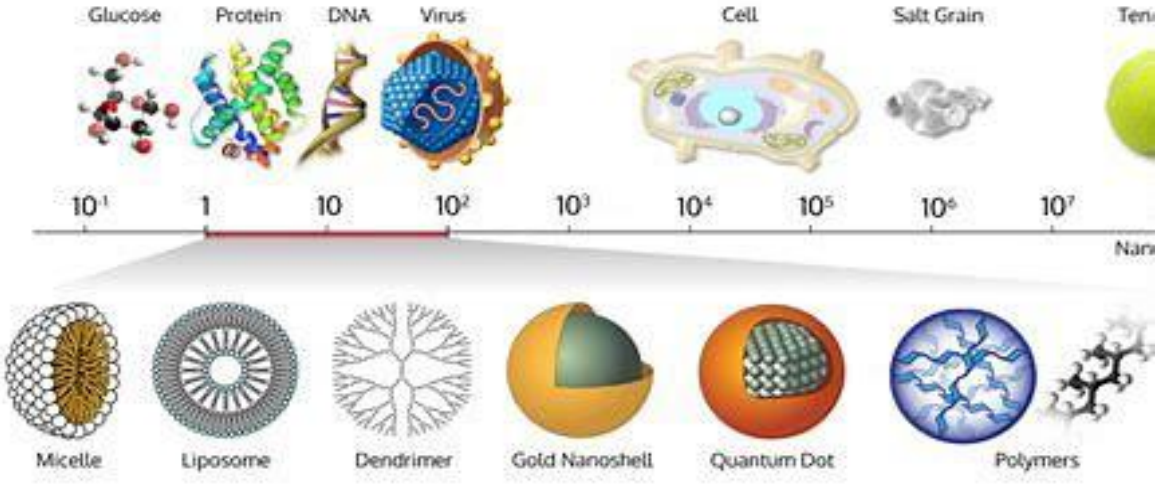

Figure 4: Shapes of nanoparticles

Crystallinity

The type of crystalline structure may affect the toxicity of nanomaterials. Polymorphs, the different crystalline structures of the same chemical composition showed different chemical and physical properties. Lai et al. reported cytotoxicity of 10-hydroxycamptothecin (HCPT) nanoparticle dispersions, which depends on the polymorph, in both *in vivo* and *in vitro* studies. The cytotoxicity results indicated that all the different HCPT nanoparticles' cellular toxicities depended on size and shape. However, the needle-shaped HCPT nanoparticles are more potent in apoptotic response in cancer cells despite similar cellular uptakes as prismatic nanoparticles. Andersson et al. also reported titanium dioxide NPs' uptake and toxicity in A549 lung epithelial cells, which were polymorph-dependent.

Surface Coating or Surface Functionalization

Surface coatings of nanoparticles are applied in order to modify its properties. The surface of a particle (the “core”) is covered with a variety of layer(s) (the “shell”). The objective of the surface coating may be to tailor its stability, wettability, dissolution, or functionality. The surface coating can convert noxious particles to be nontoxic while less harmful particles may become more toxic due to bioavailability. Xu et al. performed an *in vitro* evaluation of the toxicity of iron oxide nanoparticles coated with silica (Fe3O4/SiO2 NP) on the cells of HeLa and A549.

Dissolution

The dissolution ability of nanoparticles is a significant property that determines safety, uptakes, and associated toxic mechanisms. Two identical NPs of similar composition and size may have completely different behavior in dissolution, depending on different surface modification.

Agglomeration

Nanomaterials are likely to agglomerate in solution due to their high free surface energy. The toxicity of nanomaterials is also dependent on whether or not agglomeration occurred. The agglomeration of nanoparticles could be a potential inducer of inflammatory lung conditions in humans. The agglomeration-dependent toxicity of nanomaterials is more commonly observed in carbon nanotubes and oxide nanoparticles.

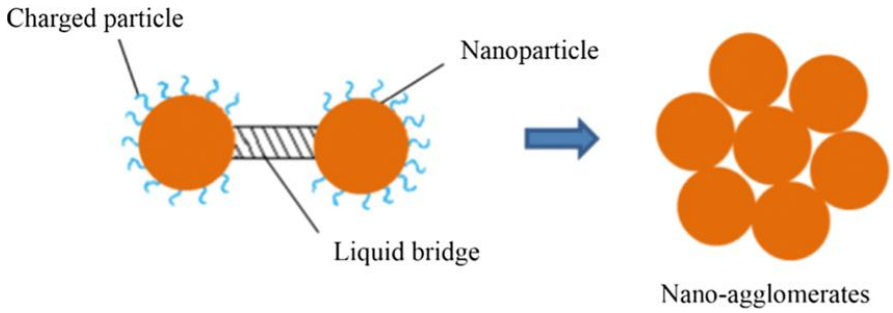

Figure 5: Agglomeration

Composition

Researchers have found that some metal and metal oxide NPs may affect cells inducing DNA breakage and oxidation, mutations, reduced cell viability, warped morphology, induced apoptosis and necrosis, and decreased proliferation. Moreover, metal nanoparticles may persist in the organisms after administration if not carefully engineered. The latest toxicology studies on mice as of 2013 involving exposure to carbon nanotubes (CNT) showed a limited pulmonary inflammatory potential of MWCNT at levels corresponding to the average inhalable elemental carbon concentrations observed in U.S.-based CNT facilities. The study estimated that considerable years of exposure are necessary for significant pathology to occur.

Routes Of Administration

Respiratory Tract

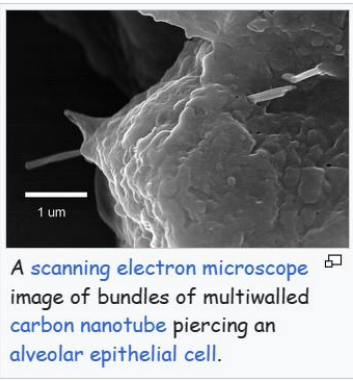

Figure 6

Exposure through inhalation is the most common route of exposure to nanoparticles as airborne particles in the workplace. The deposition of nanoparticles in the respiratory tract is determined by their size and shape or their agglomerates, and they are deposited in the lungs more than other larger respiratory particles. These nanoparticles may enter the bloodstream from the lungs and translocate to other organs like the brain. The deposition efficiency of nanoparticles depends on their diameter and aerodynamic characteristics. These particles are deposited in the entire respiratory tract from nasal cavity to alveoli through different diffusion techniques. Small nanoparticles have the ability to travel more deeply into the respiratory tree and settle and be absorbed by the pulmonary epithelium before entering circulation whereas those with larger diameter are more easily stopped at upper respiratory cavity and expelled through mechanisms of mucociliary clearance. Recent studies have shown translocation of inhaled nanoparticles to extrapulmonary sites, like circulatory system, brain, liver, and others. The toxicity of these nanoparticles depends on the number and size, surface coating, degree of aggregation or agglomeration, the surface charges and synthesis method.

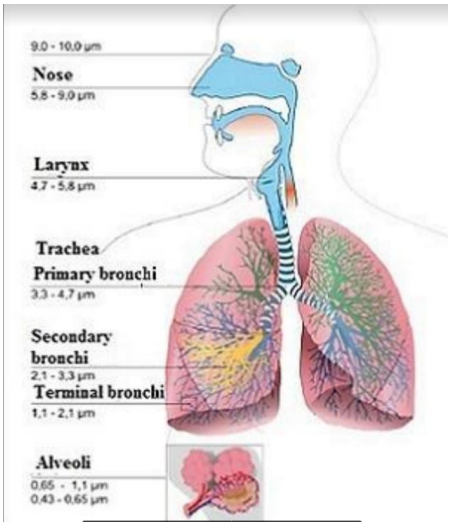

Figure 7

Dermal Exposure

Studies have shown that particles smaller than 1 µm in diameter may penetrate into mechanically flexed skin samples, and that nanoparticles with varying physicochemical properties were able to penetrate the intact skin of pigs. Factors such as size, shape, water solubility, and surface coating directly affect a nanoparticle's potential to penetrate the skin. topical application of raw SWCNT to nude mice has been shown to cause dermal irritation, and *in vitro* studies using primary or cultured human skin cells have shown that carbon nanotubes can enter cells and cause release of pro-inflammatory cytokines, oxidative stress, and decreased viability.In addition, nanoparticles may enter the body through wounds, with particles migrating into the blood and lymph nodes.

Gastrointestinal Tract

Nanoparticles may be ingested along with food and once ingested, these are subjected to the usual digestive process. In the enteric tract, the absorption kinetics is complex and occurs by diffusion through the mucus layer. The smaller the diameter of a nanoparticle, the faster its absorption is. The translocation of ingested particles by intestinal lumen to blood may be influenced by chemical-physical properties of nanoparticles like dimension, form, composition and charge. Studies both on rats and humans have shown that TiO_2 particles, once ingested, get accumulated in the liver and spleen. Ingestion may also accompany inhalation exposure because particles that are cleared from the respiratory tract via the mucociliary escalator may be swallowed.

Effects of Nanoparticles on Animals

Inhalation Exposure

The first known pathology to be caused due to inhaled nanoparticles is malignant mesothelioma (a type of cancer). Asbestos is its major cause. Asbestos mainly consists of naturally occurring fibrous silicate minerals that can easily split into long thin fibers. Two forms of asbestos are seen: long and thin fibers called amphiboles (blue asbestos) and feathery fibers called chrysotile (white asbestos). Amphibole is more toxic than chrysotile. Several processes are believed to be involved in the asbestos-induced mutations in the mesothelium. Asbestos fibers' length to width ratio facilitates their penetrations deep in the lung where they irritate the pleura. This may result in the formation of scars (plaques) or a malignant process (mesothelioma).

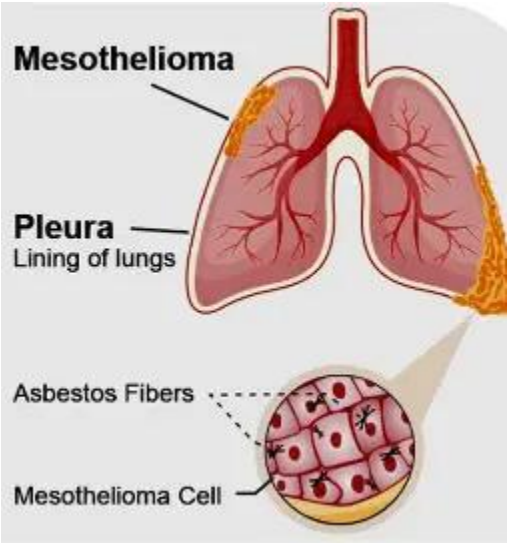

Figure 8: Effect of Asbestos

Asbestos fibers may also disturb the mitotic spindle of cells and disrupt mitosis, causing chromosomal damage and irregularities. Asbestos is also associated with ROS induction which may cause DNA damage. It may also activate the expression of early-response proto-oncogenes. When a phagocyte tries to engulf a fiber longer than it can completely enclose, it results in "frustrated phagocytosis". It is accompanied by spillage of phagolysosomal enzymes which induces inflammation and cytokine release that encourages macrophage fusion and giant cell formation.

Dermal Exposure

Data on skin penetration and permeation studies show that nanoparticles smaller than 4 nm penetrate intact skin, those between 4 nm and 20 nm potentially can penetrate intact and damaged skin , those from 21 to 45 nm can penetrate damaged skin and nanoparticles larger than 45 nm don't penetrate the skin.

An example of toxicity after dermal exposure to ultrafine particles is the disease endemic non-filarial elephantiasis known as podoconiosis. It occurs in countries in tropical Africa, Central America and northwest India, where irritating volcanic soil can be found. The detailed pathogenesis mechanism is not fully understood, but there are findings of ultrafine particles (of the oxides of aluminum, silicon, magnesium and iron) absorbed through foot skin, phagocytosed by macrophages and retained in lower limb lymph nodes. It starts with itching and burning sensations and subendothelial edema and progresses with collagenization of afferent lymphatics which narrows and obstructs the lumen. Later, two types of swelling occur: soft and fluid (water-bag type) or hard and fibrotic (leathery type). There are acute episodes with hyper-pyrexia and the foot is warm and painful. The pathological changes may progress with fusion of the interdigital spaces and ankylosis of the interphalangeal or ankle points.

Induces Hemolysis

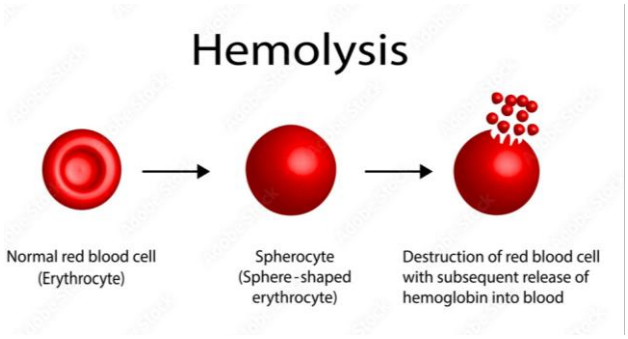

Figure 9: Hemolysis

Hemolysis occurs when erythrocytes are damaged and hemoglobin leaks out of them. A nanoparticle may induce hemolysis via direct erythrocyte membrane interactions or specific antibody-mediated mechanisms. Then, these nanoparticles may absorb hemoglobin or cell debris, changing their biological identity in a way that makes them a likely target for phagocytosis, mediated via scavenger receptor and phosphatidylserine. Surface properties (surface charge) have been recognised as the decisive factor for direct erythrocyte membrane interactions. Studies on fullerenes of similar size but different surface charge, showed that the negative charge didn't cause hemolysis, and an increased number of cationic surface groups corresponded to increased hemolysis. Neutralizing the cationic surface charge by blocking the primary amino groups resulted in a great decrease in hemolysis.

Effects Of Nanoparticles On Aquatic Organisms

QD Exposure To Algae And Microbes

Weathering of various types of QDs under acidic (pH ≤ 4) or alkaline (pH ≥ 10) conditions significantly increased bactericidal activity due to the rapid (<1 min) release of cadmium and selenite ions following QD destabilization upon loss of the organic coating.

Trophic transfer and biomagnification of QDs were assessed in experiments with reconstructed trophic chains, where living organisms were exposed to QDs and then used as food to other species of a higher trophic level (Fig. 6). In freshwater, two trophic level transfer of QDs was observed from bacteria (*E. coli*) to protozoan (*Tetrahymena pyriformis*) (TTF = ~ 5.4) (Werlin et al., 2011); from algae (*P. subcapitata*) to daphnia (*C. dubia*) (Bouldin et al., 2008); and from zooplankton.

Toxicity Of Specific Nanomaterials

Metallic Nanoparticles

Gold Nanoparticles

Gold nanoparticles are arguably the first nanoparticles that are used in commercial materials and approved by the United States Food and Drug Administration (USFDA) as nanomedicine and nanocarrier. Moreover, these nanostructures possess unique size-dependent surface plasmon resonance properties that make them utilizable in biosensor applications. In spite of these applications, gold nanoparticles are also considered to be toxic based on the administered dose and concentration via accumulation in cells, similar to heavy metals.

Senut et al. explored the size-dependent toxicity of gold nanoparticles towards human embryonic stem cells and their neural derivatives. Particle sizes such as 1.5, 4, and 14 nm of gold nanoparticles were used to evaluate its neuronal differentiation, viability, DNA methylation, and pluripotency. The result of the study revealed that the chemically synthesized gold nanoparticles of size below 20 nm are highly toxic to stem cells by altering cellular DNA methylation and the hydromethylation pattern. Recently, Jo et al. evaluated the *in vitro* and *in vivo* toxicity, as well as estimated the oral absorption and tissue distribution biokinetics of orally administered, chemically synthesized gold nanoparticles using human and rat intestinal cells for 14 days. The result revealed that the gold nanoparticles were nontoxic for 24 h in terms of membrane damage, oxidative stress, and cell proliferation inhibition. However, they also revealed that these nanosized gold particles are toxic after 14 days exhibiting long-term and high concentration

exposure dependent on toxic reactions. Semmler-Behnke et al. showed that gold nanoparticles can accumulate in the fetus of a rat via maternal blood and can lead to toxicity towards the fetus.

Silver Nanoparticles

Silver nanoparticles are toxic towards cancer cells by releasing ROS, specific to cancer cells. The nanoparticle-mediated oxidative stress and DNA damage can be reduced by the antioxidant *N*-acetylcysteine. Similarly, Ahamed et al. demonstrated that the silver nanoparticles are toxic to the cells of the skin, brain, liver, lung, and reproductive and vascular systems of mammals. de Lima et al. stated that silver nanoparticles possess the ability to trigger inflammatory reactions in human cells. Moreover, these nanosized silver particles had the ability to cross the cell membrane and reach the nucleus which causes increasing damage to the genetic material and hence genotoxicity. Gaillet and Rouanet examined the toxicity of silver nanoparticles after their exposure towards humans via the oral route. They revealed that the silver nanoparticles cause toxic side effects mainly in the intestinal tract and liver via oral exposure. It is noteworthy that the silver nanoparticles produce free radicals and induce oxidative damage via cellular oxidative stress, which leads to inflammatory reaction-triggered toxicity and death by apoptosis or necrosis. Furthermore, the accumulation of silver at an increased concentration in cells may lead to Parkinson's disease, silver-Russell syndrome, and Alzheimer's diseases. After exposing polyvinyl-pyrrolidone-coated silver NPs (6-20 nm) to human lung cancer cell line, Foldbjerg *et al*. have reported a dose-dependent cytotoxicity, and cellular DNA adduct formation.

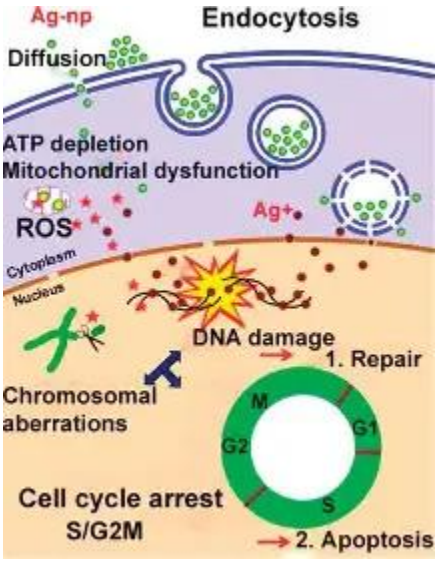

Figure 10: Effect of Ag nanoparticles on cancer cells

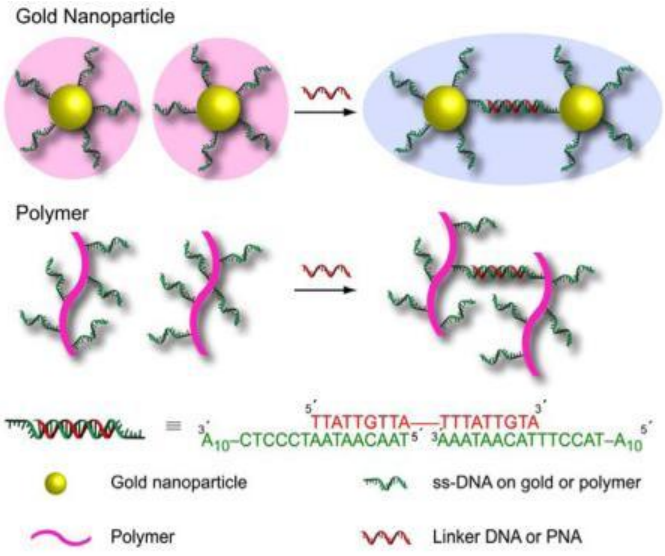

Figure 11: DNA Methylation

Copper Oxide Nanoparticles

Numerous studies have revealed that the copper oxide nanoparticles are highly toxic towards microbes such as bacteria, fungi, algae, and viruses as well as cancer cells. In spite of these exclusive biomedical properties, several reports showed that copper oxide nanoparticles are also highly toxic to normal and healthy human cells. Karlsson et al. evaluated the toxicity of metal oxide nanoparticles such as oxides of titanium, iron, zinc, and copper with carbon nanoparticles and multi-walled carbon nanotubes (MWCNTs) using the human A549 lung epithelial cell line. The result showed that the copper oxide nanoparticles are highly toxic to lung cells by causing oxidative lesions and damaging DNA, compared to other nanosized metal oxides, carbon nanoparticles, and MWCNTs. In addition, Fahmy and Cormier demonstrated that copper oxide nanoparticles exhibit cytotoxicity in airway epithelial cells by inducing oxidative stress. Furthermore, Alarifi et al. showed that copper oxide nanoparticles are cytotoxic and genotoxic towards human skin keratinocyte cells. It is noteworthy that the copper oxide nanoparticles also showed toxic reactions towards human lung epithelial cells, cardiac microvascular endothelial cells, HepG2 cells, and human skin organ culture. Moreover, Atha et al. demonstrated that copper oxide nanoparticles are toxic to terrestrial plant models such as *Raphanus sativus*, *Lolium perenne*, and *Lolium rigidum* by damaging their DNA.

In recent times, Wongrakpanich et al. stated that copper oxide nanoparticles exhibit high toxicity towards lung epithelial cells, which depends on their size. Four and 24 nm sized particles were used for the study and the result demonstrated that the 24 nm sized oxide nanoparticles of copper were highly toxic to cells, compared to 4 nm sized ones. In addition, Akhtar et al. showed that copper oxide nanoparticles induce dose-dependent genotoxicity by stimulating ROS generation in human lung epithelial cells. Likewise, Srikanth et al. showed that the copper oxide nanoparticles exhibited cytotoxicity towards Chinook salmon cells by altering their morphology and inducing oxidative stress. In addition to cytotoxicity and genotoxicity, it is noteworthy that the copper oxide nanoparticles also induce neurotoxicity and hepatotoxicity. Bulcke and Dringen examined the toxicity of copper oxide nanoparticles towards astrocytes in the brain and revealed that the nanoparticles rapidly undergo endocytosis-mediated accumulation in astrocytes, which increases cellular copper content, ROS production, reduces cell viability, and causes diseases due to metabolic disturbances in brain copper balance. In certain cases, the copper ions act as heavy metals and exhibit trojan horse–like mechanisms and bind with cell organelles including genetic material and inhibit cell development.

Zinc Oxide Nanoparticles

Kao et al. evaluated the toxicity of zinc oxide nanoparticles in broncho-alveolar lavage and white blood cells. The result shows that the nanoparticles interfere with the homeostasis of zinc ions present in

the body fluid. The disintegration of zinc oxide nanoparticles led to an increase in the zinc ions which eventually caused dysfunction of mitochondria, activation of caspase and apoptosis of cells. Similarly, Sharma et al. examined the *in vitro* cytotoxicity of zinc oxide nanoparticles towards human HepG2 liver cells. The result demonstrated that the nanoparticles exhibited apoptotic and genotoxic mediated toxicity towards liver cells. They proved that genotoxicity is due to the damages in DNA and apoptotic toxicity is due to the ROS triggered mitochondrial damage. Furthermore, Heng et al. evaluated the cytotoxicity of spherical and sheet-shaped zinc oxide nanoparticles towards RAW-264.7 mouse cells, BEAS-2B human cells, and primary bone marrow–derived dendritic mouse culture cells. Both the shapes of zinc oxide nanoparticles increased the release of ROS, upregulated the expression of CD80, CD86, and released pro-inflammatory cytokines such as IL-6 and TNF-α which inhibits the growth of cells.

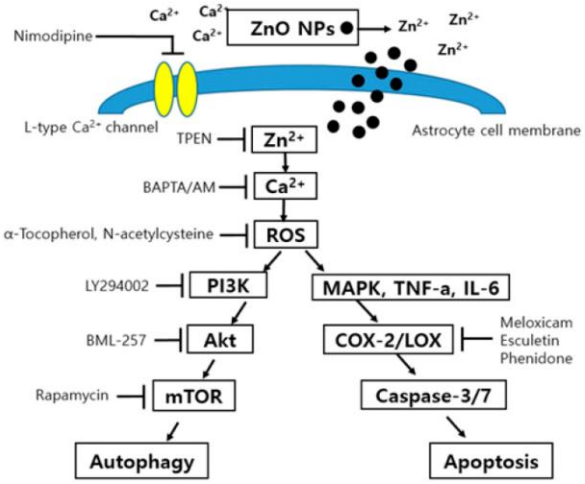

Figure 12: Apoptosis by Zn nanoparticles

Likewise, Valdiglesias et al. also proved that zinc oxide nanoparticles induce cyto- and genotoxicity in neurons which they proved by using SHSY5Y human neuronal cells. They emphasized that the nanoparticle did not enter into the cells and toxicity was due to the presence of nanoparticles in the medium, which lead to cell cycle alterations, apoptosis, micronuclei production, H2AX phosphorylation, and DNA damage mediated cyto- and genotoxicity. Furthermore, they added that the toxicity is dose- and time-dependent, whereas free zinc ions from the nanoparticles are not responsible for cytotoxicity in neuronal cells. Several studies also reported the cytotoxicity of zinc oxide nanoparticles towards rat retinal ganglion cells, human epidermal cells, human nasal mucosa cells, murine macrophages, and human bronchial epithelial cells. Pati et al. reported that the zinc oxide nanoparticles exhibited genotoxic, cytotoxic, clastogenic, and actin depolymerization effects by inducing ROS-mediated oxidative stress responses towards macrophages of mice. In addition, they examined their histopathological effects on adult mice, which revealed that these nanoparticles are highly toxic and lead to severe inflammation and damage to the liver, lungs, and kidneys. Brunner *et al.* found almost complete cell death in the cell culture. Similarly, in another *in vitro* study, zinc oxide NPs have been accounted for change in cell morphology, DNA damage, alteration in mitochondrial activity in human hepatocytes, and embryonic kidney cells. In this experiment, MTT and comet assays have been used for measuring the cell viability and DNA damage, respectively.

Iron Oxide Nanoparticles

Singh et al. reported that the superparamagnetic iron oxide nanoparticles (SPIONs) exhibited cytotoxicity via subtle cellular perturbation such as actin cytoskeleton modulation, gene expression profile alteration, iron homeostasis disturbance, impaired alterations in signaling pathways, cell regulation, DNA damage, and oxidative stress. Petri-Fink et al. examined the cytotoxicity of SPIONs coated with polyvinyl alcohol (PVA), vinyl alcohol/vinyl amine copolymer (A-PVA), and polyethyleneimine (PEI) towards HeLa cells. In addition, Magdolenova et al. evaluated the effects of surface coatings over iron oxide nanoparticles such as oleate using human lymphoblastoid TK6 cells and primary human blood cells. The result revealed that the surface-coated iron oxide nanoparticles altered their behavior and cellular uptake, and helped them to exhibit dose-dependent cytotoxicity and genotoxicity via DNA damage. Furthermore, they conveyed via *in vivo* studies that these magnetic nanoparticles possess the ability to get distributed to different organs and tissues, especially cross the blood-brain barrier in the brain, and lead to acute toxicity, immunotoxicity, reproductive toxicity, genotoxicity, and neurotoxicity.

Aluminum Oxide Nanoparticles

Yoon et al. investigated the cytotoxicity of alumina nanoparticles for concentrations of 25–200 μg/ml and an incubation time of 0–72 h using THP-1 floating cells and adherent cells such as A549, 293, and J774A.1. The results emphasized that cytotoxicity depends on the dose, time of exposure, agglomeration, sedimentation, and enhanced cellular uptake. Likewise, Lin et al. evaluated the cytotoxicity of 13 and 22 nm sized alumina nanoparticles using cultured human bronchoalveolar A549 carcinoma-derived cells and revealed that they are highly toxic than titanium dioxide and less toxic than cerium oxide nanoparticles via alteration in the cell membrane potential, surface chemistry, and exposure duration. In addition, Kim et al. demonstrated that the alumina nanoparticles induce genotoxicity towards BEAS-2B mammalian cell lines. Another study by Asztemborska evaluated and confirmed the toxicity of alumina nanoparticles towards plants via environmental transformation and bioaccumulation. In addition, it was reported that the low-dimensional alumina nanoparticles are highly toxic towards L 929 mouse fibroblast and Neuro-2a Mus musculus brain neuroblastoma cell lines via ROS production and oxidative stress. Chen *et al.* have reported that aluminum oxide NPs disturb the cell viability, alter mitochondrial function, increase oxidative stress, and also alter tight junction protein expression of the blood brain barrier (BBB).

Titanium Oxide Nanoparticles

Titanium oxide is chemically an inert compound, but studies have shown that NPs of titanium dioxide possess some toxic health effects in experimental animals, including DNA damage as well as genotoxicity and lung inflammation. Titanium dioxide NPs (<100 nm) induce oxidative stress and form DNA adducts. Besides genotoxicity, titanium dioxide NPs (5-200 nm) possess toxic effects on immune function, liver, kidney, spleen, myocardium, glucose, and lipids homeostasis in experimental animals.

Non-Metallic Nanoparticles

Carbon Nanoparticles

Magrez *et al.* have reported that carbon-based nanomaterials possess size-dependent cytotoxicity. These investigators have tested various forms of carbon NPs on lung cancer cells to assess cell viability with MTT assay. Carbon nanotubes exert size-dependent toxicity. In animals, multi-walled carbon nanotubes have produced carcinogenic effects similar to asbestos after injecting into the peritoneal cavity, as compared to single-walled carbon nanotubes, which were readily taken up by macrophages. However, long-term accumulation of single-walled carbon nanotubes in the liver has caused disturbance in certain biochemical parameters in the form of LDH, aspartate transaminases, alanine transaminase, glutathione, and malondialdehyde along with changing the organ indices in experimental animals. In case of carbon NPs, along with size, method of preparation and the presence of trace metals determine the extent of toxicity and biological response of the cells. Fullerenes are a type of carbon-based nanomaterials. They are extensively present in our environment released from fuel combustion. Non-functionalized fullerenes C60 are highly distributed in all tissues, and long-term accumulation has been observed in the liver, kidney, bones, and spleen. *In vitro* studies have shown that fullerenes exert genotoxicity in the form of DNA strand breakage, chromosomal damage, and micronucleus formation after incubating fullerenes (1 ng/mL) with Chinese hamster ovary cells, human epidermoid-like carcinoma cells and human embryonic kidney cells (HEK293) for 80 days.

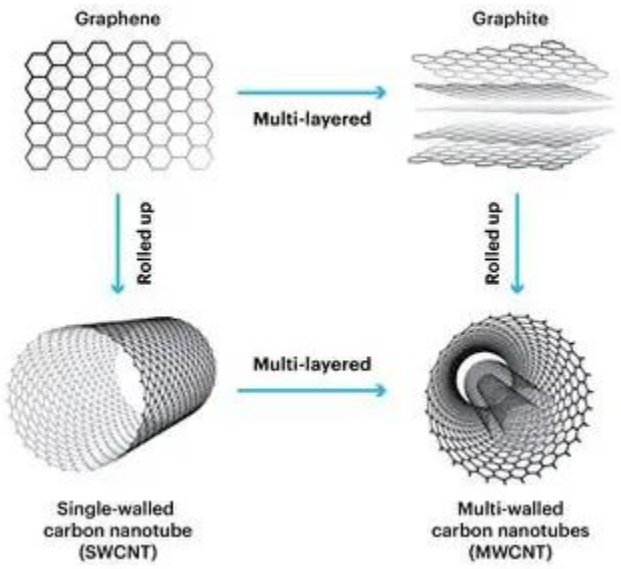

Figure 13: Carbon Nanotubes

Silica Nanoparticles

Lin *et al.* have reported an increase in the level of ROS, LDH, and malondialdehyde after treating human bronchoalveolar carcinoma cells with silica NPs (15-46 nm,) at a dosage range of 10-100 μg/mL. In this experiment, ROS has been measured with 2',7'-dichlorofluorescin diacetate, LDH, with a commercial kit. Similarly, induction of inflammatory biomarkers such as IL-1, IL-6, IL-8, TNF-α (tumor necrosis factor) and mitochondrial damage by silica NPs have been reported in various other studies. In one more *in vitro* study on liver cells, silica-based NPs (70 nm) at 30 mg/kg have been found to alter biochemical parameters along with hepatotoxic effects.

Nanoparticles Of Polymeric Materials

Up to now, poly -(D,L-lactide-co-glycolide)-based nanosystems have been reported with least toxicity, as it undergoes hydrolysis and produces biocompatible metabolites, lactic acid and glycolic acid. However, there has been recently published one report proposing that surface coating induces the toxicity of polymeric NPs towards human-like macrophages.

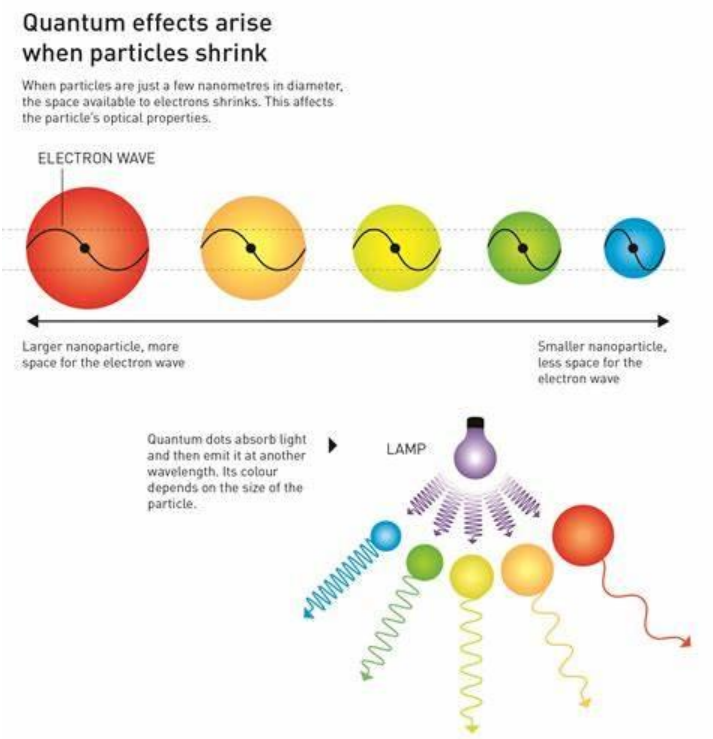

Figure 14: Quantum Dots

Quantum Dots

Zhang et al. have shown that skin penetration is one of the major routes of exposure for QDs to gain access to a biological system. Lovric et al. found that CdTe QDs coated with mercaptopropionic acid (MPA) and cysteamine were cytotoxic to rat pheochromocytoma cells (PC12) in culture at concentrations of 10 µg/mL. Uncoated CdTe QDs were cytotoxic at 1 µg/mL. Shiohara et al. have also observed QD-induced cytotoxicity. MUA-coated CdSe/ZnS QDs were observed to be cytotoxic to HeLa cells and primary human hepatocytes at concentrations of 100 µg/mL (MTT assay). Using primary hepatocytes as a liver model, Derfus et al. found that CdSe-core QDs were indeed acutely toxic under certain conditions.

Conclusion

Over the last decade, nanoparticles have found great interest among scientists and researchers working in various fields within the realm of biomedicine including drug delivery, gene delivery, diagnostics, targeted therapy and biomarker mapping. While their physical and chemical properties are impressive, there is growing concern about the toxicological potential of nanoparticles and possible adverse health effects as enhanced exposure of biological systems to nanoparticles may result in toxic effects leading to serious contraindications. Hence, the study of nanotoxicology becomes all the more important with increased emphasis on their industrial use. For environmental safety and human health, this field is very vital to be researched upon and finding new scientific breakthroughs. However, comprehensive knowledge of nanotoxicity mechanisms and mitigation strategies may be useful to overcome the hazardous situation while treating diseases with therapeutic nanoparticles. Further, it is worth noting for authorities and regulators to enforce strict laws to ensure only appropriate and suitable application of these particles while also regulating for proper disposal of these materials. Also, industrialists and researchers should be aware of its ill-effects and take required actions. All in all, the negatives of nanoparticles shouldn't mask its benefits.

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