Review Article

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Engineered Nanomaterials: Safety and Health Hazard

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Abstract

An exponential growth in nanotechnology, an enabling technology, has led to a rapid progress in diverse areas including medicine, manufacturing, personal care products, ground water remediation, removal of toxic contaminants from air streams, electronics and energy production. Due to the large production and widespread use in consumer products, it is expected that ENMs will be released into aquatic, terrestrial, and atmospheric environments through washing and disposal, where their fate and behaviour are still largely unknown. This could lead to unexpected and unanticipated consequences to environment and human health.Humans get exposed to ENMs at various steps of its synthesis (laboratory);manufacture (industry), use (consumer products, devices, medicines etc.) and the environment (through disposal).

Due to the concerns over nanomaterial risks, there has been a dramatic increase in focused safety research. The present review provides a summary of the published findings with regard to the (1) nanomaterial exposure;(2) hazard posed by nanomaterial to humans and the environment (3) the present discrepancies in our understanding of risk. Also, the nanomaterials induced pulmonary, dermal, systemic, environmental, brain, cardiovascular toxicity and carcinogenicity has been discussed in detail.

Introduction

Nanotechnology is the understanding and control of matter at the nanoscale, at dimensions between approximately 1 and 100 nanometers, where unique phenomena enable novel applications. The nano-structures exhibit significantly novel and improved physical, chemical, biological properties and processes due to their size. Also, the unique optical, magnetic, electrical and physicochemical properties of engineered nanomaterials (ENMs) arise due to higher surface-to-volume ratio and an increased number of atoms on particle boundaries than their bulk counterpart. Due to their distinctive characteristic, ENMs are widely used in cosmetics, food packaging, drug delivery systems, therapeutics, biosensors, and others. The use of nanotechnology in consumer products continues to grow on a rapid and consistent basis. The number of nano-products in 2005 and 2010 was 54 and 1317, whereas more than 1,628 manufacturer-identified, nanotechnology-enabled consumer products have now entered into the commercial market and the number is predicted to be ~3,400 by 2020 [1]. The existing inventory of the Project on Emerging Nanotechnologies (PEN) is dominated by the health and fitness items (49%), with nano-silver products (23.5%) having the maximum share.

Due to the large production and widespread use in consumer products, it is expected that ENMs through washing and disposal will be released into aquatic, terrestrial, and atmospheric environments, where their fate and behaviour are still largely unknown [2]. Humans get exposed to ENMs at various steps of its synthesis (laboratory), manufacture (industry), use (consumer products, devices, medicines etc.) and the environment (through disposal). Although ENMs provides several benefits in these applications, there are concerns about their environmental fate [3]. Hence, the concern for the adverse effects of such ENMs, both to human and the environmental health continues to grow with their diverse applications. The ENMs can be classified as hazardous pollutant due to their unique properties, such as high specific surface area, abundant reactive sites on the surface, large fraction of atoms located on the exterior face as well as mobility that can lead to unexpected risk to public and environmental health [4-6].

European Commission's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) evaluated the risk assessment of products of nanotechnologies [7]. SCENIHR evaluated the knowledge base on the release of ENMs into the environment and the subsequent exposure to humans through inhalation. It was reported that "Examples of the exposure routes for ENMs via the environment are inhalation by human and other air breathing species, and uptake by aquatic organisms from water and sediments. Assessment of exposure concentrations of dispersed nanomaterials requires detailed insight into the process that act on these materials in the environment. However, currently available

knowledge of these processes is insufficient to allow quantitative prediction of the environmental fate of nanomaterials" [7].

The critical questions in relation to ENM exposure are how much (intensity/concentration), how long (duration/frequency) and how many (number) [8]. The main routes by which one can get exposed to the ENMs are inhalation, ingestion and dermal. Since the size of ENMs is approximately equivalent to the biological macromolecules, their interaction behaviour with the biomolecules is quite different. For example, the interaction of ENMs with plasma proteins in the blood could lead to the exposure of novel epitopes that are normally encrypted inside the proteins. Also, the biomolecules such as proteins present in the cell forms a layer over the ENMs, named as "corona" which play important role in their cellular fate. It has also been shown that it is not only the ENMs alone but the "corona" governs the properties of the "particle-plus-corona" compound in the biological system [9,10].

The present review is therefore aimed towards ENM exposure assessment, cellular and organismal to toxicity and its mechanism.

Nanomaterials Exposure Assessment

Exposure assessment of the ENMs can be done based on the data available for the production volume of ENMs, expected use in everyday products, potential industrial applications, pathways for their disposal/recycling, behaviour in the different ecosystems, environmental transport, cellular and biological fate as well as distribution. Unfortunately, there is a lack of sufficient data for any kind of ENMs in this regards. This is probably due to the fact that the, ENMs represent a very diverse group of compounds, produced from different substances such as carbon, silver, zinc, silica, titanium and gold, in multiple forms such as different shapes (oval, square, round, triangle and others) and sizes, as well as with a variety of surface coatings. It is really difficult to conclude a generalized statement about the behaviour of any specific kind of ENMs. Additionally, it has also been reported that, ENMs behave differently after their release into the environment [2]. Studies have been demonstrated that ENMs may undergo agglomeration, aggregation, adhesion, diffusion, dissociation, degradation, bioconcentration, bioaccumulation as well as can be biomagnified in the trophic pyramids [3,11]. These properties of the ENMs are not only dependent on their size/shape, but the local environment and cellular conditions also play an important role. Therefore the physical (i.e., size, shape, surface area and properties, agglomeration state), chemical (i.e., chemical composition, charge, chemical reactivity), biological (i.e., route of administration, metabolism, excretion, inhibition) and environmental (i.e., presence of natural organic matter, microbes, temperature, pH, salinity, acidity, viscosity) factors should be properly be considered for the ENMs exposure assessment.

Exposure of ENMs to the human can occur at various stages of the life cycle (of synthesis, manufacture, product and through the environment). However, the major concern in relation to ENM exposure are how much (intensity/concentration), how long (duration/frequency) and how many (number). The main routes by which one can get exposed to the ENMs are inhalation, ingestion and dermal. Inhalation is the primary route by which air breathing species including humans get exposed to the ENMs suspended in air. Once these ENMs are inhaled, they are likely to get deposited in different regions of the respiratory tract. However, the location and the extent of the deposition depend on the particle size [12]. Additionally, the translocation of the ENMs from lung to different organs is a big concern. Ingestion exposure of ENMs may arise through hand to mouth contact or by consuming contaminated water/food [13,14]. It may also be caused by swallowing mucous which contains deposited particle cleared from the lungs. Dermal exposure of the ENMs can occur by handling or touching the materials or surfaces coated with ENMs. It also occurs by the use of cosmetics and other personal care/protective equipment containing ENMs. The following discussion on nanomaterial exposure assessment will be focussed on various routes by which one can get exposed to the ENMs as well as a few exposure studies that have been conducted for the same.

Inhalation Exposure

Inhalation is considered to be the primary route of exposure to ENMs suspended in air. Nanoparticles are abundant in nature, as they are produced in many natural processes, including photochemical reactions, volcanic eruptions, forest fires, and simple erosion, and by plants and animals, e.g. shed skin and hair. Additionally, human have also created ENMs by chemical manufacturing, welding, ore refining & smelting, combustion in vehicle and airplane engines, combustion of treated pulverized sewage sludge, combustion of coal and fuel oil for power generation and others. Human and their activities has also created considerable amount of particulate matter indoors. A quantitative determination of ENMs emissions from the selected indoor sources is given in table 1.

Nanoparticle source	Concentration (nanoparticles/cm^3)	Estimated source strength (particles/min x 10^{11})
Pure wax candle	241,500	3.65
Radiator	218,400	8.84
Cigarette	213,300	3.76
Frying meat	150,900	8.27
Heater	116,800	3.89
Gas stove	79,600	1.3
Scented candles	69,600	0.88
Vacuum cleaner	38,300	0.38
Air freshener	29,900	2.34
Ironing a cotton sheet	7,200	0.007

Table 1: Measured concentrations of nanoparticles resulting from various common indoor household activities [15].

The behaviour of ENMs in air is majorly governed by diffusion, agglomeration and potential re-suspension of aerosol from deposited nanomaterials. It is also reported that in traditional aerosol science, particle size, inertia, gravitational and diffusion

forces govern aerosol behaviour in the environment [16]. As the particle size decreases, diffusional forces become increasingly important and nanoscale particles are thus likely to behave in a manner more alike to a gas or vapour [16,17]. Particle diffusion coefficient is inversely proportional to the particle diameter. Particle with a high diffusion coefficient such as ENMs have high mobility and mix rapidly in an aerosol. After their release in the environment, atmospheric diffusion facilitates the ENMs to migrate rapidly from a higher to a lower concentration, thus resulting in rapid dispersion and potential for particles to travel a great distance from the source [18].

These migrated ENMs may enter into the human respiratory tract via inhalation. The respiratory tract of human has a larger internal surface area and a thin air-blood tissue interface to facilitate the gaseous exchange and blood oxygenation functions [19,20]. Human respiratory tract consists of nasopharyngeal, tracheobronchial and the pulmonary regions. Each of these regions have structural and functional barriers to protect the lung from the inhaled particulates. However, this system cannot always deal adequately with the wide range of airborne particles that may occur in the occupational and environmental settings [19].

During inhalation, the particles tend to continue to travel along their original path into the lungs. However, the inertia of the particles get altered in the various regions of the lung due to the presence of airway junctions, bending air streams etc, leading to their deposition at various sites. The larger particles are deposited in the nasopharyngeal region (5–30 μm) while, smaller particles (1–5 μm) that fail to be captured in the nasopharyngeal region will be deposited in the tracheobronchial region, mainly due to sedimentation. Finally, the remaining submicron particles (<1 μm) and nanoparticles (<100 nm) penetrate deeply into the alveolar region where removal mechanisms maybe insufficient [21]. Depending upon the physicochemical characteristics of inhaled ENMs and their absorption, different regions of the respiratory system can be considered as a site of toxicity for pulmonary toxicants [22].

Very few exposure studies with airborne ENMs have been conducted [23-25]. However, it is established that the major confounding factor in such studies is the high level of background incidental nanoparticles. For example, while measure the airborne metal working fluid mist, it was observed that the background incidental nanoparticles from direct-fire, natural gas furnace exhaust was higher than the machining processes itself [26]. Further, Maynard et al. (2004) used an enclosed system to eliminate the background nanoparticles and observed that the airborne mass concentration of carbon nano tube (CNT) in a simulated production environment was lesser than 53 $\mu g/m^3$ [27]. They also observed that the majority of the observed particulates were CNT agglomerates having more than 1 μm diameter and were not of respirable size [27].

Translocation of nanoparticles from lung

Deposition of ENMs in the various parts of lung and their translocation (absorption, distribution, metabolism, excretion) to different organs is a major hurdle in assessing the risk of ENMs to human. The ENM characteristics and kinetic behaviour, can help in identifying the target organs, ENM-bio-interactions, molecular mechanisms, and ENMs protein coronas.

Mucocilliary escalator and phagocytosis are the most reported clearance mechanisms of the alveolar macrophages. It has been demonstrated in the rodents that nanoparticles deposited in the lungs can translocate to the pulmonary interstitium [28]. It is also reported that the interstitialzed concentration intensity and the concentration of ENMs exposure is directly proportional to the exposure time [28]. However, the interstitializations of nanoparticles observed at doses used are not relevant to normal human exposure conditions. It is also expected that, compared with rodents, humans would tend to interstitialize a greater proportion of an inhaled nanoparticle dose in an equivalent exposure scenario [29].

The movement of particles from the lung to the circulation and secondary organs is also of a big concern, since studies support a direct role for inhaled nanoparticles in systemic disease, such as cardiovascular disease. Studies showing the rates and mechanisms of deposition, bio-accessibility, route-specific absorption, blood and/or lymph flow dependent distribution into cells and tissues, the breakdown and the excretion of ENMs are limited.

Dermal exposure

Skin is the largest exposed organ of the human body. It acts as a barrier between the organism and the external environment. Skin functions include protection against UV radiation, physical and chemical damage and microbiological attack, homeostasis among others. Nanotechnology is used to modify the drug permeation/penetration by controlling the release of active substances and increasing the retention period on the skin besides ensuring a direct contact with the stratum corneum and skin appendices and protecting the drug against chemical or physical instability. The increased use of ENMs in consumer product has also increased the likelihood for their exposure to human. Human are getting dermal exposure through the handling of nanoparticles suspensions or dry powders. As with many subjects involving nanoparticles, dermal penetration is still controversial [30]. Studies have shown that ENMs are able to penetrate the stratum corneum [30-33]. The skin penetration is majorly via hair follicles and flexed and broken skin [32,33].

MWCNTs are internalized by human epidermal keratinocytes (the major cell type of the epidermis) in cytoplasmic vacuoles and induce the release of pro-inflammatory mediators [34,35]. Spherical particles with diameter between 750 nm and 6 microns selectively penetrate the skin at hair follicles to a maximum penetration depth of 2400 microns. Broken skin facilitates the entry of a wide range of larger particles (500 nm-7 μm) [33]. While stationary unbroken skin has been shown to resist the penetration, nanoparticles have been observed to penetrate when the skin is flexed. Thus mechanical deformation is capable of transporting particles through the stratum corneum and into the epidermis and

dermis. Hence, it can be summarized that the ENMs penetration in the skin is dependent on size, shape/structure and the texture of the skin.

Gastro intestinal exposure

Wide application of nanoparticles in food (such as colorants - titanium oxide), pharmaceuticals, water purification, cosmetics and personal care products (toothpaste, lipstick) and dental prosthesis debris have increased the chances of their exposure to human body via oral route [36,37]. It has now been established that the retention and half-life of the ENMs is different organs is much longer than their bulk counterpart. Due to this, the likelihood for their adsorption and accumulation in different cells are also very high [38,39]. This has also enhanced the probability for the transportation of ENMs within the body through the circulatory system.

It has also been reported that the clearance of the ingested ENMs is also rapid and majorly from the feces (98%) after 48 hours and the remaining via urine [33]. However, the accumulation of ENMs in the blood, spleen, liver, bone marrow, lymph nodes, kidneys, lungs, and brain and small intestine has also been reported in mammals [40,41]. This has also been shown that the ingested nanoparticles may be absorbed through the intestinal lining and enter into the blood stream where they undergo first pass metabolism in the liver [42].

Nanomaterials toxicity and its mechanism

ENMs have been shown to produce cytotoxic, genotoxic, inflammatory and oxidative stress responses in different mammalian cells in vitro [8,43-51]. The harmful effects of ENMs have also been studied in vivo [52,53]. In spite of the presence of voluminous data (Table 2-5), knowledge about the interactions of ENMs with biological systems, their behaviour in mammals and biological responses is still in its infancy. This is due to the improper characterization of ENMs, inferences with the assays system, lack of regulatory guidelines and standard operating protocols and many others. ENMs display several unique physicochemical properties that can interfere with or pose challenges to the use of classical toxicity assays. They require much more extensive particle characterization (size, shape, solubility, agglomeration, elemental purity, surface area, etc.) than other chemical compounds. Incomplete characterization will hinder attempts to find a correlation between various biological effects and particle properties. Their high adsorption capacities, different optical properties, and increased catalytic activities can influence the results of many in vitro toxicity assays, leading to the misinterpretation of results. Also, an absence of standardized methodologies and guidelines makes it difficult to compare the safety/toxicity assessments from different research groups. This impedes nanotoxicology and results in much apprehension regarding the possible adverse health and environmental implications of nanomaterials.

Oxidative stress, genotoxicity, Inflammation and activation of different apoptotic pathways have been identified as possible mechanisms for ENMs associated toxicities in mammals [5,54-57]. However, this is not true for all kind of nanoparticles. ENMs also have the potential to interact with the proteins involved in essential cellular pathways such as DNA replication, transcription and repair; mitotic spindle apparatus, centrioles and their associated proteins. The binding efficiencies of ENMs with different essential proteins have been investigated by some in silico and in vitro studies. An in silico study by Baweja et al. (2011) showed that C60 fullerene interact at the ATP binding domain of human DNA topoisomerase II alpha and could inhibit the enzyme activity [58]. Another in silico study showed that C60 fullerene might interact with PMS2, RFC3 and PCNA proteins involved in the DNA mismatch repair pathway [59]. It has also been proposed that ENMs bind to the active site of the protein leading to their structural/conformational changes. This could also result in the competitive inhibition of the enzyme due to the inability of the substrate to bind [60-62]. Jugan et al (2012) have shown that titanium dioxide nanoparticles exhibit genotoxicity and impair DNA repair activity in A549 cells [61]. The inactivation of the DNA repair protein activity has been attributed to the ROS generation [61].

ENMs of various kinds are reported to induce ROS and oxidative stress under in vitro and in vivo conditions [63-70]. Low concentrations of ROS can activate the signalling pathways [71]. However, at higher concentrations, ROS induces lipid peroxidation, damage to mitochondria, macromolecules and cell membrane. Mitochondria are the major source of the oxygen free radicals and also a major target of ROS induced oxidative stress and damage. Under oxidative stress, mitochondria release various pro-apoptotic factors due an increased permeabilization of outer membrane and the depolarization of the inter-membrane potential [72].

Pulmonary toxicity of the nanomaterial

Inhalation is one of the most common routes of human exposure to nanomaterials. Several research studies have been designed to understand the toxic effect of nanomaterials in lung (Table 2). The practical basis for this research is the potential for inhalation of nanomaterials particularly in regard to the worker exposure through handling and managing the nanomaterials.

Results from in vivo studies reported within the past few years have provided some of the evidence that exposure to nanomaterials could cause injury in the lung of experimental animals. The in vivo studies reported primarily focussed on the effect of metal oxide and carbon based particle such as SWCNT and MWCNT. Studies by Lam et al (2004) and Warheit et al (2004) used intratracheal instillation as a method to deliver SWCNT to the lungs of rats and mice, respectively [73,74]. From their short term toxicity studies Lam et al (2004) reported that the SWCNT produced granuloams, small nodules of the cells in the lung tissues and their appearance increased with dose [73]. Warheit and colleagues also reported the presence of granulomas in a number of areas in the lung tissues of exposed rat but the appearance was not dose dependent [74]. Also, Shvedova et al (2005) used pharyngeal aspiration to deliver SWCNT to the lungs of mice [75]. They reported that their exposure

produces not only a strong inflammatory reaction shortly after treatment, but also progressive and dose dependent development of fibrotic changes in the lung tissues. They have also shown that the inflammatory effects of SWCNT in mice were mitigated by vitamin E and antioxidants [76]. The results of different in vivo pulmonary toxicity data have been reviewed in table 2.

Table 2: Pulmonary toxicity studies of nanomaterials.

NPs Type & size	NPs dose & Time	Animal model	Exposure route	Test Method	Results/ observation	Reference
CeO_2	0.04, 0.4, 4 and 40 µg for 1, 7, and 28 days	CD-1 mice	Intratracheal instillation	Bronchoalveolar lavage fluid (BALF) analysis, cell counts, biochemical analysis of lung homogenate, histopathology and lactate dehydrogenate activity	The present finding suggest that exposure to nanoceria at the current levels in the ambient air may not cause respiratory toxicity	[23]
	24 hrs, 48 hrs and 14 days	Rats	Head and nose inhalation route	Cytotoxicity, oxidative stress and inflammation, LDH release assay	Cytotoxicity via oxidative stress and chronic inflammatory responses were observed. Decrease in the cell viability, increase of lactate dehydrogenase, alkaline phosphatase levels in the bronchoalveolar lavage fluid (BALF).	[24]
	2 mg/m^3 for 0, 7, 14 or 28 days exposure with 14 or 28 days of recovery time	Male CD1 mice	Nose inhalation	Lung injury markers, pro-inflammatory cytokines (such as interleukin-1β, tumour necrosis factor-alpha, interleukin-6 and macrophage inflammatory protein-2) in bronchoalveolar lavage fluid (BALF), oxidative stress, bio-accumulation, and histopathology of pulmonary and extra-pulmonary tissues	CeO_2 NPs can induce pulmonary and extra pulmonary toxicity.	[25]
Cd doped Silica NPs	1mg for 1, 7 and 30 days	Rats	Intratracheal instillation	Histopatholoy immunocytochemistry apoptosis, cell proliferation, inflammation, fibrosis and metabolism	This study suggests that Cd doped SiNPs produce significantly greater pulmonary alterations than either SiNPs or $CdCl_2$ alone.	[77]
	500 µg/mouse for 7 days & 24 hrs	BALB/c mice	Intranasal	in vivo transmission electron microscopy analysis, hematological examination and coagulation tests	The exposure of silica NPs could induce abnormal activation of the coagulation system through the activation of an intrinsic coagulation cascade	[78]
	0, 2, 10 and 50 mg/kg & sacrificed at 24 hrs, 1, 4 and 14 weeks after exposure	A/J mice	Intratracheal instillation	Gomori's trichrome staining, the mRNA and protein levels of IL-4, IL-10, IL-13, IFN-gamma, matrix metalloproteinases (MMP-2, MMP-9 and MMP-10) and tissue inhibitor of MMP-1	This study demonstrated that cytokines (IL-4, IL-10, IL-13 and IFN-gamma), MMPs (MMP-2, MMP-9 and MMP-10) and TIMP-1 play significant roles in the fibrosis induced by the intratracheal instillation of silica NPs	[79]
CdSe/ZnS Quantum dots	12.5, 5.0, or 1.25 µg/rat & 0, 1, 3, 5, 7, 14, and 28 days	Male Sprague-Dawley rats	Intratracheal instillation	Cd metal analysis, microscopy, histopathological analysis, injury and inflammation	Lung injury and inflammation was dose-dependent, QD lost their fluorescent properties	[80]
	6 µg cadmium (Cd) equivalents/ kg body weight & 30 min, 1, 3, 8 or 24 hrs	Male Gclm +/+, +/−, and −/− mice	Nasal instillation	neutrophil counts in bronchoalveolar lavage fluid (BALF), metalloproteinase activity	QDs have in vivo pro-inflammatory properties and the inflammatory response is dependent on GSH synthesis status and no change in MMP activity	[81]

	0.52 mg Cd/ m^3 & head-nose exposed for 6 hrs/ day for 5 days	Male Wistar rats	Inhalation	Histological examination, Cd organ burden determinations, Clinical parameters in blood, bronchoalveolar lavage fluid and lung tissue	The study indicates that QD caused local neutrophil inflammation in the lungs which partially regressed after the 3-week. QD were not found translocated to the central nervous system	[82]
TiO_2	9.35 mg/m^3 aerosol for 6 and 42 hrs	Brown Norway rats	Inhalation	Leukocytes in bronchoalveolar lavages (BAL). Plasma and BAL cytokines were measured and lung histological analysis.	Exposure to TiO_2 decreased BAL leukocytes. Plasma and BAL IL-4, IL-6, and INF-γ levels were also decreased in the TiO_2 group. TiO_2 decreases lung inflammation in asthmatic rats	[83]
	0.5, 5 or 50 mg/ kg	Sprague–Dawley rats	Intra-tracheal instillation	Nitric Oxide and Tumor Necrosis Factor- Assays, Neutral red dye uptake, Migration assay, NO activity assay, EA rosettes formation	Upon TiO_2 exposure pulmonary alveolar macrophage dysfunction may occur, which lead to reduction in both non-specific and specific immune responses in exposed individuals.	[84]
	1 µg/g body wt & 4, 7, and 10 days	C57BL/6 mice	Intranasal instillation	Lung function, inflammation measurement, bronchoalveolar lavage fluid for cytokines detection by PCR array and multiplex analysis	The exposure of TiO_2 in developing lungs may lead to ineffective clearance by macrophages and persistent inflammation and may possibly impact the risk of respiratory disorders in later life	[85]
AgNPs	20 mg/L to 200 mg/L and 5 hrs	Rats	Inhalation	Pulmonary inflammation, alveolar air/blood barrier damage, alveolar macrophage activity, blood cell differentiation, tail artery response to vasoconstrictor or vasodilatory agents	Changes in pulmonary parameters were nonsignificant at 1 or 7 days postexposure except increased blood monocytes 1 day after high-dose Ag exposure. Thus a short-term inhalation of AgNPs did not produce apparent marked acute toxicity	[86]
	1.93×10^7 particles/cm^3 & 6 hrs	Male C57BL/6 mice	Nose-only exposure chamber system	Bronchoalveolar lavage fluid analysis, Western blotting, changes in histopathology and silver burden in major organs	The single inhaled AgNPs caused mild pulmonary toxicity, which was associated with activated MAPK signalling.	[87]
	Low-dose (0.94×10^6 particle/ cm^3, 76 µg/m^3, middle-dose (1.64×10^6 particle/ cm^3, 135 µg/m^3, and high-dose (3.08×10^6 particle/cm^3, 750 µg/m^3 & 4 hrs	Sprague-Dawley rats	Whole-body inhalation chamber	Clinical parameters, body weights, food consumption, and pulmonary function tests were recorded weekly, necropsy, and the organ weights measurement	No significant change in body weight and clinical parameters were observed during the 2-week period. The lung function tests also showed no significant difference between the fresh air control and AgNPs exposed groups	[88]
Amorphous SiO_2	29.5, 103.5 and 177.5 mg/kg for 14 days	Mouse	Intravenous injection	ICP-OES analysis, TEM imaging, serum LDH, AST and ALT levels, histological examination, CD68 immuno-histochemical staining	Elevated serum LDH, AST and ALT levels. Macrophage proliferation in the liver and spleen. Mononuclear phagocytic cells played significant role in overall injury process.	[89]
	400 mg/m^3 & 2 hrs of exposure followed by analysis after 24 and 48 hrs	Sprague Dawley Rats	Inhalation	Total protein, LDH assay, bronchoalveolar lavage fluid (BALF) assessment	The total protein and LDH in BALF increased firstly and then reduced. The visible part of pulmonary alveolus showed gaps of varying degree proliferation accumulation	[90]

Fullerene (C60) NPs	Single dose of 0.5 or 2.5 mg/kg or repeated dose of 0.1 or 0.5 mg/kg, once a week for 5 weeks	Male Rats	Intratracheal instillation	Histopathological examinations, comet assay, inflammation study	C(60) NPs induced hemorrhages in alveoli and the cellular infiltration of macrophages and neutrophils. Comet assays showed, no increase in % Tail DNA after C(60) NPs exposure. These findings indicate that C60 NPs do not have potential to damage DNA even at doses causing inflammation.	[91]
	0.12 mg/m^3, 4.1x10^4 particles/cm^3 & for 6 hrs a day, for 4 weeks (5 days a week)	Rats	Whole-body inhalation exposure	Gene expression profiles of involved genes in inflammatory responses, oxidative stress, apoptosis, and metallo-endopeptidase activity	C(60) were not found to have severe pulmonary toxicity under the inhalation exposure condition	[92]
	2.22 mg/m^3, & 3 hrs a day, for 10 consecutive days	Male rats	nose-only exposure system	Hematology, serum chemistry, bronchoalveolar lavage fluid parameters, lung deposition rate	It was found that C60 nanoparticles caused minimal changes in the toxicological endpoints examined	[93]
Iron oxide & 15-20 nm	640 mg/m^3 for 4 hrs exposure and assessment after 24 hrs, 48 hrs, and 14 days	Wistar rats	Inhalation exposure	Lung injury markers and pro-inflammatory cytokines such as interleukin-1β, tumor necrosis factor-α, and interleukin-6, in bronchoalveolar lavage fluid and blood, oxidative stress in lungs, and histopathology	It was found that upon inhalation iron oxide NPs induce cytotoxicity via oxidative stress and lead to biphasic inflammatory responses.	[94]
	30 and 100 µg/m^3, exposure at 10 days and again at 23 days of age was for 6 h/day for 3 day.	Neonatal rats (10 days old)	Inhalation exposure	Oxidative stress, Fe concentrations measurement, GSSG/ GSH ratio, reducing antioxidant power in bronchoalveolar lavage, LDH assay and interleukin measurements	A significant decrease in cell viability, increase in oxidative injury, cytotoxicity and pro-inflammatory responses. Using chelator bio-availability of iron was found.	[95]
	0, 4.7, 16.6 and 52.1 mg/m^3 & 6 hrs per day, five days per week for 13 consecutive weeks	Rats	Inhalation	body weights, food and water consumption or systemic toxicity, clinical pathology and urinalysis, hematology, neutrophil counts in peripheral blood	Pulmonary inflammation was evidenced by BAL, histopathology, increase in lung and lung-associated-lymph node (LALN) weights at 16.6 and 52.1 mg/m3. Increased septal collagenous fibers were observed at 52.1 mg/m3. Particle translocations into LALN were also found.	[96]
Carbon Nanotubes (CNTs)	0.2, 1, and 5 mg/kg & at 24 hrs, one week, one month, and three months	Male Rats	Intratracheal instillation	Bronchoalveolar lavage (BAL) fluid analysis, lactate dehydrogenase, alkaline phosphatase, lipid peroxidation products; malondialdehyde), and total microprotein levels were analyze	Dose dependant increase in BAL fluid LDH, ALP, MDA, and MTP values than control were found at all post exposure periods	[97]
	0.37 mg/m^3 aerosols & 3 days to 6 months	Male Rats	Inhalation and intratracheal instillation	neutrophil infiltration, pulmonary inflammation and presence of small granulomatous lesions	It was found that well-dispersed CNTs can create pulmonary lesions, including inflammation.	[98]
	0.03 ± 0.003 and 0.13 ± 0.03 mg/m^3 & 3 days, 1, and 3 months	Wistar rats	Inhalation exposure	total cell or neutrophil counts in the bronchoalveolar lavage fluid (BALF), concentration of cytokine-induced neutrophil chemo attractant	In this study, pulmonary infiltration of neutrophils was not observed in exposed group during the observation period	[99]

The results of all these studies suggest that nanomaterial are capable of producing fibrotic alterations in the lung, similar to those reported for the other fibrous material. However, there are numerous uncertainties in the dosing of nanomaterial which affect the interpretation significantly.

Dermal toxicity of nanomaterials

Metal oxide nanoparticles are the most commonly used nanoparticles in cosmetics, sunscreens, toiletries, textiles and wound dressing materials and are thus accessible to the human organism via the skin. Since ENMs have a tendency to agglomerate/aggregate, it cannot be ruled out that, even before exposure, large particles may be formed that can barely be absorbed through the skin. Maynard et al 2004 while measurement of aerosol concentration in air observed that the gloves of workers were contaminated with SWCNT [27]. This indicated the importance of dermal contact as a source of worker exposures to nanomaterials. These findings were followed by a number of in vitro and in vivo studies to determine the potential effect of nanomaterial exposure on dermal cell system. The limited in vivo studies that have been conducted to address the issue of cutaneous toxicity (Table 3) have identified only mild irritation as an adverse response to topical nanomaterial application. There are large discrepancies in the data regarding the penetration and toxicity of nanomaterials in dermal cells. This can be attributed to the improper physicochemical characterization of the nanomaterial such as size and form of the particle in the exposure phase, surface properties and others. Due to the methodological problems, the dermal penetration data of ENMs is still insufficient. Further data of well-designed studies and the development of new analytical methods are required for the quantification of dermal penetration of ENMs and for the risk assessment.

Table 3: Dermal toxicity studies of nanomaterials.

NPs Type & size	NPs dose & Time	Animal model	Exposure route	Test Method	Results/observation	Reference
TiO_2 NPs	5% by weight in sunscreen & 2 mg cream/cm2 skin (4 applications/day, 5 days/week, 4 weeks	Minipigs	Topical application	Skin (multiple sites), lymph nodes, liver, spleen, and kidneys were removed, and the TiO_2 content was determined (as titanium) using inductively coupled plasma mass spectroscopy (ICPMS)	Presence of titanium was found in spleen, epidermis, abdominal and neck dermis, stratum corneum and upper follicular lumens. However, titanium levels in lymph nodes and liver in treated animals were not found increased than control	[100]
	10% by weight in sunscreen & exposed skin was observed after 4, 24, 72 and 168 hrs	Hairless rats	Topical application	Exposed skin was investigated by light microscopy (LM), confocal laser scanning microscopy (CLSM) and electron microscopy (EM) with energy-dispersive X-ray spectrometry (EDX)	LM did not show any morphological and immunohistochemical changes. EM showed that the most TiO_2 particles were localized in the interfollicular stratum disjunctum and the keratinized layer of follicular infundibulum. Thus, TiO_2 particles neither found penetrating into viable cell layers nor cause any biologically or cellular perturbation	[101]
	10% TiO_2 in sunscreen & 24 and 48 hrs	Pigs	Skin application of sunscreen	Light microscopy, scanning (SEM) and transmission electron microscopy (TEM), and time-of-flight secondary ion mass spectrometry (TOF-SIMS) and skin histology	TEM showed TiO_2 NPs in 13 layers into UVB damaged stratum corneum (SC) and 7 in normal. TOF-SIMS showed epidermal penetration TiO_2	[102]
Fullerenes	200 µg for 72 hrs and 24 weeks	Mouse	Topical application	Epidermal DNA synthesis and induction of ornithine decarboxylase activity in epidermis	This study suggests that repeated administration of the fullerenes (up to 24 weeks) post-initiation did not result in either benign or malignant skin tumor formation	[103]
	0.5 g, 6 cm^2 area & 24 hrs exposure and evaluated after 0-48 hrs	Male white Japanese Rabbits & Male Hartley Guinea pigs	Dorsal application	Skin irritation, skin sensitization, skin photosensitization	This study shows that exposure did not induce primary or cumulative skin irritation, skin sensitization, skin photosensitization	[104]
	50 mg (dermal irritation), 40 mg (skin sensitization) & 1 hrs and 24 hrs	Rabbits	Topical application	Skin sensitization study	No dermal responses, neither irritation nor sensitization was caused on the skin were observed	[105]

ZnO NPs	5% ZnO in sunscreen & 24 and 48 hrs	Pigs	Topical application	Light microscopy, scanning (SEM) and transmission electron microscopy (TEM), and time-of-flight secondary ion mass spectrometry (TOF-SIMS) and skin histology	Zn NPs were localized to the upper 1-2 SC layers in the skin. SEM revealed that, NPs were localized as agglomerates in formulation on the skin surface and base of hair. TOF-SIMS analyses showed that Zn was limited to SC and upper epidermis	[102]
	20% w/w in sunscreen, 12 g of sunscreen was applied to 6 cm^2 area & 5 days	Human	Topical application	Venous blood and urine samples were collected 8 days before exposure, twice daily during the trial and 6 days post-exposure	Urine and blood samples exhibited small increases in levels of Zn	[106]
	20% w/w in sunscreen, 2 mg/cm^2 & sunscreens were applied in the morning and afternoon at the first 2 days, and in the morning only at day 3 and 4.	Female, immune-competent, hairless SKH: QS mice	Topical application	Major organs were assessed for Zn concentration, serum amyloid A in blood, whole-genome transcriptional profiling on livers	Zn tracer were detected in internal organs, however, did not observed any adverse biological response following short-term topical applications in mice	[107]
AgNPs	100, 1000 and 10000 ppm for 13 weeks	Male guinea pigs	Dermal application	Uptake in kidneys, muscle, bone, liver, heart, skin and spleen, histopathological studies, cardiocyte deformity, congestion and inflammation	The tissue distribution among various organs were found as kidney s>muscle>bone>skin>liver>Heart> spleen. Separated lines and marrow space narrow were considered as the two major signs of bone toxicities	[108]
	1000 and 10,000 µg/mL & dermal application was performed once daily for five days per week over a period of 13 weeks	Male guinea pigs	Dermal application	Toxic responses were assessed by clinical and histopathologic parameters	No significant changes in organ weight or major macroscopic changes, however dose-dependent histopathological abnormalities were observed in skin liver, and spleen	[109]
	1000 and 10,000 µg/mL & dermal application was performed once daily for five days per week over a period of 13 weeks	Male guinea pigs	Dermal application	Toxic responses were assessed by clinical and histopathologic parameters	No significant changes in organ weight or major macroscopic changes, however dose-dependent histopathological abnormalities were observed in skin liver, and spleen	[110]
CNTs	75 mg/kg MWCNTs & 26 week	RasH2 mice	Subcutaneous implantation	Neoplasm development	MWCNTs showed lower carcinogenicity than carbon black (control)	[111]
	Daily doses of 40, 80 and 160 µg/mouse for 5 days	Immune-competent hairless SKH-1 mice	Topical exposure	Oxidative stress, GSH level, protein thiols oxidation status and carbonyls, myeloperoxidase activity, dermal cell numbers, and skin thickening and accumulation of polymorphonuclear leukocytes (PMNs) and mast cells	Topical exposure of unpurified SWCNT, may induced free radical generation, oxidative stress, elevated myeloperoxidase activity, oxidation of GSH and inflammation, thus causing dermal toxicity	[112]
Qds	2 mg/cm^2 for 24 to 48 hrs	Mouse	Transdermal exposure	QD penetration into skin was monitored in organs such as liver and regional draining lymph nodes, using inductively coupled plasma mass spectrometry for the presence of cadmium	No significant cadmium level elevation was found in sentinel organs. Cd accumulation in the liver was approximately 2.0% of the applied dose. It was suggested that transdermal absorption of nanoscale materials depends on skin barrier quality.	[113]

	0.32 nmol QDs on 1.5 cm^2 & 0.32 nmol QDs	Male ICR mice	Topical application	Fluorescence microscopy and transmission electron microscopy and inductively coupled plasma-mass spectrometry (ICP-MS) was used to measure the Cd content to indicate the concentration of QDs in plasma and organs	This study indicates that QDs can penetrate into the dermal layer and are limited to the uppermost stratum corneum layers and hair follicles. Through blood circulation, QDs deposit mostly in liver and kidney and are difficult to clear. Cd concentration was found 14 ng/g in kidney after 120 h after 0.32 nmol QDs was applied to a mouse	[114]

Systemic toxicity of nanomaterials

The safety concerns derived from ENMs route of entry and their potential bio distribution are mainly controlled by shape, size, dose, route of administration, stability in dispersing media, aggregation, size of protein corona etc. The size of ENMs greatly affects the lung deposition (when injected intravenously) and rapid systemic translocation wherein smaller size particles cause various inflammatory, oxidative and other cytopathic effects on test models than larger particles. Further, ENMs surface molecules too play important role and their ionic interactions with mammalian cell surface may cause undesirable accumulation in vital organs. Among these surface ligands, PEG has gained popularity due to its aqueous solubility and amphiphilic nature [115,116].

Probably these properties increase the circulation period of AuNPs in blood stream and thus the bio distribution pattern. In a study by Patra et al. 2010 reported a large percentage of PEG modified AuNPs in the blood than unmodified particles over the same period of circulation [116]. Exposure route also plays important role in bio-distribution of ENMs. Silver nanoparticles (AgNPs) administered by oral gavage did not show any sign of hepatotoxicity, immunotoxicity or change in body weight. However, intra-treacheal inhalation showed dose dependent lesions, chronic alveolar inflammation and small granulomatous lesions. This effect could be due to the interaction of administered AgNPs with digestive and other enzymes in gastrointestinal track, which could delay the release in blood stream; however, in inhalation AgNPs get exposed to blood circulation and get deposited to vital organs thus lead to toxicity. Several reports discuss the bio distribution of cerium oxide nanoparticles (nanoceria), administered by inhalation or intra venous injection. Controversial reports have been published on toxicity of nanoceria. Few reports describe lung injury and expression of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6 and macrophage inflammatory protein-2) in bronchoalveolar lavage fluid (BALF), oxidative stress in lungs, bio-accumulation, and histopathology of pulmonary and extra-pulmonary tissues [25]. However, others report no accumulation of nanoceria in major organs except extra-pulmonary tissues. Tissue clearance was found slow but nanoceria were found eliminated out from body and did not observe any acute or long-term negative effects [117-119]. Further, it has been shown that the plasma half-life of particle decreases with increase in size (>50 nm) due to recognition by reticuloendothelial system in liver and spleen. However, extremely smaller particles (<5 nm) have found low retention in body due to renal elimination [120]. More detail about ENMs types and their related systemic toxicity has been provided in the table 4.

Table 4: Systemic toxicity studies of nanomaterials.

NPs type & Size	Dose & Time	Animal model	Exposure route	Test Method	Results/Observation	Reference
TiO_2 NPs	One time injection of 50 µg TiO_2	Oncorhynchus mykiss	Intravenous caudal-vein injection	Accumulation in vital organs	94% of injected TiO_2 was accumulated in kidney, however, no toxicity was observed	[127]
	25 mg/kg	Wistar rats	Intraperitoneal injection	Body weight, organ coefficient, blood biochemistry panel assay (AST, ALT, LDH, uric acid, creatinine, and glucose content) and emotional behavior	Decrease in body weight but increase in brain weight, increased uric acid concentration and AST activity, decrease in creatinine level, but no effect on glucose concentration, ALT and LDH activity	[128]
	1 mg/kg for 28 days	ICR mice	Intratracheal exposure	Histopathological lesions and secretion of inflammatory cytokines	Increase in the formation of autophagosome like vacuoles and decrease in mitochondrial calcium concentration	[129]

ZnO NPs	1, 5, 10, 20, 50, and 100 mg/L for 144 hrs post-fertilisation	Zebrafish embryos	Whole body	Superoxide dismutase, catalase, glutathione peroxidase activity and malondialdehyde contents, the genes related to oxidative damage, reactive oxygen species generation and DNA damage	Dose-dependent toxicity to zebrafish embryos and larvae, increase in ROS, and DNA damage and related proteins expression	[130]
	300 mg/kg for 14 days	Mice	Oral exposure	ALT and ALP levels in serum, pathological lesions in the liver, lipid peroxidation and Fpg-modified Comet assay, TUNEL assay	ZnO NPs were found accumulated in the liver causing oxidative stress thus DNA damage and apoptosis	[52]
	600 mg/kg and 1 g/kg body weight for 5 consecutive days	Rats	Oral exposure	troponin-T, creatine kinase-MB (CK-MB), and myoglobin, pro-inflammatory biomarkers level, cardiac calcium concentration	Increase in cardiac calcium concentration and the consequent oxidative DNA damage, as well as the increase in cardiac caspase-3 activity of intoxicated rats	[131]
Fullerenes	1, 10, 100, or 1000 mg/kg/day daily for 29 days, followed by a 14-day recovery period	Rats	Oral gavage	clinical observations, body weights, food consumption behaviour and histopathological changes	Fullerenes were not detected in the liver, spleen or kidney at the end of the administration period and also at the end of the recovery period	[132]
	0.1, 1 and 10 mg/kg body weight daily for 92 days	Rats	Intragastric administration	biochemical, physiological, hematological, immunological indicators	Observed significant increase in the absorption for antigenic proteins in the digestive tract in animals treated with fullerene at 10 mg/kg dose	[133]
	10 mg/kg for 8, 24, and 48 hrs	Sprague-Dawley rats	Intravenous administration	Complete histopathology and clinical chemistries	Minor changes in histopathological studies. No clinically significant chemical changes were observed	[134]
SiO_2 NPs	2, 20, and 50 mg/kg/day for 4 weeks	BALB/c mice	Intraperitoneal	Clinical toxicity, lymphocyte population, serum IgG/IgM levels, and histological changes	No sign of clinical toxicity, increases in liver and spleen weight and splenocyte proliferation, altered lymphocyte populations, increased serum IgG and IgM levels	[135]
	20, 40 and 80 mg/kg for 14 days	Mice	Intravenous	Biodistribution and clearance study	NPs were accumulated in mononuclear phagocytic cells in liver and spleen. The entire clearance time of the particles was found 4 weeks	[136]
	500 µg/mouse for 7 days	BALB/c mice	Intranasal	In vivo transmission electron microscopy analysis, hematological examination and coagulation tests	NPs were absorbed through the nasal cavity and distributed into liver and brain, platelet counts decreased and the activated partial thromboplastin time was prolonged	[137]
AgNPs	90 mg/kg body weight for 29 days and after a wash-out period of 1 or 8 weeks	Rats	Oral gavage	Biochemical markers, antibody levels in blood, lymphocyte proliferation, cytokine release, and NK-cell activity	Any sign of hepatotoxicity or immunotoxicity was not observed	[138]

	30 mg/kg, 300 mg/kg, and 1000 mg/kg for 28 days	Sprague-Dawley rats	Oral gavage	Blood biochemical and hematology, along with histopathological examination and silver distribution study	Rats did not show any significant changes in body weight, dose more than 300 mg was slightly toxic to liver, twofold increase in the weight of female rat kidneys was observed than male kidneys	[138]
	0.6 x 10^6 particle/cm^3, 49 µg/m^3, 1.4 x 106 particle/cm^3, 133 µg/m^3, 3.0 x 10^6 particle/cm^3, 515 µg/m^3 for 6 hrs/day, 5 days/week, for 13 weeks	Sprague-Dawley rats	Inhalation	Mortality, clinical observations, body weight, food consumption, and pulmonary function tests were recorded weekly	Bile-duct hyperplasia in the liver increased dose, histopathological examinations indicated dose-dependent increases in lesions, including mixed inflammatory cell infiltrate, chronic alveolar inflammation, and small granulomatous lesions	[139]
CNTs	Single dose of 0.05 or 0.2 mg/animal	ICR mice	Intratracheally instilled	DNA damage by comet assay, 8-oxo-7,8-dihydro-2'-deoxyguanosine and heptanone etheno-deoxyribonucleosides	DNA damage was increased in a concentreation dependent manner, Transversions were predominant, and G:C to C:G was increased. NO synthase and nitrotyrosine were also observed in the lungs of MWCNT-exposed mice	[140]
	0.2 or 1.0 mg/kg body weight (single dose), 0.04 or 0.2 mg/kg body weight once a week for 5 weeks (multiple dose)	Rats	Intratracheally instilled	Histopathological, inflammation, comet assay,	This study indicated that a single intratracheal instillation of SWCNTs induced a clear inflammatory response but no DNA damage	[141]
CeO_2 NPs	2 mg/m^3 for 0, 7, 14 or 28 days with 14 or 28 days of recovery time	CD1 mice	Nose-inhalation	Lung injury and pro-inflammatory cytokines (IL-1β, TNF-α, IL-6 and macrophage inflammatory protein-2) in BALF, oxidative stress in lungs, bio-accumulation, and histopathology of pulmonary and extrapulmonary tissues	Induction of pulmonary inflammation and pro-inflammatory cytokines, bio-accumulation of these particles in the pulmonary and extrapulmonary tissues, even after one month of post-inhalation exposure.	[25]
	1 µM or 0.344 ng to 1 mM or 344 ng in saline solution for 1 hour to 120 days	Sprague-Dawley albino rats	Intravitreal Injection	Temporal and spatial distributions of nanoceria after a single intravitreal injection, and toxic effects in healthy rat retina	Did not observe any acute or long-term negative effects of nanoceria on retinal function or cytoarchitecture even after this long-term exposure	[117]
	7.65 g/cm^3 & 6 hrs per day till 28 days	Male Wistar rats	Inhalation study	Distribution to tissues such as lungs, liver, kidney, and spleen and also brain, testis, and epididymis and clearance study	No accumulation of NPs in various major organs except extra-pulmonary tissues. Tissue clearance was slow but CNPs were found eliminated out from body.	[119]
Qds	500 µg/L for single exposure	Zebrafish (Danio rerio) embryo and larvae	Aqueous whole body exposure	Developmental toxicities to zebrafish	QDs caused higher mortality, lower hatch rate, and more malformations, embryo cell apoptosis appeared in the head and tail region	[142]

	0.05-31.25 mg/L	Zebrafish embryo and larvae	Aqueous whole body exposure	Developmental and behavioral toxicities to zebrafish under continuous exposure to low level CdSe QDs	QDs exposure from embryo to larvae stage affected overall fitness, lower hatch rate, abnormal vascular of zebrafish larvae appeared including vascular junction, bifurcation, crossing and particle appearance	[143]
	305.6 ng/kg body weight, 7.6 ng/ kg body weight and 15.3 ng/kg body weight for 6 weeks for 48 hrs after last injection	Male Kunming mice	Intraperitoneal	Organ accumulation, cytotoxicity as characterized by a significant increase of the malondialdehyde level within hepatocytes	Increase in the MDA level, production of ROS and related cellular damage, hepatotoxicity cause were similar to Cd ions	[144]
Iron oxide NPs	15 mg of Fe/kg body weight for 0.5, 1, and 4 hrs as well as for 1, 4, 7, 14, and 28 days	ICR mice	Tail vein	Biodistribution and accumulation by ex vivo optical imaging and fluorescence signal generation at various time points over 28 day	Iron-oxide NPs showed highest accumulation in the lung at 0.5 h post-injection and decreased rapidly over time. Density in liver and spleen was maintained over 28 days.	[145]
	640 mg/m^3 for 24 hrs, 48 hrs, and 14 days	Wistar rats	Acute inhalation exposure	Expression of IL-1β, TNF-α, and IL-6 in bronchoalveolar lavage fluid (BALF) and blood, oxidative stress in lungs, and histopathology	Increased inflammation, malondialdehyde concentration, while reduced glutathione and antioxidant enzyme activities, lung histology showed an early activation of pulmonary clearance	[94]

Environmental toxicity of nanomaterials

The applications of nanotechnology in diverse areas will lead to their inadvertent release in surface and sub-surface environments through landfills and other waste disposal methods. It is likely that some of these ENMs may induce adverse/toxic effects in both lower and higher trophic organisms (Table 5) [4,121,122]. At the safety level, it is well known that the high surface area to volume ratio of ENM leads to increased surface reactivity and associated risk. However, the mechanisms involved in reactivity and toxicity are not well understood yet. There is also a great deal of uncertainty about the environmental fate, behaviour and bioavailability of ENMs in the ecosystem. Also, lack of reliable and validated schemes for assessing the ecotoxicological risk is a big concern [3,123]. The major constraints in risk assessment of ENM are the lack of appropriate methods for characterization in exposure media, bioavailability, mobility, biopersistance, and bioaccumulation [124,125]. The impact of ENMs on various ecosystems will be significant because their distribution depends on a number of factors such as Brownian motion, inertia, gravitational influences, thermal influences, pH, and ionization. As the ENMs have high mobility, they can easily move in the air, water and soil and can contaminate the flora and fauna. This may also result to the transfer of ENMs in the food chain, leading to the creation of non-biodegradable pollutants [126]. Also, ENMs can affect the bioavailability of the other toxicant/pollutant by facilitating their transportation [125]. ENMs may also elicit a negative physical, chemical and biological impact on different strata of the ecosystem (air, water and soil).

The behaviour and bioavailability of ENMs in freshwater/marine ecosystem depends on their interaction with the aquatic colloids, such as natural organic matters (NOMs), humic substances, and salt ions [125]. NOMs usually get adsorbed on the surface of the ENMs by different electrostatic, hydrogen bonding and hydrophobic interactions, which affects their dispersity and bioavailability. ENMs in aqueous suspension are dispersed due to the electrostatic and steric repulsion of the surface charge (positive/ negative) present on the particle. Apart from NOMs, salt ions, protein content, presence of molecular clusters enable nucleation leading to agglomeration/aggregation thereby modulating the bioavailability of ENMs in the environment. Also, the biomolecules such as proteins or polymers present in the ecosystem form a layer over the ENMs, named as "corona" which plays important role in their biological fate. It has also been shown that it is not only the ENMs alone but the "corona" governs the properties of the "particle-plus-corona" compound in the biological system [9,10]..

Table 5: Environmental toxicity studies of nanomaterials.

Organism	Particle type/size	Concentration	Assay	Results/Outcome	Reference
Bacteria					
Escherichia coli	Zinc Oxide nanoparticles/ size not reported	15.63 - 1000 µg/ml	Microtiter plate–based method using resazurin indicator dye solution for cytotoxicity assay, disk diffusion method	Antibacterial activity was observed at 500 µg/ml by resazurin dye method whereas for disk diffusion assay it was 400 µg/ml	[146]
	Zinc Oxide nanoparticles/ 20-30 nm	5 - 45 µg/ml	Spectroscopic methods (OD600) for cytotoxicity assay	Antibacterial activity was observed at 15 µg/ml	[147]
	Zinc Oxide nanoparticles/ 10-20 nm	1 x 10^{-3} - 10 x 10^{-3} M	Cell viability assay (standard plate count method)	Nanoparticles were internalized in the cells and induced toxicity at concentrations higher than 1.7 x 10^{-3} M	[148]
	Zinc Oxide NPs/ size not reported	2 – 40 mg/L	Spectroscopic methods (OD600) for cytotoxicity assay	No toxicity was observed	[149]
	Silver nanoparticle/10-15 nm	20 – 100 µg/ml	Bacterial growth kinetics by surface spread plate method and bacterial phosphotyrosine profile for signalling transduction	Exhibited antibacterial activity with a minimum inhibitory concentration (MIC) of 25µg/ml	[150]
	Silver nanoparticles/ < 12 nm	10 – 60 µg/ml	Cell viability assay (standard plate count method)	Exhibited antibacterial activity with a MIC of 20 µg/ml	[151]
	Silver nanoparticle/ truncated triangular plates, rods, and polyhedral plates/ average size 39 nm	1 – 100 µg/ml	Cytotoxicity assay by surface spread plate method	Truncated triangular particles completely inhibited cell growth at 10 µg/ml whereas 100 µg/ml of rod and polyhedral plates shaped particles induced inhibition after prolonged exposure.	[152]
	Titanium dioxide (TiO_2)/25nm Degussa	0.01 – 1 mg/ml	Cell viability assay (standard plate count methods), lipid peroxidation assay, determination of cellular respiration using 2,3,5-triphenyltetrazolium formazan (TTF) dye	UV light activated particles exhibited higher toxicity than inactivated particles at concentration 0.1 mg/ml. Activated nanoparticles also induced significant membrane damage and lipid peroxidation at the same concentration.	[153]
	TiO_2/(66 nm, 950 nm, and 44 µm), SiO_2/ (14 nm, 930 nm, and 60 µm) ZnO/ (67 nm and 820nm)	10 – 5000 ppm	Spectroscopic methods (OD600) and standard plate count methods for cell viability assay	The antibacterial activity of TiO_2 was significantly higher in the presence of light.	[154]
	Al_2O_3/Degussa/ spherical 11-13 nm, TiO_2/ spherical/17 nm, MWCNT/ elongated/L:1.5 µm and D: 44.0 nm	10 – 500 mg/L	Live/Dead Viability Assay using flow cytometry and reactive oxygen species generation by DCFDA dye	Al_2O_3, TiO_2, MWCNT exhibited bactericidal effects at concentration 100 mg/L and also able to induce significant ROS at same concentration.	[155]
	Magnesium oxide/3.6 µm	100 mg/ml	Halo Test and conductance assay for cytotoxicity	MgO powder has antibacterial against E. coli and The zone of inhibition for E. coli was smaller than that of S. aureus.	[156]
	Multiwalled carbon nanotubes/ D: 17-35 nm, L: 2.3-91 µm	4 mg/ml	Dead discrimination Assay using PI dye by flow cytometry	Different shapes of MWCNT exhibit growth inhibition.	[157]

Salmonella typhimurium	Zinc Oxide nanoparticles/ 20 – 30 nm	5 – 45 µg/ml	Spectroscopic methods (OD_{600}) for cytotoxicity assay	Bacterial growth was inhibited at 15 µg/ml.	[158]
	Zinc oxide nanoparticles capped with tetramethylamonium hydroxide	39.1 – 5000 µg/plate	Ames Test	Mutagenicity was negative in all strains at all concentrations.	[159]
	TiO_2/<25nm and ZnO/30nm	0.008 – 8 µg/ plate	Ames Test	ZnO NPs induced frameshift mutation with metabolic activation system whereas TiO_2 NPs induced frame shift mutation with and without metabolic activation system. Both NPs were also found to induce oxidative mutation in E. coli strain WP2uvrA.	[160]
	Al_2O_3/<50nm, Co_3O_4/<50nm, CuO/<50nm, TiO_2/<100nm, ZnO/<100nm	100 – 1600 µg/ plate	Ames Test	TiO_2 and ZnO NPs induced reverse mutants to E. coli strain WP2uvrA with metabolic activation.	[161]
	MWCNT/ 0.2 – 1 µm	50 – 5000 µg/ plate	Ames test	Mutagenicity was negative in all strains at all concentrations.	[162]
	MWCNT/D: 110–170 nm L: 5–9 µm	0.01 – 9 µg/ plate	Ames Test	Mutagenicity was negative in all strains at all concentrations.	[163]
	Zinc Oxide nanoparticles/ size not reported	15.63 – 1000 µg/ml	Microtiter plate–based method using resazurin indicator dye solution for cytotoxicity assay, Disk diffusion method	Antibacterial activity was observed at 500 µg/ml by resazurin dye method whereas for disk diffusion assay it was 400 µg/ml.	[146]
Staphylococcus aureus	Zinc Oxide nanoparticles /150 nm	0.0006 – 0.12 M	Cytotoxicity assay by surface spread plate method and disk diffusion assay	Exhibited antibacterial activity with a MIC of 0.12.	[164]
	Zinc Oxide nanoparticles/ 20-30 nm	5 – 45 µg/ml	Spectroscopic methods (OD_{600}) and standard plate count methods for cell viability assay	Microbial growth was inhibited at 15 µg/ml.	[158]
	MgO, TiO_2, Al_2O_3, CuO, CeO_2 and ZnO nanoparticles/ size not reported	1 – 10 mM	Spectroscopic methods (OD_{600}) and standard plate count methods for cell viability assay	MgO, TiO_2, CuO and CeO_2, did not show any significant growth inhibition up to 10 mM suspension, whereas Al_2O_3 and ZnO showed significant growth inhibition at 2 mM concentration.	[165]
Saccharomycs cerevisiae	TiO_2 NPs: 25 – 70 nm, ZnO NPs: 50 – 70 nm and CuO: 30 nm	TiO_2: 1-20000 mg/L ZnO: 10-1000 mg/L CuO: 1-10000 mg/L	Growth responses by viable cell count method	Growth was inhibited with an EC50 of 131–158 mg/L for ZnO, 873 mg/L for CuO and for TiO_2 it was even more than 20000 mg/L.	[166]
Algae	Zinc oxide nanoparticles/D: 6.3 - 15.4 nm, L: 242nm to 862 nm	10 – 80 mg/L	Cytotoxicity assay by algal cell count method	ZnO NPs stopped the growth of T. pseudonana and C. gracilis, whereas P. tricornutum was the least sensitive.	[167]
Thalassiosira pseudonana, Chaetoceros gracilis, Phaeodactylum tricornutum	Nickel oxide/ 20 nm	10 – 50 mg/L	Growth-inhibition test	Exhibited toxic responses with an EC_{50} of 32.28 mg/L for 72 hour.	[168]

Chlorella vulgaris	Zinc Oxide NPs/ size not reported	2 – 40 mg/L	Spectroscopic methods (OD_{600}) for cytotoxicity assay	Cyanothece in aquatic media was inhibited at 40 mg/L	[149]
Cyanothece	TiO_2/ 34 nm and Al_2O_3/17 nm	5 – 100 mg/ml	Cytotoxicity assay by algal cell count method	TiO_2 and Al_2O_3 inhibited the growth of C. dubia and the EC_{50} values were 42 and 45 mg/L for TiO_2 and Al_2O_3, respectively.	[169]
Pseduokirchneriella subcapatitata and Ceriodaphnia dubia	CuO/30 nm, ZnO/50-70 nm TiO_2/25-70 nm	ZnO: 0-0.5 mg/L TiO_2: 0-120 mg/L CuO: 0-7 mg/L	Cytotoxicity assay by fluorescence measurement assay and algal cell count method	Inhibit the algal growth with EC_{50} of 0.04 mg/L for ZnO, 5.83 mg/L for TiO_2 and 0.71 mg/L for CuO.	[68]

Effect of nanomaterials on blood and cardiovascular system

Air pollution is recognised as an important factor for cardiovascular disease in urban communities [170]. Toxicological studies have demonstrated that the combustion and model NPs can gain access to the blood following inhalation and instillation and can enhance the thrombosis. However, it is not clear that whether this was an effect of pulmonary inflammation or the particles translocated to the blood [170-172]. High exposure of diesel exhausts particles (DEP) by inhalation caused altered heart rate in hypertensive rats [173,174]. This has interpreted as a direct effect of DEP on the pacemaker activity of the heart. Ultrafine carbon black instilled into the blood has been reported to induce platelet accumulation in the hepatic microvasculature of healthy mice in association with the prothrombotic changes on endothelial surface of the hepatic microvessels [175]. Also, the effect of various kinds of carbon nanomaterials (single wall carbon nanotubes, multi wall carbon nanotubes) on platelet aggregation was compared with the C60 fullerene and urban particulate matter by Radomski et al. (2005) [176]. It was observed that the mixed carbon nanoparticles, single wall carbon nanotubes, multi wall carbon nanotubes were stimulating the platelet aggregation and accelerating the vascular thrombosis in a ferric chloride model of thrombosis in a specific rat model. However, the C60 fullerenes that are used as a building block for these CNT were inert [176]. In contrast for several nanomaterials designed for drug delivery purposes, no or limited effects on platelet function in vitro was noted including alcohol/polysorbate nanoparticles, gadolinium nanoparticles and nanostructured silica hydroxyethyl methacrylate biocomposites [177-179].

Based on the present observation, it can be summarized that there is concern on the possible effect of ENMs on the cardiovascular system. However, the available information is not enough to conclude their limited/safe use.

Nanoparticles uptake and effects in the brain

Nanoparticles can get access to the brain by two major mechanisms (a) transsynaptic transport after inhalation through the olfactory epithelium, and (b) uptake through the blood brain barrier [180]. The first pathway has been studied primarily with the particles such as carbon, gold and MnO_2 in experimental inhalation models in rats [12,33]. However, the second pathway is an outcome of the extensive research and particle surface manipulation in drug delivery [178,181].

It has been reported that the physiological barrier may limit the distribution of some protein and viral particles after transvascular delivery to the brain [180]. This suggests that the healthy BBB contains defence mechanisms protecting it from blood borne nanoparticles exposure. When nanoparticles with different surface characteristics were evaluated, neutral nanoparticles and low concentration of anionic particles were found to have no effect on BBB whereas high concentration of the cationic nanoparticles was toxic to BBB. Nanoparticles have been reported to induce the ROS and the oxidative stress, which is an established factor for pathogenesis for the neurogenerative diseases such as Parkinson and Alzeimers disease.

Carcinogenicity of nanomaterials

In vitro and *in vivo* studies have revealed that ENMs induce DNA damage and mutations through different mechanisms. The association between genotoxicity and cancer is also well established in the literature. Therefore the studies with genotoxic potential of ENMs provide invaluable information in predicting the carcinogenicity of ENMs. For example, the carcinogenic effects of ionizing radiation, UV radiation and many chemical carcinogens are due to their ability to cause DNA damage and gene mutations. The correlation between the metals, metal oxides, oxidative stress and cancer has been extensively reviewed [182-186].

It is well accepted that excessive generation of ROS, overwhelms the antioxidant defence mechanism of the cells through oxidation of biomolecules. The role of oxygen-derived species in causing cell injury or death is increasingly recognized. ROS is involved in a large number of degenerative changes, leading to tissue degradation, a hallmark in carcinogenesis, aging and other diseases [187]. It also compromises the immune system leading to an increased microbial load resulting in cell and tissue damage. It is now well established that free radicals produce different types of genetic damage which could lead to cancer. Among oxidative DNA damage products, 8-OHdG is the most studied due to its relative ease of measurement and premutagenic potential. Elevated 8-OHdG has also been reported in numerous tumors, strongly implicating such damage in the etiology of cancer [188]. Several studies has shown that the ENMs have capability to

induce the level of 8-OHdG in different cell models suggesting the carcinogenic potential.

ENMs can induce oxidative stress and subsequently can elicit inflammatory responses, which could act as an initiator of carcinogenesis. ENMs are highly reactive because of the presence of electrons on their boundary. They are also more likely to adsorb endogenous substances, react with proteins and enzymes, and trigger cytokine release. This could mediate inflammatory responses and potentially initiate a series of toxic responses far from the initial site of deposition [21]. C60 fullerene, for example, was reported to cause photo induced DNA damage by interacting with NADH, which is an endogenously present reducing agent [189]. Similarly, carbon nanotube exposure has been associated with adverse cardiovascular effects by causing aortic DNA damage, platelet aggregation and enhanced vascular thrombosis through inflammatory events [176,190]. TiO_2 ENMs are reported to induce inflammatory cytokines and apoptosis in cell lines derived from different organs as well as in vivo [191].

Biopersistence of ENMs also poses a certain degree of adverse health effect to human and environment. As the ENMs size is very small, the likelihood for their entry into the cell as well their persistence into the cell is also higher. For instance, when the clearance rate of ENMs is slower than the accumulative rate, the presence of ENMs will remain in the organ. Additionally the exposure and persistence of those ENMs, which can induce the mutation, will increase the risk of developing cancer. To address this concern, the mutagenic potential of the ENMs were assessed by bacterial reverse mutation assay [68,156,192] and extrapolated with the carcinogenic properties. Kumar et al. have demonstrated the frame shift mutagenic potential of the ZnO and TiO_2 ENMs in Ames test [156]. Whereas, Sera et al. demonstrated the potential of C60 fullerene ENMs to exert mutagenic activity due to the oxidized phospholipids in rat liver microsoms [192].

Based on the epidemiological studies conducted among the male production workers at TiO_2 industry from Western Europe and North America it was predicted that the workers were on high risk for lung and kidney cancer with comparison to general population [190]. However, the data were not enough to conclude the association between occupational exposure of TiO_2 ENPs and cancer risk. In contrast, sufficient in vitro reports are available to exhibit the genotoxic potential (such as micronucleus formation, DNA damage) of TiO_2 ENPs. Several in vivo experiments were also demonstrated that exposure of TiO_2 ENPs increases the probability for the tumor incidence in the experimental animals. The shortcoming with these tests and reports are the short term treatment of ENMs. It is suggested to perform the in vitro and in vivo genotoxicity testing for longer periods to observe if there are long-term effects of ENMs such as tumour formation and carcinogenesis. It will also be useful to look at the clearance of ENMs from the body and to study if there is a preference for accumulation in certain organs and any effect from biopersistence of such ENMs.

Conclusion

The safety/toxicity aspects of nanomaterials have lagged far behind the rate at which they are being produced. To unravel the potential risks of ENMs in human, it is prudent to undertake in-depth studies using particle characterization, uptake, computational modelling, exposure assessment, omics approaches and others will be helpful in improving knowledge for better study designs for risk modelling and assessment. A multidisciplinary team effort from material scientists, molecular biologists, toxicologists and physicists is necessary as it will facilitate the interlinking of different facets of nanotoxicology thus aiding in the understanding of cellular responses to nanomaterials exposure and mechanisms involved. The lack of data on concentration and quantitative exposure of ENMs currently hampers the prediction of their risk fate.

There is also a need for more comprehensive studies to fully understand and address the potential risks of engineered nanomaterials to human health and the environment. This will help in creating environment friendly and biologically safe nanoparticles.

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