Review

Interaction of engineered nanoparticles with various components of the environment and possible strategies for their risk assessment

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ABSTRACT

Nanoparticles are the materials with at least two dimensions between 1 and 100 nm. Mostly these nanoparticles are natural products but their tremendous commercial use has boosted the artificial synthesis of these particles (engineered nanoparticles). Accelerated production and use of these engineered nanoparticles may cause their release in the environment and facilitate the frequent interactions with biotic and abiotic components of the ecosystems. Despite remarkable commercial benefits, their presence in the nature may cause hazardous biological effects. Therefore, detail understanding of their sources, release interaction with environment, and possible risk assessment would provide a basis for safer use of engineered nanoparticles with minimal or no hazardous impact on environment. Keeping all these points in mind the present review provides updated information on various aspects, e.g. sources, different types, synthesis, interaction with environment, possible strategies for risk management of engineered nanoparticles.

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Contents

1. Introduction

A remarkable progress has been noted in the area of nanotechnology in recent years as evident from its widespread use in textile, electronics, pharmaceuticals, cosmetics, foods and environmental remediation (Dunphy Guzman et al., 2006; Royal Society, 2004). Despite tremendous benefits, the inevitable release of engineered nanoparticles (ENPs) in the environment with the development of nanotechnology is a serious case of concern of environmental biologists worldwide. However, a few studies have already demonstrated the toxic effects of nanoparticles on various organisms, including mammals (Handy et al., 2008a). But scanty and fragmentary information are available on probable inputs, fate and interactions of these nanoparticles with various components of the environment. Thus the present review is aimed to address the current understanding of the structure, fate, behaviour, ecotoxicity test methods and environmental risks assessment of ENPs.

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2. Natural vs engineered nanoparticles

The existence of naturally occurring nanoparticles in water, air and soil is known from the beginning of earth's history as they have been recorded from 10 000 years old glacial ice cores. These nanoparticles are assumed to be derived from natural combustion processes and deposited into the ice core via atmospheric deposition (Murr et al., 2004). Likewise, the presence of natural nanoparticles has also been recorded from the sediments of Cretaceous–Tertiary (K–T) boundary at Gubbio, Italy (Verma et al., 2002). Many geological and biological processes are known to produce natural nanoparticles. Geological mechanisms include physico-chemical weathering, authigenesis/neoformation and volcanic eruptions (Handy et al., 2008a).

Many biological molecules/entities, e.g. protein, nucleic acids, ATP, viruses are typically nano-sized. Some of these are clearly released into the environment directly from the organisms (e.g. nucleoprotein exudates from algae, dispersion of viruses from animals). Additionally degradation of biological matters also produces nanoparticles, e.g. humic and fulvic acids.

3. Production of engineered nanoparticles

Theoretically ENPs can be produced from any chemical but usually most of the ENPs are synthesized from transition metals, silicon, carbon/single-walled carbon annotates, fullerenes and metal oxides (Drobne, 2007). Top-down and bottom-up fabrication are two distinct methods for the production of ENPs. In top-down method, lithographic techniques are used to cut larger pieces of a material into NPs. Particles with sizes lesser than 100 nm and 30 nm can be produced using extreme UV photolithography and electron beam lithography, respectively (Borm et al., 2006). In some cases, grinding of a macro-material in a ball mill can also be used for the production of NPs with sizes lesser than 30 nm. Bottom-up fabrication is more convenient method for producing ENPs and also relevant to the chemical industry. In this approach, different techniques are used to increase the size of very small units, often individual molecules or even atoms, up to the size of NPs (Borm et al., 2006; Christian et al., 2008). Controlling the size of the nanoparticles is very important step in the bottom-up approach of nanoparticle production. The regulation of size of nanoparticles varies with the media of their synthesis, in gas-phase concentration of precursors and temperature of the reaction, whereas in wet-phase temperature and reaction time control the size of ENPs (Borm et al., 2006).

Synthesized nanoparticles are also processed further to prevent the agglomeration (tendency to form aggregates with neighbouring molecules). Mechanical milling of the particles powder with addition of dispersing additives, which form a layer around the particle, is widely used practice to inhibit agglomeration (Borm et al., 2006). The surface of the nanoparticles can also be modified depending on their applications.

4. Classes of engineered nanoparticles

ENPs can be divided into several classes, such as, carbonaceous nanomaterials; metal oxides; semi-conductor materials; zero-valent metals and nanopolymers (Fig. 1). Carbonaceous nanomaterials include fullerene compounds, nanotubes, nanowires, etc. The discovery of first fullerene (C_{60}-atom hollow sphere, also known as the buckyball) in 1985 marked the origin of this class (Kroto et al., 1985). C_{60} fullerenes possess a regular truncated icosahedron, the vertices of which bear the carbon atoms (Kroto et al., 1985). The smallest fullerene has a diameter of 0.7 nm (Mauter and Elimelech, 2008). Fullerenes are naturally non-ionogenic, but acquire charge under selective conditions. They have a negative zeta potential (Brant et al., 2005) and exhibit optical, mechanical, elastic and thermal properties. Later in 1991, cylindrical fullerene derivative, the carbon nanotube (CNT), was synthesized using vapour–liquid–solid (VLS) method (Kukovitsky et al., 2000). CNTs are formed of sheets of carbon atoms covalently bonded to form one-dimensional hollow cylindrical shape (Smart et al., 2006). There are two classes of CNTs:
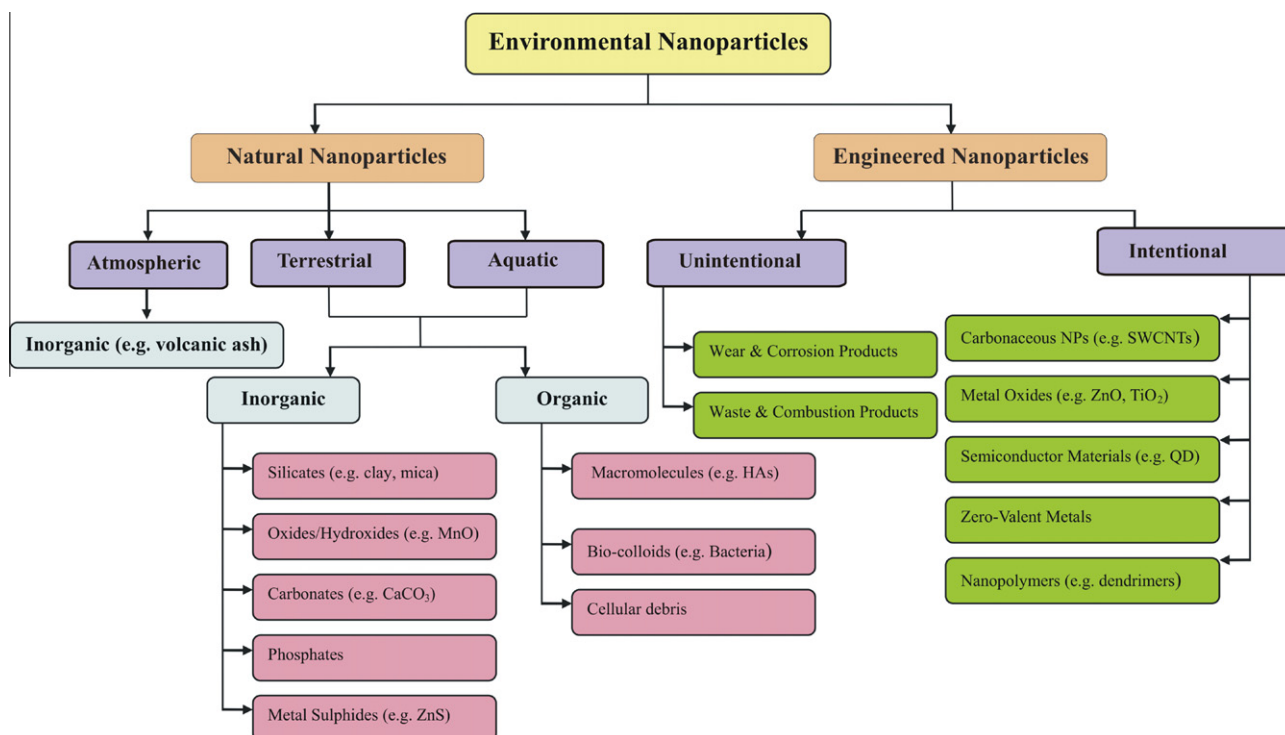

Fig. 1. Categories of nanoparticles present in the environment.

single walled (SWNTs) and multiwalled nanotubes (MWNTs). SWCNTs are structurally single-layered graphene sheets rolled up in cylindrical shapes of approximately 1 nm diameter and several micrometers of length, whereas MWCNTs possess two or more concentric layers with varying length and diameters (Gao, 2004). CNTs also possess mechanical, thermal, photochemical and electrical properties (Arepalli et al., 2001). Apart from these, they have a distinctive electron transport property (Watts et al., 2002). The agglomeration tendency of CNTs in aqueous solution can be prevented by covalent modification (attachment of polyethylene glycol to SWCNTs; Holzinger et al., 2004) or non-covalent modifications (e.g. the self assembly of SWCNTs and the phospholipids lysophosphatidylcholine and C_{60} and lysophosphatidylcholine; Qiao et al., 2007). CNTs and fullerenes offer a wide application in human health area, plastics, catalysts, battery and fuel electrodes, super capacitors, water purification system, orthopedic implants, conductive coating, adhesives and composites, sensors, and components in the electronics, aircrafts, aerospace and automotive industries (Klaine et al., 2008).

Metal containing materials, particularly metal oxides, belong to the second class of ENPs. Both individual, such as, Zinc oxide [ZnO], Titanium dioxide [TiO_2], Cerium dioxide [CeO_2], Chromium dioxide [CrO_2], Molybdenum trioxide [MoO_3], Bismuth trioxide [Bi_2O_3] and binary oxides such as, Lithium Cobalt dioxide [$LiCoO_2$], Indium Tin oxide [InSnO] are included in this class. Stabilized precipitation and flame pyrolysis are the two most common methods used for the synthesis of metal oxides (Christian et al., 2008). Amongst the metal oxides, TiO_2 has received considerable attention and extensive application due to its photo-catalytic properties and ultra-violet blocking ability (Klaine et al., 2008). TiO_2 particles are crystalline in nature and exist in three main structures viz. anatase, rutile and brookite (Li et al., 2004; Arami et al., 2007) of which anatase is most stable with particle diameters below 14 nm (Zhang and Banfield, 2000). In general TiO_2 has a large energy gap (E_g = 3.2 eV) and hence is considered as an excellent band-gap semi-conductor (Bellardita et al., 2007; Lihitkar et al., 2007; Reijnders, 2008). Like TiO_2, ZnO also has extensive application in skin care products (Christian et al., 2008). ZnO particles are 20–100 nm in size and possess a band gap energy of 3.36 eV, high exciton binding energy (60 meV) and high dielectric constant (Singh et al., 2007). Another metal oxide, CeO_2 is widely used as a combustion catalyst in diesel fuels to improve emission quality (Corma et al., 2004). CeO_2 can exist as both Ce(III) and Ce(IV) forms (Robinson et al., 2002) and the ratio of the two oxidation states appears to be size dependent showing increased Ce(III) form with decrease in size.

The third class of ENPs constitutes nanometer sized semi-conductor nanocrystals, known as quantum dots (QDs) with their size ranges between 2 and 10 nm (Schmid, 2004). QDs possess a reactive core consisted of metal or semi-conductor, such as, Cadmium selenide (CdSe), Indium phosphide (InP), or Zinc selenide (ZnSe), which controls their optical and electrical properties (Logothetidis, 2006; Klaine et al., 2008). The cores are produced from a nucleation reaction of the metal/semi-conductor materials and strict regulation of the growth of the crystals subsequently (Murray et al., 2001). To protect the core from oxidation and enhance the photoluminescence yield, a shell, made up of Silica or ZnS (Murray et al., 2001) surrounds the reactive semi-conductor cores. QDs possess unique magnetic and catalytic properties (Wang et al., 2007); high luminescence and stability against photobleaching (Hoshino et al., 2004). Relatively inexpensive and simple synthesis has broadened their use in experimental medicines, biomedical imaging and targeted therapeutics (Klaine et al., 2008; Logothetidis, 2006). They can be attached to a variety of surface ligands and inserted into various organisms for in vivo research (Roszek et al., 2005). They are also used in the production of solar cells and photovoltaics,

security inks, photomics and telecommunications (Alivisatos et al., 2005).

The fourth class of ENPs includes zero-valent metals, which are usually prepared by reduction of metal salts, e.g. zero-valent iron is made through the reduction of ferric (Fe^{3+}) or ferrous (Fe^{2+}) salts with a sodium borohydride (Li et al., 2006). Similarly, the chemical synthesis of gold and silver NPs involves dissolution of the metal salt in an appropriate solvent and its subsequent reduction to the zero valency. Metal ENPs exhibit an important phenomenon called as Surface Plasmon Resonance (SPR), which is caused by interaction of incident light and free electrons in the materials (Noguez, 2007). This imparts metal ENPs unique optical properties, e.g. 13 and 56 nm gold nanoparticles are rose-red and purple-red coloured in solution due to their large surface plasmon extinction bands. Zero-valent iron, has found usage in nitrate removal from water, soil and sediments and also for detoxification of organochlorine pesticides and polychlorinated biphenyls (Zhang, 2003). Metallic silver NPs or electrochemically generated ionic silver (diameter of ∼10 nm) is used widely in a broad range of products, e.g. textile products, baby-products, vacuum cleaners, washing machines, toothpastes, etc. (Klaine et al., 2008). However, its short half-life (results in aggregate formation) has limited its use in biological and other aqueous applications (Doty et al., 2005). Non-particulate gold (3–20 nm diameter) is used extensively as vector in tumor therapy and more recently, being used in electronics and also as catalyst (Klaine et al., 2008).

The fifth class of ENPs is made by dendrimers, which are complex, multifunctional polymers with 1–10 nm diameter. They assume highly asymmetric shapes and with increase in branching they adopt a globular structure (Caminati et al., 1990). The presence of numerous chain-ends confers high solubility and miscibility to dendrimers (Frechet, 1994). Physico-chemical and biological properties of dendrimers are comparable to traditional polymers, and their size, topology, flexibility, and molecular weight can be controlled (Logothetidis, 2006; Klaine et al., 2008). Dendrimers are generally prepared by emulsion polymerization where an emulsion of monomer (e.g. styrene) is prepared using a non-solvent (e.g. water) and a surfactant (e.g. sodium dodecyl sulphate) (Shim et al., 2004) and a water soluble initiator (e.g. ammonium per sulphate) is used to initiate free-radical polymerization. The branched tree-like structure of dendrimers facilitate the attachment of a variety of molecules including drugs thereby, broaden their use in areas ranging from biology, material sciences, and surface modification to enantioselective catalysis (Klaine et al., 2008). The applications of some commonly used ENPs are summarized in Table 1.

5. Physico-chemical properties of engineered nanoparticles

Physico-chemical properties of ENPs are one of the most important factors that regulate the behaviour of ENPs in the environment. Engineered nanoparticles are synthesized for a particular application therefore, the physico-chemical properties of each nanoparticles vary considerably. However, universally agreed and essentially required properties for ENPs are chemical composition, mass, particle number and concentration, surface area concentration, size distribution, specific surface area, surface charge/zeta potential, stability, solubility, nature of ENPs shell (Klaine et al., 2008).

Variation in composition, size or surface composition of ENPs, considerably change their physico-chemical properties (Borm et al., 2006). Properties like solubility, transparency, colour, conductivity, melting points and catalytic behaviour mainly depend on the particle size. Similarly, surface composition of the ENPs affects the dispersibility, optical properties, conductivity and cata-

Table 1
Commonly used Engineered Nanoparticles and their applications.

Class of ENPs	Application
Carbonaceous compounds	
1. CNTs and their derivatives	For sorption of metals such as copper, nickel, cadmium, lead, silver, zinc, americium and rare earth metals
	In electronics and computers
	In plastics, catalysts, battery, fuel cell electrodes, supercapacitors, water purification systems, orthopedic implants, conductive coatings, adhesives and composites, sensors, and components in the electronics, aircrafts, aerospace, and automotive industries, sporting goods.
2. C_{60}	In cosmetics
3. Fullerenes	Sorption of organic compounds (e.g. naphthalene)
	For the removal of organometallic compounds
4. Nanowires	For early detection of cancerous tumor
Metals and metal oxides	
1. TiO_2	In cosmetics, skin care products, sunscreen lotions
	In solar cells, paints and coatings
	In bioremediation, for the removal of various organics (phenol, p-nitrophenol, salicylic acid)
2. ZnO	In skin care products
	In bottle coatings
3. CeO_2	As combustion catalyst in diesel fuels to improve emission quality, I gas sensors, solar cells, oxygen pumps
	In metallurgical and glass/ceramic applications
Semi-conductor devices	
Quantum dots (QDs)	In medicine, e.g. medical imaging and targeted therapeutics
	In solar cells, photovoltaic cells, security inks, photonics and telecommunications
Zero-valent metals	
1. Zero-valent iron	Remediation of water, sediments, and soils to remove nitrates and organic contaminants
	In detoxification of organochlorine pesticides and polychlorinated biphenyls
	In bioremediation for the decomposition of molinate (a carbothionate herbicide)
2. Nanoparticulate silver	In wound dressings, socks, and other textiles
	In air filters, toothpastes, baby-products, vacuum cleaners, and washing machines
3. Colloidal elemental gold	As vector in tumor therapy
	As catalyst
	In flexible conducting inks or films
Polymers	
Dendrimers	In manufacture of macrocapsules, nanolatex, coloured glasses, chemical sensors, modified electrodes
	As DNA transfecting agents, therapeutic agents for prion diseases
	In drug delivery and DNA chips
	In tumor treatment (used as a powerful anticancer drug)

lytic behaviour of the particle. Metallic ENPs are usually coated with inorganic or organic compounds or surfactants to maintain their stability as colloidal solutions (Mafune et al., 2000). Thus, surface properties of ENPs strongly depend on composition of these coatings also. Further surface properties of ENPs decide their fate in the environment, e.g. formation of colloidal solution or aggregation. In colloidal solution, ENPs remain dispersed and maintain their reactivity and catalytic behaviour and thereby easily interact with various components of the environment. Whereas, in aggregation where ENPs tend to agglomerate with neighbours due to high surface energy, ENPs loose their reactivity as well as catalytic nature by forming large sized particles. However, aggregation is a kinetic process; size may easily change with passage of time or due to natural geothermal weathering processes (Klaine et al., 2008). But reverting back to nano-sized is time taking process and also influenced by variety of environmental factors, e.g. pH, ionic strength, salinity, etc. (Klaine et al., 2008). ENPs also show tendency to interact with the natural organic matter (NOM) (Buffle et al., 1998) or artificial organic compounds, which further direct their stability or aggregation. Aggregation of ENPs is enhanced in presence of high-molecular weight NOM compounds, whereas, low-molecular weight NOM compounds increase the mobility of ENPs in colloidal solution (Navarro et al., 2008).

6. Release of engineered nanoparticles in the environment

Increased production and widespread use ENPs in various industries cause their frequent release into the environment. ENPs enter the environment through intentional as well as unintentional releases such as atmospheric emissions and solid or liquid waste streams from production sites. Intentional release of ENPs in the environment includes the uses of nanoparticles for remediation of contaminated soil and water (Klaine et al., 2008). A proportion of nanoparticles used as one of the components of paints, fabrics, personal health care products, cosmetics, etc. also find route to enter the environment (Biswas and Wu, 2005). Several routine human activities, e.g. wear of car tyres, urban air pollution also significantly release the nanoparticles in the environment.

As stated earlier, the natural nanoparticles are present in the environment from the beginning of the earth's history, so why the release of ENPs in nature is becoming an increasing concern of regulatory agencies. Natural nanoparticles often disappear from the environment by dissolution or become larger enough through aggregation. But ENPs may persist in the environment due to their stabilization by capping or fixing agents (Handy et al., 2008b). Additionally, ENPs may contain chemically toxic components in concentration of structural forms that do not occur naturally.

7. Biological uptake of engineered nanoparticles

The probable mechanisms of uptake of ENPs by living organisms are not well known so far. But, it has been believed that animals incorporate NPs in their bodies mainly via gut (Baun et al., 2008). NPs can enter in the gut cells by diffusing through cell membranes (Lin et al., 2007), through endocytosis (Kim et al., 2006) and adhesion (Geiser et al., 2005) as shown in Fig. 2. The uptake of NPs
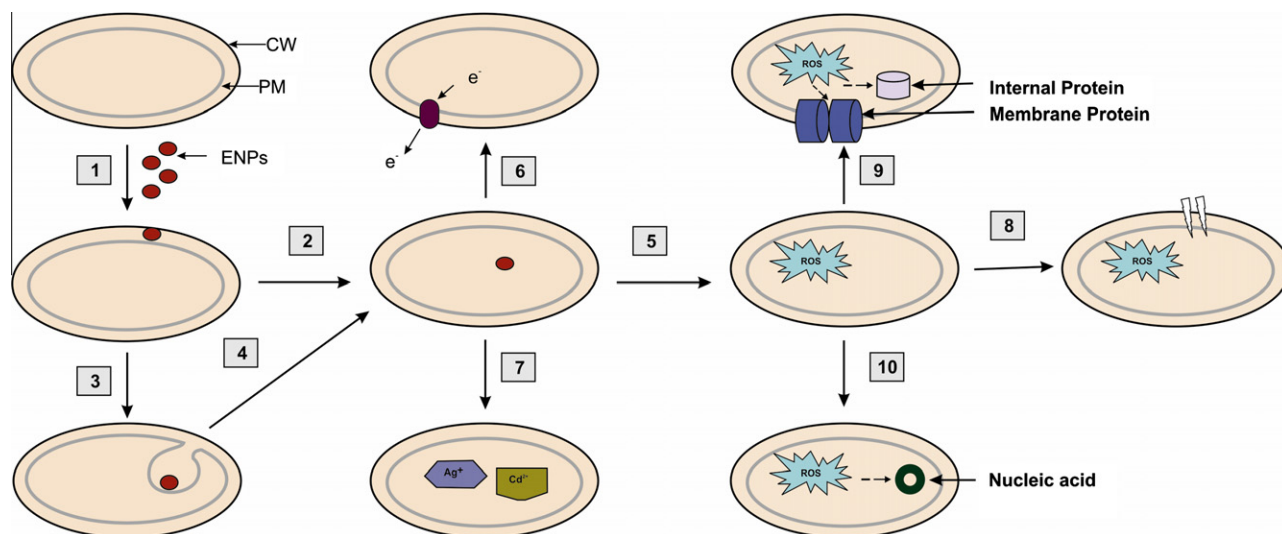
Fig. 2. Possible mode of entry in the cell and mechanism of toxicity of engineered nanoparticles (ENPs). ENPs enter the cell through the semi-permeable cell wall (1), attach to the plasma membrane and enter the cell via diffusion (2) or endocytosis (3 and 4). Once inside the cell, they interrupt the energy transduction (6), release toxic elements inside the cell (7) or produce ROS by altering and interfering with the metabolic activities of various organelles (5). The ROS thus produced disrupt the cell membrane (8), destabilize and oxidize proteins (9) or damage the nucleic acids (10).

via ion transporters seems unlikely considering the larger size of NPs than ions (Handy et al., 2008a). Moore (2006) supported the theory of uptake of NPs via endocytosis by demonstrating the uptake of polyester NPs via lysosomes and endosomes of mussels. But in cases of plants, algae, and fungi cell wall acts as a barrier for the entry of ENPs. But pores (with size ranging from 5 to 20 nm) present in cell wall allow the entry of small molecules like ENPs (Zemke-White et al., 2000). It has been suggested that the interaction of ENPs with cells might induce the formation of new and bigger pores and thereby increase the internalization of ENPs through the cell wall (Navarro et al., 2008). After passing through the cell wall, ENPs enter the bilipid plasma membrane by similar mechanisms as described for animals (Moore, 2006). A cavity-like structure (Fig. 2) forms during the endocytic process, which results in the internalization of ENPs into the cell (Navarro et al., 2008). The internalized ENPs may bind with different organelles and interfere with various metabolic processes.

Compared with algae and fungi, plants due to their large size and high leaf area frequently interact with ENPs. Airborne ENPs get attached to the leaves and reach into the leaf tissue through stomata, cuts or wounds present on leaf surface. Root parts of the plant also encounter with water or soil-borne ENPs and easily accumulate and transfer them to different plant parts (Navarro et al., 2008).

8. Ecotoxicity of engineered nanoparticles

Though a detailed mechanism of toxicity caused by ENPs is not yet elucidated, a few mechanisms like damage to membrane integrity, protein destabilization and oxidation, damage to nucleic acids, production of reactive oxygen species (ROS), interruption of energy transduction, release of harmful and toxic components are likely involved in the damage caused by ENPs (Klaine et al., 2008).

Cell membrane is a potential target of damage from ENPs. Carboxyfullerene and gold NPs have been reported to cause death by puncturing cell membrane of gram-positive bacterial stain (Tsao et al., 1999). ENPs induce heat shock responses in *Escherichia coli* in weakening the membrane (Hwang et al., 2007). Gold NPs have been known to alter the structure and activity of enzymes like glucose oxidase (Liu et al., 2004). Fullerenes interact with DNA and

affect the DNA stability and functions by strand deformation (Zhao et al., 2005). Scientific Committee of Emerging and Newly Identified Health Risks (SCENIHR) (2007) has suggested a probable involvement of ENPs in causing tumor formation through DNA damage in lungs.

ENPs stimulate the production of ROS in organisms and cause damage in possibly every cell component. These ROS oxidize double bonds of fatty acids in cell membrane resulting in increased permeability rendering it more susceptible to osmotic stress. ENPs, like TiO_2, with photocatalyst properties (Khus et al., 2006) upon exposure to UV light (Zhao et al., 2007) generate ROS and can nick supercoiled DNA. ROS production can also lead to DNA strand breaks, cross-linking, and adducts of the bases or sugars (Cabiscol et al., 2000). Other photosensitive ENPs like fullerenes and silver NPs are shown to cleave double stranded DNA upon light exposure (Badireddy et al., 2007). ENPs like QDs are known to release harmful components, such as heavy metals or ions, which cause toxicity to the cells (Klaine et al., 2008). The metals released from ENPs have a long retention time in the cell where they cause damage at various levels. Ionic silver (Ag^+) released from silver-containing ENPs interact with the thiol groups of vital enzymes resulting in their inhibition (Matsumura et al., 2003). It has been reported that Ag^+ is inhibitory to certain respiratory enzymes and induces ROS production (Kim et al., 2007). Ag^+ also has a tendency to bind with the sulphur- and phosphorus-containing antioxidant molecules (Pappa et al., 2007), thereby depleting their concentration and weakening the antioxidant defense system of cells (Hussain et al., 2005). Certain reports show the involvement of Ag^+ in prevention of DNA replication and permeability of cell membrane (Feng et al., 2000). The alteration of membrane structure and porosity results in disruption of electron transport phosphorylation and energy transduction processes. It is suggested that the cytotoxic effects of CeO_2 NPs are mainly due to oxidization of membrane components involved in the electron transport chain (Thill et al., 2006).

Recently, a few mammalian models have been studied (Handy and Shaw, 2007) with primary focus on respiratory toxicology and inflammation reactions to ENP exposure (Lam et al., 2004). Zhu et al. (2006) reported generation of oxidative stress in the gills of adult fathead minnow (*Pimephales promelas*) upon exposure to nC_{60} fullerene prepared by water stirring. Upon ingestion of

water-containing SWCNTs, gill irritation and brain injury was seen in rainbow trout (*Oncorhynchus mykiss*) (Smith et al., 2007). Federici et al. (2007) also reported the sensitivity of gills of *O. mykiss* towards TiO$_2$ NPs. Fullerenes and SWCNTs have also been reported to cause neurotoxicity in fishes (Oberdorster et al., 2006; Smith et al., 2007). Furthermore, *Daphnia magna* (Lovern and Klaper, 2006) and *Hyallela azteca* (Oberdorster et al., 2006) have also been studied for toxicity caused by ENPs. Recently, effect of C$_{60}$ on the mobility, moulting and feeding behaviour in *H. azteca* (Oberdorster et al., 2006) and locomotory behaviour in *D. magna* (Lovern et al., 2007) has been studied. A few ecotoxicity studies have also been performed on marine organisms. Kashiwada (2006) reported that the toxicity, in terms of mortality rate, in Japanese medaka exposed to fluorescent NPs (30 mg L^{-1}) increased with increase in salinity. A reduction in the life cycle molting success was reported in the estuarine copepod *Amphiascus tenuiremis* exposed to SWCNTs (Templeton et al., 2006). Literature elucidating the effect of ENPs on photosynthetic organisms is also limited (Navarro et al., 2008). Exposure of carbon black NPs on marine macroalga *Fucus serratus* resulted in a reduction in fertilization success (Nielsen et al., 2008). High concentrations of TiO$_2$ NPs have been shown to inhibit the growth of green alga *Desmodesmus subspicatus* (Hund-Rinke and Simon, 2006). Yang and Watts (2005) reported the toxicity of alumina (Al$_2$O$_3$) NPs at high concentrations to root growth in cabbage, carrot, corn, cucumber and soybean. Similarly, Lin and Xing (2007) showed a negative effect on the growth in raddish, rape and ryegrass upon exposure to Zn and ZnO NPs. Multi-walled carbon nanotubes were also shown to reduce the root growth of the ryegrass (Goodman et al., 2004). A detailed knowledge of ecotoxicity of ENPs on bacteria and other microorganisms is also lacking. SWCNTs are antibacterial to *E. coli* and result in cell membrane damage (Wei et al., 2007), whereas, fullerene derivatives with pyrrolidone groups inhibit growth of *E. coli* by interfering with energy metabolism (Mashino et al., 2003). Bosi et al. (2000) reported the growth inhibition of Mycobacteria exposed to a few C$_{60}$ derivatives. QDs have also been reported to cause oxidative damage and toxicity to *E. coli* and *Bacillus subtilis* (Hardman, 2006). A summary of deleterious impact of ENPs on various organisms is given in Table 2.

Besides these toxic effects, some reports show positive effects of ENPs on organisms. Wang et al. (1999) reported that fullerenes may help in preventing lipid peroxidation induced by superoxide and hydroxyl radicals, thus, emphasizing or its antioxidant property. TiO$_2$ NPs have also been found to increase the dry weight, chlorophyll synthesis, and metabolism in photosynthetic organisms (Navarro et al., 2008). At low concentrations, TiO$_2$ is also reported to stimulate spinach seed germination and seedling growth (Zheng et al., 2005). Due to their antimicrobial properties, it is suggested that ENPs may increase strength and resistance of plants to stress. Furthermore, ENPs with high specific surface area may also help to sequester nutrients on their surface, thus serving as a nutrient stock to the organisms (Navarro et al., 2008).

9. Ecotoxicity test strategies and biological hazard assessment

Frequent release and interaction of ENPs with various components of environment as well as probable risks necessitate the development of certain strategies to test the potential hazards of ENPs. The toxicity of ENPs vary with their size and shape and other basic properties, therefore, it is essential to study these basic properties while assessing its biological hazards. But studying the basic properties of ENPs becomes difficult because of two reasons. First, their relevant concentration (in ng L^{-1} to pg L^{-1} range) in environment is lower than the detection limits for most test methods. Second, environmental samples also carry natural and unintentionally produced NPs in addition to intentional ENPs (Lead and Wilkinson,

Table 2
Summary of deleterious impacts of ENPs on various organisms based on available data.

Particle type	Concentration	Exposure time	Test organism	Effect	References
Carbon-containing fullerenes					
Filtered C$_{60}$ suspended in tetrahydrofuran	0.5–1 mg mL^{-1}	48 h	*Micropterus salmoides*	Significant lipid peroxidation	Oberdorster, 2004
	260 µg L^{-1}	60 min	*Daphnia magna*	Increased hopping frequency, appendage movement and heart rate	Noack et al., 2000
C$_{60}$ (prepared with benzene and acetone)	1.5 mg L^{-1}	96 h	*Danio rerio*	Delayed embryo and larval development, decreased survival and hatching rates	Gimbert et al., 2007
Carbon nanotubes					
MWCNTs	>1 mg L^{-1}	5 d	*Stylonychia mytilus*	Inhibition of growth	Zhu et al., 2006
	0.5–5 mg rat^{-1}	3–15 d	Rat	Generation of collagen rich granulomas	Muller et al., 2005
SWCNTs	0.1–0.5 mg L^{-1}	>10 d	*Oncorhynchus mykiss*	Oxidative stress linked effects, increase in ventilation rate, gill pathologies and mucus secretions	Gimbert et al., 2007
	0.1–0.5 mg mouse^{-1}	7–90 d	Mouse	Weight loss, lung lesions, necrosis, macrophages and granuloma, intestinal and peribronchial inflammation	Lam et al., 2004
Metal-containing ENPs					
Titanium oxide	0.1–1 mg L^{-1}	14 d	*Oncorhynchus mykiss*	Gill pathologies	Kretzschmar et al., 1995
Ultrafine cadmium oxide	70 µg m^{-3}	1–7 d	Rat	Increased percentage of neutrophils and multifocal alveolar inflammation	Takenaka et al., 2004
Ultrafine metallic nickel	0.15–2.54 mg m^{-3}	5 h–21 d	Rat	Increased lung weight, accumulation of foamy alveolar macrophages	Serita et al., 1999
Silver nanoparticles	5–50 µg mL^{-1}	24 h	Rat	Decreased cell glutathione level, malfunctioning of mitochondria and ROS generation	Hussain et al., 2005
Copper nanoparticles	100 µg L^{-1}	48 h	*Danio rerio*	Gill injury and lethality	Seaman and Bertsch, 2000
Quantum dots					
Cadmium telluride	8 mg mL^{-1}	24 h	*Elliptio complanata*	Significant increase in lipid peroxidation, DNA damage and reduced phagocytic activity	Karathanasis, 1999

2006). Therefore, it is required to develop or update the already existing methods to achieve a better screening capability and high selective detection. The properties of the material of which the sampling vessel is made should also be kept in mind. The possible charges on the walls of vessel and other physico-chemical properties should be checked to ensure minimum adsorption and contamination of ENPs. Hall (1998) suggested the use of plastics (especially fluoroplastics) for inorganic ENPs or metal analysis and glass for analysis of organic trace constituents for the water samples containing ENPs. The ENPs in soils and sediments have much higher quantities of natural colloids than water systems, because of which they enhance the complication in different analyses. To reduce the mixture of particles in the environmental samples, pre-fractionation can be done by using stirring, centrifu-gation or filtration (Hassellov et al., 2008). Fractionation is most commonly used for pre-fractionation. Size fractionation can either be done using membranes as in ultra-filtration, nano-filtration and dialysis or by using chromatographic techniques such as size exclusion and hydrodynamic chromatography (Hassellov et al., 2008). Once the pre-fractionation is complete, the size of ENPs can be determined by using various techniques, light scattering being a very commonly used method amongst them (Schurtenber-ger and Newman, 1993). Photon correlation spectroscopy (also called dynamic light scattering, DLS), multi angle (laser) light scat-tering (also called static light scattering, SLS), nephelometry and la-ser induced breakdown detection (LIBD) are used to determine the size and sometimes shape of the ENPs (Hassellov et al., 2008). Cer-tain classes of ENPs, e.g. QDs exhibit strong fluorescence. The
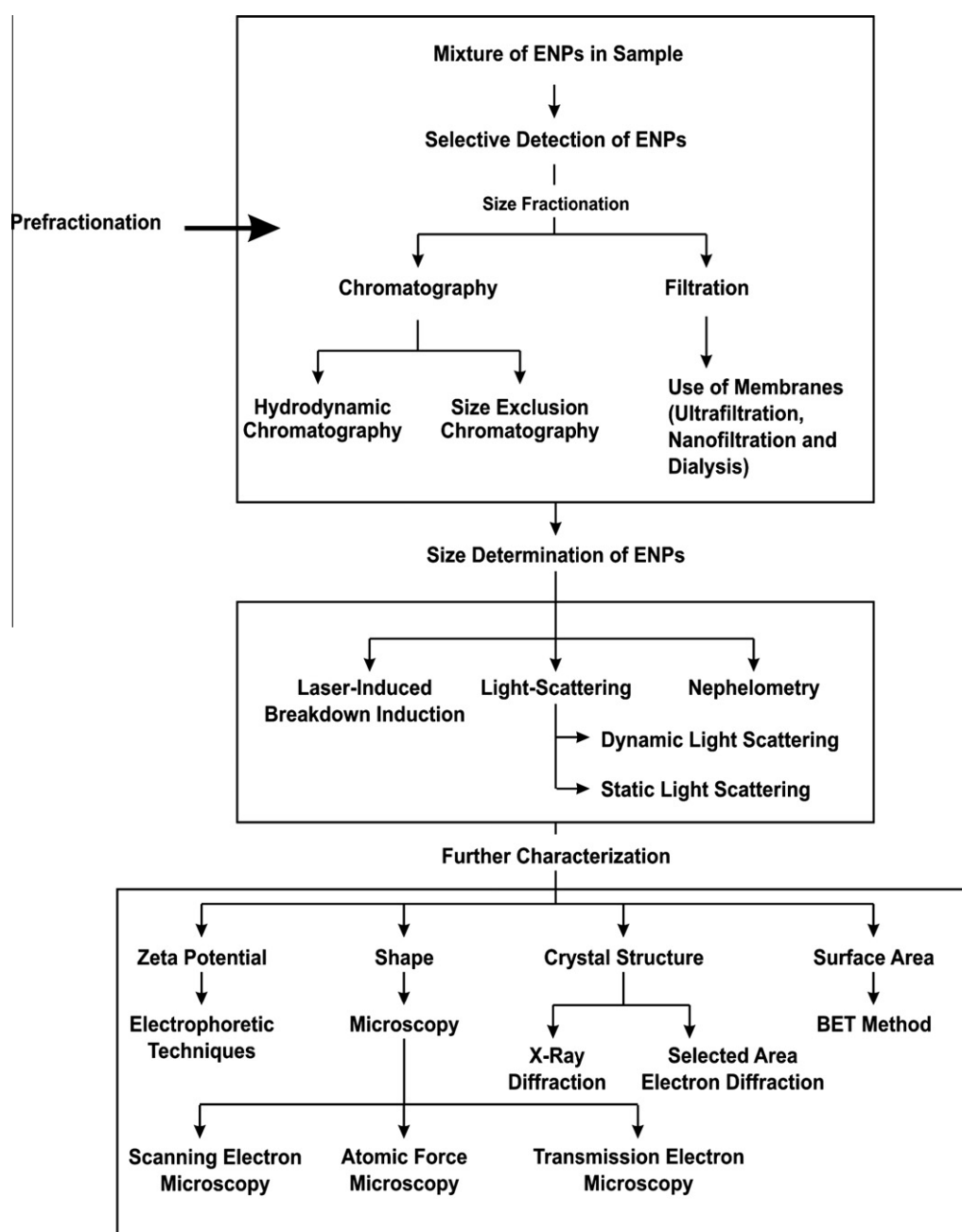

Fig. 3. Size determination of ENPs and the methods of their characterization in the environment.

absorption or fluorescence emission spectra of these particles can help in their categorization. Further, to enhance the information about the composition, structure and charges or force measurements, microscopy techniques like electron microscopy (transmission electron microscopy [TEM] and scanning electron microscope [SEM]) and atomic force microscopy (AFM) are practiced (Hassellov et al., 2008). AFM is one of the most common methods for the study of nanometrics (Viguie et al., 2007). ENPs have a tendency to develop surface charges or surface potentials in aqueous solutions, which primarily determines their stability and dispersion thus influencing their fate and behaviour (Guzman et al., 2006). The direct measurement of surface potential is not easy; however, the measurement of its zeta-potential can be done easily by electrophoretic techniques (Hassellov et al., 2008). Finally the surface area can be measured by using Brunauer, Emmet, Teller (BET method) (Brunauer et al., 1938) and the crystal structure of ENPs can be determined by using X-ray diffraction (XRD). TEM can also be used to study the diffraction patterns of single particles of NPs using a method called selected area electron diffraction (SAD or SAED) (Hassellov et al., 2008). The methods for size-determination and further characterization are illustrated in Fig. 3.

After the analysis and characterization of ENPs, the effects of ENPs on organisms are studied. The first step in the studies of ENPs is the sampling and sample handling. Being dynamic non-equilibrium systems, ENPs are often sensitive to physical or chemical disturbances (Filella, 2007), which usually affect the dispersion state of ENPs. Any change in the dispersion state results in either further aggregation or partial disruption of existing aggregation (Navarro et al., 2008). Interaction of ENPs with the natural organic material present in the sample leads to further complication in maintaining the original state of ENPs. Therefore, it becomes essential to ensure proper dispersion of ENPs in the media used for sample preparation. Use of dispersants, e.g. surfactants (Jiang et al., 2003) and biopolymers, including humic and fulvic acids (Hyung et al., 2007) facilitate even dispersion of ENPs. For ENPs like CNTs, which have a tendency to form aggregates, techniques like sonication may be practiced. It further requires use of strong acid, which hydroxylates and repairs the ends and damaged regions of the tubes (Nowack and Bucheli, 2007). Sonication and use of chemical dispersants often result in changes in their physio-chemical state (Gee and Bauder, 1986). Other than use of surfactants and sonication, prolonged stirring may be used to form a uniform stock solution without causing much damage to the NP test substance (Crane et al., 2008). The dispersant should also be selected keeping its properties in mind. Few good dispersants, e.g. tetrahydrofuran (THF) are toxic to organisms (Zhu et al., 2006). Therefore, it is suggested to compromise on using a less powerful dispersant rather than imposing the risk of solvent toxicity on the test organism.

The present standard toxicity tests can assess both short- and long-term impacts of NPs. For an effective study of the impact of NPs, a dose–response relationship (exposing the organism to different concentrations of NPs) should also be studied (Navarro et al., 2008). Standard ecotoxicity tests used to assess the environmental hazards associated with chemical substances focuses on the species and measurement endpoints that are used in ecotoxicity tests, and the methods of test substance dosing and exposure, as these are the most important aspects of the tests for understanding whether they are suitable for assessing the toxicity of NPs. The endpoints that are measured in these tests are usually Lethal Concentrations (LC), Effective Concentrations (EC) or No Observed Effect Concentrations (NOECs). In most cases, these are for survival, growth or reproduction, but for microbes and algae the endpoint is population growth because of their rapid growth. These are widely accepted endpoints for use in chemical risk assessment, even if their utility for assessing NP toxicity has yet to be elucidated (Crane et al., 2008).

10. Legislations on the management of risk related to engineered nanoparticles

Due to serious environmental risk posed by the release of ENPs in the environment, it becomes essential to set specific standards for the manufacture, use, and disposal of ENPs (Handy and Shaw, 2007). The Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR, 2005) reports that the existing legislations do not mention or define ENPs. However, a number of organizations have evolved development-, description- and usage-associated standards for ENPs (Bard et al., 2009). The potential hazard by ENPs and the subsequent protection of environment and human health is also taken into consideration in these standards. Some of the legislations set by European Union deal with the health and safety regulations for the protection of workers, e.g. General Directive 89/391/EEC (on the improvements in the safety and health of workers at work); Directive 98/24/EC, Directive 2004/37/EC, Directive 1992/92/EC (on the protection of the health and safety of workers from the risks related to chemical agents, carcinogens or mutagens, and explosive atmospheres, respectively); Council Directive 89/655/EEC (concerning the minimum safety and health requirements for the use of work equipments by workers at work). Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) legislation as applicable from 1st of July 2007 also notifies for the inclusion of hazardous data for nano-sized materials. However, some ENPs cannot be classified as new substances on the basis of REACH as the threshold value for the registration of a new chemical substance is 1 ton (Bard et al., 2009; Handy and Shaw, 2007). Therefore, some ENPs by-pass the testing and safety evaluations as their concentration seldom reach this limit. Another interesting point to be noted is that some ENPs have identical or similar chemical formula to an existing compound. This results in another by-pass of ENPs from chemical abstracting service (CAS) which provides a series of unique identifying numbers for existing chemicals (Handy and Shaw, 2007).

A few organizations have established specific committees for developing standards on the usage and manufacture of ENPs. Few of these are CEN/TC 352 "Nanotechnologies" established by European Committee for Standardization CEN; ISO/TC 229 "Nanotechnologies" established by ISO and TC 113 "Nanotechnology standardization for electrical and electronic products and systems" established by IEC (Bard et al., 2009). Other than these non-specific rules, not only there are no specific regulations for ENPs, but also, the gaps in knowledge regarding their toxicological and exposure data prevail. It is therefore an important matter of concern to build up specific legislations and guidelines for ENPs in order to avoid the risk imposed by ENPs on the environment.

11. Future research

Considering the wide application of ENPs and their entry into the environment, the study of their impact on the ecosystem, at biotic as well as abiotic level, has become mandatory. Only a limited number of areas have been covered as far as ecotoxicity tests and assessment of the hazardous effects of ENPs are concerned. Therefore, it is required to study their release, uptake, and mode of toxicity in the organisms. Furthermore, to understand the long-term effect of ENPs on the ecosystem, substantial information is required regarding their persistence and bioaccumulation.

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