The Bioscan 8(3): 975-982, 2013 (Supplement on Toxicology)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

NANOTOXICITY AT VARIOUS TROPHIC LEVELS: A REVIEW

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KEYWORDS
Biosafety
Nanotoxicology
Oxidative Stress
Reactive Oxygen Species

Received on :
13.05.2013

Accepted on :
16.08.2013

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ABSTRACT
Nanotoxicity refers to the physiological and metabolic interruptions caused by engineered nano-particles that may differ at various trophic levels of ecological pyramids. This review focuses on the nanotoxicity events that are reported in literature in a wide array of living organisms such as algae, microbes, plants, fishes, rodents and humans. Literature survey reveals that even adaptive organisms such as algae which have proved to tolerate adverse and fluctuating environmental conditions are prone to nanotoxicity as a result of defective photosynthetic system. The microbes such as plant growth promoting rhizobacteria and other beneficial soil microorganisms have been reported to be inhibited in its functionalities by nanoparticles but their relative toxicities are quite inconclusive and warrant further investigations. Despite plants have evolved tolerance mechanisms to deter xenobiotics, they expressed their sensitivity to some of the nano-particles as a consequence of physical and chemical routes of action. In animal models (rodents), the data have vividly shown that the nanoparticles have caused significant inflammatory effects while in aquatic system (fish) nanoparticles are found to accumulate in various organs besides causing morphological dysfunctions. In the case of humans, nano-particles enter primarily through inhalation which causes inflammation and ultimately cancer. Overall, the nanotoxicity in biological systems is mainly caused by the excessive production of reactive oxygen species that damages the living cells. Despite this mechanism has been unequivocally demonstrated in some case studies, scientists are still working harder to establish a clear relationship between nanoparticles and its toxicity impacts.

INTRODUCTION

Nanotechnology is advancing rapidly and the engineered nanoparticles (ENPs) are finding applications in a wide spectrum of disciplines such as electronics, energy, environment, agriculture and health sectors (Subramanian and Tarafdar, 2011). Engineered nanoparticles are defined as manufactured particles with at least one dimension below 100nm (Nowack and Bucheli, 2007). Although humans have been exposed to airborne nanosized particles throughout their evolutionary stages, such exposure has increased dramatically over the last century due to anthropogenic sources. The production of ENPs was 2000 tonnes in 2004 and it is expected to increase to 58,000 tonnes in 2011 - 2020 (Maynard, 2006). And it is predicted that increased manufacture and use of nanomaterials closely coincides with human and environmental exposure (Rajkishore *et al*., 2011a).

Nanotoxicology is an emerging discipline that can be defined as "science of engineered nanodevices and nanostructures that deals with their effects in living organisms" (Oberdorster *et al.,* 2005). Results of older biokinetic studies with nano-sized particles and newer epidemiologic and toxicologic studies with airborne ultrafine particles can be viewed as the basis for expanding the field of nanotoxicology. The nanomaterials commercially desirable can also make them more toxic than their normal size counterparts. Only limited information is available to the public because of statutory protections afforded to manufacturers who claim that even basic data are "confidential business information."

Basically, nanotechnology is about developing products and process that behave differently through controlling their makeup at the nanoscale. The particles at the nano-scale exhibit very large surface to mass ratio, which is a distinctive property and it will challenge the way we identify, understand and address potential risks (Rajkishore *et al*., 2011b). Current research into the risks presented by engineered nanomaterials is rather limited. However, it is sufficient to alert us to the fact that some ENPs do indeed behave differently to their more conventional counterparts and may present new and unusual risks. Despite the fact that it is challenging to evaluate the risks of ENPs before commercial products are well defined, proactive research is critical to ensure safety and sustainability (Colvin, 2003).

Biological responses to nanoparticles

Several studies have documented the toxic effects of nanoparticles in biological systems at various trophic levels (Table 1).

Algae

The prokaryotic and eukaryotic algae serve as a base for primary productivity and food web chain equilibria, and thus the nanotoxicity assessment becomes inevitable (Herrero and Flores, 2008). A few studies confirmed that exposure to nanoscale TiO_2 affects algal growth (Aruoja *et al.,* 2009) and photosynthetic activity (Navarro *et al*., 2008a). The single-celled microalgae (*Pseudokirchneriella subcapitata*) treated with CeO_2 nanoparticles has exhibited toxicity (Hoecke *et al.,* 2009). AgNPs reported to be more toxic than Ag^+ to *Chlamydomonas reinhardtii,* mobile single-celled algae (Novarro *et al.,* 2008 a,b). The abundance and unique

metabolic strategies used by cyanobacteria (blue-green algae) to tolerate adverse and fluctuating conditions often make them a good model for evaluating environmental stresses (Apte *et al.,* 1998). Cherchi *et al*. (2011) investigated the impact of $nTiO_2$ exposure on the cellular structures of the nitrogen-fixing cyanobacteria *Anabaena variabilis*. They observed alteration in various intracellular structures and $nTiO_2$ induced a series of recognized stress responses, including production of ROS (Reactive Oxygen Species) that increases the abundance of membrane crystalline inclusions, membrane mucilage layer formation, opening of intra-thylakoidal spaces and internal plasma membrane disruption. This study demonstrated that the internalization of $nTiO_2$ particles through multilayered membranes in algal cells may ultimately impact the ecological food web. In a recent study, it is reported that SiO_2 NPs were toxic to *Pseudokirchneriella subcapitata* in standard OECD (Organization for Economic Cooperation and Development) test medium, however SiO_2 NPs coated with a thin layer of alumina onto the surface were found to be less toxic (Hoecke *et al*., 2011). This result highlights the point that coating formulations also should be taken into account when performing risk assessments of ENPs.

Microbes

The increased applications of nanotechnology will inevitably lead to the accumulation of ENPs in soil and has raised concerns about their ill-effects on soil microbial activity and diversity. Silver nanoparticles (AgNPs) are the most widespread metallic nanomaterials found in consumer products due to their antimicrobial activity (Klaine *et al*., 2008). AgNPs damaged the cell wall of *Escherichia coli,* leading to increased cell permeability and ultimately cell death (Sondi and Salopek-Sondi, 2004). Moreover, the toxicity of AgNPs has been reported in heterotrophic (ammonifying/nitrogen fixing/plant growth promoting rhizobacteria) and chemolithotrophic, soil formation bacteria (Throback *et al.,* 2007). Fullerenes have been found to inhibit the growth of commonly occurring soil and water bacteria (Oberdorster *et al.,* 2005). Nano zerovalent iron particles (nZVI) particles exhibited a bactericidal effect on *Escherichia coli,* but the toxic effects were not observed with other types of iron-based compounds, such as iron oxide nanoparticles, microscale ZVI and Fe^{3+} ions (Lee *et al*., 2008). Copper oxide NPs showed antibacterial activity against plant growth promoting strains such as *Klebsiella pneumonia, P. aeruginosa, Salmonella paratyphi and Shigella* (Mahapatra *et al*., 2008). Metal nanoparticles like fullerens, gold, silver, aluminium caused toxicity to plant growth promoting rhizobacteria (Mishra and Kumar, 2009). They also reported that plant growth promoting rhizobacteria (PGPR) like *P.aeruginosa, P.putida, P.fluorescens, B.subtilis* and soil N cycle bacteria *viz*., nitrifying bacteria and denitrifying bacteria showed varying degrees of toxicity when exposed to ENPs in controlled conditions. Emami-Karvani and Chehrazi (2011) reported that the ZnO nanoparticles showed antibacterial activity on both Gram-positive (*Escherichia coli*) and Gram-negative bacteria (*Staphylococcus aureus*). The reports on the relative toxicities of metal and metal oxides ENPs on microbes are contradictory and inconclusive (Dinesh *et al*., 2012). For example, Jiang *et al*. (2009) reported that ZnO NPs was highly toxic causing 100% mortality of *B. subtilis, E.coli and P. fluorescens* while CuO NPs was more toxic to beneficial rhizosphere isolate *P. chlororaphis O6* than ZnO NPs (Dimkpa *et al*., 2011). Though it is claimed that nanoparticles produced through biological synthesis is environmentally safer, the results from the study conducted by Jaidev and Narasimha (2010) disagree this notion. They reported that Ag NPs biosynthesized by fungi showed potent activity against fungus like *Aspergillus niger* and bacterial strains such as *Staphylococcus* sp., *Bacillus* sp. and *E. coli*. In spite of several investigations on nanotoxicity in microbial systems, still results are elusive. For instance, Shah and Belozerova (2009) registered no significant negative effect of Si, Pd, Au and Cu NPs on soil microbial communities. In contrast, Ge *et al*. (2011) reported that metal oxide NPs may measurably and negatively impact soil bacterial communities. This emphasizes the need for research that generates dataset on the effects of ENPs on microbial communities.

Higher plants

Plants interactions with nanoparticles and their associated impacts have been reported extensively in literature (Table 2). There are two likely modes of nanotoxicity in plants namely physical and chemical. Physical nanotoxicity is closely associated with the restricted flow of nutrients as a direct consequence of apoplastic or symplastic trafficking (Ma *et al.,* 2010). Nanoparticles interfere with the plant transport pathways as a physical barrier rather *i.e.,* by inhibiting through the blockage of the intercellular spaces in the plant cell wall or cell wall pores. On the other hand, the chemical nanotoxicity is related to the excessive production of reactive oxygen species (Nel *et al*., 2006). Most of the studies with ENPs indicated certain degree of phytotoxicity, especially at higher concentrations. Zinc and ZnO nanoparticles inhibited seed germination and root growth (Yang and Watts, 2005; Lin and Xing *et al*., 2007). Single-walled carbon nanotubes (SWCNTs) affected root elongation of tomato, cabbage, carrot and lettuce (Canas *et al.,* 2008) and caused programmed cell death in *Arabidopsis thaliana* and *Oryza sativa* (Shen *et al.,* 2010). In addition, it is also reported that the SWCNTs caused adverse cellular responses including cell aggregation, chromatin condensation, plasma membrane deposition and H_2O_2 accumulation in rice and *Arabidopsis thaliana* protoplasts. Silver nanoparticles (AgNPs) disrupted cell division process causing Chromatin Bridge, stickness and cell disintegration (Kumari *et al*., 2010). A few studies also showed inconsequential effects of nanoparticles on plants. For instance, SWCNTs promoted the growth of onion and cucumber (Canas *et al.,* 2008). Aluminium nanoparticles did not exhibit any toxic effects on kidney bean (*Phaseolus vulgaris*) and rye grass (*Lolium perrene*) at concentrations up to 17mg L^{-1} (Doshi *et al*., 2008). Recently, Lee *et al*. (2010) reported that Al_2O_3 nanoparticles up to 4000mg L^{-1} did not have any detectable effects on root elongation and development of *Arabidopsis* even though slight inhibition of seed germination was detected. It is evident that for most nanoparticles, relatively high concentrations are required to cause observable toxicity on plants and the toxicity threshold is species dependent (Lin and Xing, 2007; Lee *et al*., 2008).

Fishes

Li *et al*. (2008) recorded that nanoscale Selenium caused

hyper-accumulation in medaka fish liver, which was six fold higher than selenite. They demonstrated that liver was the main target organ of Se toxicity. It was indicated that high levels of Se accumulation (up to 35.3mg Se/kg) in the fish liver exposed to Nano-Se may pose more serious threat to Medaka fish compared to the relatively lower levels of accumulation (5.5mg Se/kg) induced by selenite. Moreover, nano-Se also caused more efficient accumulation of selenium in gills and muscles compared to selenite, with the differences ranging from two to fourfold. This research clearly indicated that the toxicity of Nano-Se is higher than that of selenite based on LC_{50} values. Another study showed that nZVI's were toxic to medaka fish (*Oryzias latipes*) and their embryos (Li *et al*., 2009b). At exposures of 5 and 50μg/mL of nZVI's gill samples were observed with swollen epithelium cells, missing scales, black particles deposited on the surface and few tactic pillar cells. Morphologic changes were also observed in the gills and resulted in swelling of the gill arches leading to diminished microridges.

Rodents

Nanoparticles have the ability to cross biological barriers (*i.e.,* alveolar, intestinal, dermal) when ingested or inhaled and can migrate within the body to various organs and tissues where they have the potential to cause oxidative stress (Oberdorster *et al.,* 2005). Different forms of nZVI (*i.e.,* fresh, aged, and surface modified) are differentially toxic to rodent nerve cells (Phenrat *et al*., 2009). In rats, 20nm sized titanium dioxide nanoparticles exhibited inflammation (Oberdorster *et al.,* 1992; Baggs *et al.,* 1997). Nano-scale alumino oxide produced significant inflammatory effects in the rat brain (Li *et al*., 2009a). They reported that nanoparticles are small enough to cross the blood brain barrier (BBB) and reside in the brain parenchyma, or interact with the BBB, inducing dysfunction. Several studies indicated that SWCNTs are toxic to mice (Lam *et al.,* 2004; Shvedova *et al.,* 2005) causing death, necrosis, inflammation and cell injury. In rats, the gold nanoparticles moved from mother's placenta to fetus (Warheit, 2004). Importantly, the quantum dots, semiconductor nano crystals may also pose health risks as determined by rodent animal models and *in vitro* cell cultures (Hardman, 2006; Yong *et al.,* 2013).

Humans

Throughout the evolutionary stages, man has been exposed to nanoscale airborne particles (Oberdorster *et al*., 2005). For instance, biogenic magnetite, a naturally occurring nanoparticle has been found in human brains (Kirschvink *et al*., 1992; Dunn *et al*., 1995) and has been associated with neurodegenerative diseases (Dobson, 2001; Hautot *et al*., 2003). But the cause for concern is that the rapidly developing field of nanotechnology dramatically increases the anthropogenic production and exposure of nanoscale particles. The potential routes of nanoparticle exposure to humans include inhalation (respiratory tract), dermal (skin), ingestion (gastrointestinal tract) and injection (blood circulation). Among all these portals, the inhalation is an important route of nanoparticle exposure, since NPs can travel great distances in air by brownian diffusion and are respirable, depositing within the alveolar regions of the lung (Oberdorster *et al*., 2005).

Literature currently available with animal models and human mesothelial cells suggests that Carbon Nanotubes (CNT) may have toxic effects beyond those anticipated for their mass exposure (Lechner *et al*., 2003; Lam *et al*., 2004; Shvedova *et al*., 2005; Donaldson *et al*., 2006; Fisher *et al.,* 2012). The world has not forgotten the mass spread of lung cancer (mesothelioma) in humans following asbestos exposure (Poland *et al*., 2008). Apprehensions has been raised over the safety of CNT because they have three properties (nanoscale, needle-like shape and biologically persistent) that are clearly associated with pathogenicity in particles, moreover there are similar to asbestos (Donaldson *et al*., 2006). Researchers have revealed that the exposure of long multiwalled carbon nanotubes in mice resulted in asbestos-like, length dependent, pathogenic behaviour. This includes inflammation and the formation of lesions known as granulomas (Poland *et al*., 2008). CNT fibers could protrude through the cell wall and result in frustrated phagocytosis (Dostert *et al*., 2008) which signifies that their indestructibility could lead to a pouring of oxygen radicals. When this process takes place in the pleural cavity or the peritoneum, it could result in chronic granulomatous inflammation, which could be the forerunner of mesothelioma. Treatments of human keratinocytes, mimicking potential dermal exposure have shown that both single walled carbon nanotubes and multi walled carbon nanotubes are capable of localizing within and causing cellular toxicity (Shvedova *et al*., 2003; Monteiro-Riviere *et al*., 2005). Additionally, the studies so far suggest that CNTs may have an unexpected ability to cause granuloma formation and fibrogenesis.

Wiwanitkit *et al*. (2007) found that the motility of spermatozoa was affected by the presence of gold nanoparticles. Moreover, they observed that the gold nanoparticles penetrated into the sperm head and tails causing fragmentation.

Underlying mechanisms

The results of older biokinetic studies (mostly ambient ultra fine particles) and some new toxicology studies with nanoscale particles can be viewed as the basis for the expanding field of nanotoxicology. These investigations indicated that the greater surface area per mass renders nanoparticles more active biologically than larger-sized particles of the same chemistry and that appear to be better predictors for nanoparticle induced inflammatory and oxidative stress responses (Oberdorster *et al*., 2005). The principal mechanism that contributes for nanotoxicity in most of the biological systems is the excessive generation of reactive oxygen species (ROS), resulting in oxidative stress (Foley *et al.,* 2002; Li *et al.,* 2008; Rodoslav *et al*., 2003; Oberdorster *et al.,* 2004; Shvedova *et al.,* 2010; Oberdorster *et al*., 2005). ROS play central roles in the initiation of numerous signal transduction pathways that are linked to apoptosis, inflammation and proliferation (Shukla *et al*., 2003). The characteristics or reactions that contribute to the generation of ROS after nanoparticle interaction are briefly discussed below.

ROS are generated through presence of transition metals or redox cycling organic chemicals on the nanoparticle surface. On the other hand, the transition metals can also generate hydroxyl radicals through the Fenton reaction (Nel *et al*., 2001). The Fenton chemistry is one of the mechanisms by which

metal impurities like ferrous iron on the CNT surface can react with hydrogen peroxide and produce hydroxyl radical. Iron based ENPs are presumed to react with peroxides in the environment generating free radicals.

The formation of electron-hole pairs as result of photoactivation effect during UV exposure of nanoscale TiO_2 has been associated with the generation of ROS leading to oxidative stress and inflammation (Long *et al*., 2006). Upon irradiation, the electrons in the valence band of nanoparticles are promoted to conduction band, leaving a hole. These holes at the valence band will have an oxidation potential of + 2.6 V in comparison with normal hydrogen electrode and therefore can oxidize water or hydroxide into hydroxyl radicals.

The surface of nanoparticles that possess discontinuous crystal planes or material defects creates active electronic state and favors reactive oxygen radical generation (Xia *et al*., 2009).

In some cases, the particle dissolution (e.g., ZnO, CdSe, Cu) can produce free ions that are capable of inducing ROS production (Derfus *et al.,* 2004; Meng *et al*., 2007).

Thus depending upon the nature and type of nanoparticles, ROS are generated through different reactions and ultimately can result in cellular and tissue injury responses such as inflammation, apoptosis, necrosis, fibrosis, hypertrophy, metaplasia and carcinogenesis (Nel *et al*., 2006). In a review carried out by Kahru and Dubourguier (2010) to assess the currently existing information on toxicity of ENPs on organism groups representing main food chain levels (bacteria, algae, crustaceans, ciliates, fish, yeasts and nematodes), the most harmful were NPs of Ag and ZnO that were classified as "extremely toxic", followed by C60 fullerenes and CuO NP that are classified as "very toxic". SWCNTs and MWCNTs were classified as "toxic" and TiO_2 NP was classified as "harmful".

Factors affecting nanotoxicity

Dose

Earlier the toxicological studies were governed by the saying "Dose makes the poison". But this perspective is questioned in nanotoxicology and the most appropriate dose metric for nanoparticles has been debated (Moss and Wong, 2006; Oberdorster *et al*., 2007). Toxic effects of nanoparticles do not always appear to correlate with particle mass dose. Indeed, paradoxically, a high concentration of nanoparticles may promote particle aggregation and could therefore reduce toxic responses compared to lower concentrations of the same particles (Buzea *et al.,* 2007).

Surface area

The relative portion of surface atoms to bulk atoms is considerably different in nano-sized when compared to microsized particles of same chemistry. For example, less than 1% of atoms of a microparticle occupy surface positions, while 10% of the atoms in a 10-nm particle reside on its surface. Thus, when size of the materials is reduced, it contributes to changes in surface physical and chemical properties (Jones and Grainger, 2009). For instance, following inhalation exposure of rats to 20-nm or 250-nm TiO_2 particles, the half-times for alveolar clearance of polystyrene test particles were proportional to the titanium dioxide particle surface area per million macrophages.

Size

The comparing the various cytotoxicity studies involving different sized gold nanoparticles provides a great scope to understand the size dependent toxicity. Gold nanoclusters (1.4nm) were shown to be toxic to cells owing to their specific interaction with major grooves of DNA, whereas smaller or larger gold particles did not behave in this way (Pan *et al.,* 2007). The gold nanoparticles of 35nm size were non-toxic to a murine macrophage-like cell line (Shukla *et al.*, 2003). Furthermore, transcriptomic studies using primary human umbilical vein endothelial cells observed no toxic effects of gold nanoparticles (5nm) on the global gene expression program (Esther *et al*., 2005). Overall, gold particles with a size of 13nm and above, commonly typified as colloids, may thus be viewed as non-toxic (Jahnen-Dechent and Simon, 2008). By contrast, gold particles below 2nm have shown an unexpected degree of toxicity in different cell lines (Schmid, 2008). Furthermore, quantum dots were reported to localize to different cellular compartments in relation to their size. Others have suggested that silica nanoparticles of 40–80nm in diameter can enter the cell nucleus and localize to distinct subnuclear domains in the nucleoplasm, but do not colocalize with nucleoli. Moreover, these nanoparticles induced the formation of nucleoplasmic protein aggregates. In contrast, fine and course (0.5–2μm) silica particles located exclusively in the cytoplasm (Chen and Mikecz, 2005).

Crystalline structure

The cytotoxic properties of titanium dioxide nanoparticles appear to correlate with their phase composition (Shvedova *et al*., 2010). Titania exists in a variety of crystal structures and the most researched forms are rutile, anatase and brookite (Fadeel and Bennett, 2010). In a study with titanium dioxide nanoparticles of size ranging between 3-10nm, demonstrated that anatase titanium dioxide was 100 times more toxic than an equivalent sample of rutile titanium dioxide (Sayes *et al.,* 2007). They reported that the generation of ROS under UV illumination correlated well with the observed biological responses. In addition, the pulmonary toxicities of fine and ultrafine (nano-sized) quartz particles appeared to correlate better with surface activity than with particle size and surface area. Interestingly, the crystal structure of titanium dioxide also dictates the mode of cell death. Anatase TiO_2 nanoparticles, regardless of size, were reported to induce necrosis, whereas rutile TiO_2 nanoparticles triggered apoptosis through the formation of reactive oxygen species.

Surface coating

The surfaces of ENPs make contact with cells and a thorough understanding of its surface composition is therefore vital to understand the interactions of nanoparticles with biological systems (Jones and Grainger, 2009). The contaminants on the surface of ENPs do contribute to toxicity. For instance, the surface of CNTs when contaminated with ferrous iron can induce the production of ROS through Fenton's reactions inside biological system (Nel *et al*., 2001). The frequent problem with all biomaterials is the possible adsorption of the ubiquitous bacterial endotoxin, lipopolysaccharide which can also contribute to the cellular responses, in particular immunological responses (Shevoda *et al*., 2010). Hence, it is

Table 1: Nanotoxicity at various trophic levels

Biological system	Toxicity causing nanoparticle	References
Algae	TiO_2	Navarro *et al*. (2008a); Aruoja *et al*. (2009)
	CeO_2	Hoecke *et al*. (2009)
	Ag	Novarro *et al*. (2008 a,b)
Microbes	ZVI	Lee *et al*. (2008)
	CuO	Mahapatra *et al*. (2008); Dimkpa *et al*. (2011)
	Ag	Sondi and Salopek-Sondi (2004); Throback *et al*.(2007)
	ZnO	Jiang *et al*. (2009)
Plants	CNT	Shen *et al*. (2010)
	ZnO	Yang and Watts (2005); Lin and Xing *et al*.(2007)
Fish	Se	Li *et al*. (2008)
	ZVI	Li *et al*. (2009b)
Rodents	Al_2O_3	Li *et al*. (2009a)
	TiO_2	Oberdorster *et al*. (1992) and Baggs *et al*. (1997)
	ZVI	Phenrat *et al*. (2009)
	CNT	Lam *et al*. (2004); Shvedova *et al*. (2005)
	Quantum dots	Hardman (2006); Yong *et al*. (2013)
Humans	Magnetite	Kirschvink *et al*. (1992); Dunn *et al*. (1995)
	CNT	Donaldson *et al*. (2006); Fisher *et al*. (2012)
	Au	Wiwanitkit *et al*. (2007)

Table 2: Published data on phytotoxicity of nanoparticles

Nanoparticle	Concentration	Effect	Reference
Quantum Dots	0.25 – 1 ml/mL	No germination of *Oryza sativa* seeds	Nair *et al*. (2011)
Silver	40 mg/L0.01 – 10 mg/L	Completely inhibited root hair formation, deformation of roots in *Allium cepa*Oxidative stress to *Lemna gibba*	Yin *et al*. (2011)Oukarroum *et al*. (2013)
Cerium oxide	10 mg/L	Trans-generational impact on *Lycopersicon esculentum*	Wang *et al*. (2013)
ZnO	100 - 1000 mg/L15 mg/L	Stunted root growth in *Oryza sativa*50 % inhibitory concentration to root growth of *Allium sativum*	Boonyanitipong *et al*. (2011) Shaymurat *et al*. (2011)
SWCNT	25 μg/mL	Programmed cell death in protoplasts of *Oryza sativa*	Shen *et al*. (2010)

also crucial to distinguish between undesirable cellular responses to nanoparticles themselves and residual materials associated with the nanoparticle such as surfactants or transition metals as a product of the synthetic process.

Opsonization

ENPs are seldom utilized as a sole active agent and in most cases it is encapsulated within a host system or requires functionalization of their external surface *i*.e. chemical modification through the use of tethering or coupling agents (Fadeel and Garcia-Bennett, 2010). The rationale behind these modifications is to enable the ENPs to interact in a suitable manner with the biological environment. These modifications will easily disperse in biological media or to protect the nanoparticle against degradation. In addition, the nanoparticles may also bind to proteins in biological fluids, which in turn could affect their biological performance. Researchers have pointed out that adsorbed proteins could play a vital role in modulating uptake and toxicity of nanomaterials (Dutta *et al*., 2007; Cedervall *et al*., 2007). As a whole it is proposed that the opsonized proteins constitute a major element of the biological identity of the nanoparticle (Fadeel and Garcia-Bennett, 2010). The surface chemistry of ENPs pertains to the protein adsorbing capacity and directly determines the cellular binding of nanoparticles (Ehrenberg *et al*., 2009).The contamination of gold nanoparticles with the endotoxin, lipopolysaccharide (LPS) results in the activation of dendritic cells and ultimately interferes with the assessment of biological (immuno-modulatory) effects of these nanoparticles (Vallhov *et al*., 2006)

Screening assays for nanoparticle toxicity

There are three major categories of assays namely cytotoxic, genotoxic and alterations in gene expression assays which helps in evaluating the toxicity of nano particles in *in vitro* system (Subbulakshmi, 2011). Fadeel and Garcia-Bennett (2010) reviewed the effectiveness and validity of assays for determining the toxicity and concluded that more than one assay may be required for nanotoxicity risk assessment. Monteiro-Riviere *et al*. (2009) reported that the classical dye-based assays such as MTT assay produced invalid results with certain carbonaceous nanomaterials due to nanomaterial/ dye interactions. In addition the MTT assay failed to report toxicity of some porous silica micrparticles due to spontaneous redox reactions where the MTT is reduced and nanoparticle surfaces are oxidized simultaneously (Laaksonen *et al*., 2007). Hence, it is concluded that risk assessment of nanoparticle toxicity should be carried out in case-to-case studies involving several accepted toxicity assays.

The Cytotoxic assays like Trypan Blue Exclusion Assay, In Vitro cell viability assay – WST 1, Lactase Dehydrogenase Assay (LDH) Assay focuses on cell viability, plasma membrane integrity and cellular metabolism. Genotoxicity assays namely Ames Assay, Comet Assay and 8-Oxo-dG Assay facilitates to study the DNA structure breakage, mutagenicity and chromosomal aberration. In 8-Oxo-dG Assay, the 8-hydroxy-2-deoxy Guanosine (8-OH-dG) is a product of oxidative damage of DNA by reactive oxygen and nitrogen species and serves as an established marker of oxidative stress. Gene expression assays (gene profiling) like Northern blot analysis,

quantitative real-time polymerase chain reaction (qRT-PCR), PCR arrays and micro arrays are important tools to assess the alterations in the gene expression as a result of nanoparticle interaction. Xia *et al*. (2009) formulated the hierarchical oxidative stress model as an integrative method to screen the NP toxicity. They recorded that at the lowest level of oxidative stress (tier 1), the induction of antioxidant and protective responses is mediated by the transcription factor (*Nrf2*) which regulates the activation of the antioxidant response element in the promoters of phase II genes (Li *et al*., 2003; Xiao *et al*., 2003). At the higher levels of oxidative stress (tier 2), this protective response may further yield to proinflammatory responses because ROS induces redox-sensitive signaling pathways such as the mitogen activated protein kinase (MAPK) and nuclear factor-kappa B (*NF-ºB*) cascades (Xiao *et al*., 2003). At the highest level of oxidative stress (tier 3), a perturbation of mitochondrial inner membrane electron transfer and the open/close status of the permeability transition pore can trigger cellular apoptosis and cytotoxicity. This outcome is also called as toxic oxidative stress. By employing this three-tier screening platform, they conducted several experiments with ENPs and concluded that potentially safe NPs (such as carbon black and polystyrene) induced either no response or only a tier 1 response, whereas potentially hazardous NPs (such as metal oxides and ambient UFP) induced proinflammatory (tier 2) or cytotoxic (tier 3) effects (Xia *et al*., 2006). Apart from these assays and oxidative stress model, the emerging field of nanotoxicogenomics which deals to correlate global gene expression profiles of cells or tissues exposed to ENPs with the biological responses using technologies like cDNA microarray is expected to facilitate better assessment of nanotoxicity. Furthermore, the mass spectrometry methods (proteomics) and two-dimensional electrophoresis could also improve the understanding of the biological responses induced by nanoparticles (Sheehan *et al*., 2007).

Conclusion and future perspectives

Expanding the knowledge base of nanotechnology for wide range of applications and commercialization of nano-products increases the risk to environment. It is imperative to establish a scientific basis for understanding the toxic potential of these unique and novel materials. There are many unanswered questions when it comes to biosafety concerns. However, the current knowledge is sufficient to indicate that some nanotechnologies will present new risks. Investigations carried out so far unanimously reveals the fact that the principal mechanisms of nanotoxicity are the generation of reactive oxygen species and oxidant injury. Exploring the toxic effects of nanoparticles, not only provide data for safety evaluation of ENPs but also will help to advance the field of nanotechnology by providing dataset about their undesirable properties and means to avoid them. Indeed, nanotoxicological studies may pave ways for a wide array of avenues and opportunities to explore and address all associated issues well before nano-based processes and products are flooded in the market.

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