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Toxicological studies of engineered nanoparticles in biological systems

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A B S T R A C T

Recently the ability of engineering of nanoparticles and to produce nanomaterials at the nano or near-atomic scale has triggered their rapid production due to their interesting properties that were not previously seen at scales above the micrometers. The exponential demands of nanosized materials with use of nanoparticles have been increased in industrial applications. With increasing use of nanoparticles in a variety of consumer goods, biological systems are constantly exposed to such nanomaterials besides exposure at production sites. This unintended exposure to nanomaterials may occur *via* inhalation, dermal exposure or gastrointestinal tract absorption and may pose a great risk to environment. However, there are still widespread controversies and ambiguities with respect to the toxic effects and mechanism of action of enginnered nanoparticles in biological systems. The present study reviewed toxicological studies of engineered nanoparticles in biological systems.

Introduction

Nanoparticles (NPs) or Nanomaterials are the materials having the size less than 100nm that can be nanoscale in one dimension (e.g. films), two dimensions (fibres and tubes) or three dimensions (particles). Many theories have been proposed to explain the toxicity phenomenon of nanoparticles including, generation of Reactive Oxygen Species (ROS), which can disrupt cell structures, interference with normal metabolism (Nel *et al.* 2006, Choi and Hu, 2009), binding with macromolecules making them dysfunctional (Gogoi *et al.* 2006). However, the real impact of nanoparticles and their mechanism of reactions against biological system are still not known. Researchers have provided evidence for NP-mediated production of ROS and generation of oxidative stress as a possible mechanism of toxicity (Oberdorster, 2005, Pickering and Wiesner, 2005, Zeng *et al.* 2015) especially for

carbonanceous nanoparticles (*i.e.* fullerenes, fullerols and carbon nanotubes) and nanoparticulate like titanium dioxide and zinc oxide (Reeves *et al.* 2008). NPs of TiO_2 and ZnO are widely used in sun care products (Serpone *et al.* 2007), as well as on self cleaning coatings (Cai *et al.* 2008) that may released in to environment and become a threat to ecosystems.

Toxicity of NPs in Animal Model System

Federici *et al.* (2007) observed series of physiological effects induced by TiO_2-NPs in Rainbow trout *Oncorhynchus mykiss*. Further, Baun *et al.* (2008) observed toxic effects of TiO_2 and ZnO-NPs on algae *Pseudokirchneriella subcapitata*, Crustaceans *Daphnia magna* and *Thamnocephalus platyurus* and bacteria *Vibrio fischeri* (Heinlaan *et al.* 2008). Toxicity of nanoparticles of silver, platinum, carbon nanotubes on terrestrial animals was also observed by Dani *et al.* (2008); Johansen (2008); Petersen *et al.* (2009) and Reeves *et al.* (2008). Invertebrates are able to intake nanomaterials dispersed in the environment by different ways: *direct ingestion*; *from contaminated preys; water filtration*; *inhalatio*n and *surface contact*. Some degree of bio-modification was also observed in daphnids (Oberdorste*r et al.* (2005); Roberts *et al.* (2007) and Bau*n et al.* (2008). Compartmentalization of nanosized contaminants in selected tissues and in organelles has been documented by Moor*e et al.* (2006); Li *et al.* (2011); Jaiswal *et al.* (2003); Kohle*r et al.* (2008) and Oberdoster *et al.* (2002).

The Rotifer *Brachionus Calyciflorus* was studied under exposure to nanomaterials as a component of simplified food web, algae and bacteria. Those were predated by a protozoans which in turn was the rotifer prey and both were fed by fish (Holbrook *et al.* 2008). Acute and chronic toxicity was observed by Borgmann *et al.* (2005) in *Hyalella azteca* (Family Hyalellidae) and *Leptocheirus plumulosus* (Family Aoridae) shrimps inhabited on fresh water and feed on sediment or suspended particles. Griffitt *et al.* (2008), reported lethality in three different aquatic organisms namely, algae (*Pseudokirchneriella subcapitata*), Daphnids *(Daphnia pulex, Ceriodaphnia dubia*) and fish (*Danio rerio*) on exposure of Ag-NP and Cu-NP. They also demonstrated that copper and silver nanoparticles are acutely toxic to wide spectrum of aquatic species including zebra fish. This toxicity is largely manifest at the gills and were not affected simply by particles dissolution. Moore (2006) stated that engineered nanoparticles are likely to be deposit in aquatic systems and represent a possible danger to aquatic life. Both dissolved and particulate nanoparticles enhanced deposition of metals in gills. Branchial uptake of ionic silver and copper nanoparticles has been well documented in freshwater fish by Bury and Wood (1999) and proposed several mechanisms to enhanced silver levels in gills. NPs may be trapped in the mucus layer of the gill as demonstrated for larger particles by Dani *et al.* (2008) and Connor *et al.* (2005).

The sediment particles less than 500 nm were observed intracellularly in salmonid gill epithelial cells by Martens and Seruizi, (1993). Morgan *et al.* (2004) observed significant increase in whole body silver content on exposure of silver NPs. However, it not clear either is due to translocation of silver through gills or due to ingestion of particulates and gastro Intestinal (GI) absorption. Soluble copper is a well known gill toxicant in freshwater fish species (Mazon *et al.*, 2002) and causes significant thickening in gill filament (Griffitt *et al.*

2008). Morgan *et al.* (1997) reported silver as most potent gill toxicants in freshwater fish and caused highly specific inhibition of Na+/K± ATPase. Dabbousi *et al.* (1997) observed change in morphology in spiny dogfish, a marine elasmobranch on exposure of Ag-NPs.

Common Animal Model System used for Nanotoxicity Test

Usually Daphnids *(Daphnia magna, Daphnia pulex and Ceriodaphnia dubia*) preferred as first *choice* for eco-toxicological tests of nanoparticles (Joncxyk and Gilron, 2005). Benthic organisms (cnidarian *Hydra attenuate*) are also used (Dabbousi *et al*., 1997; Blaise and Kusui 1997; Holdway, 2005) for long term toxicity studies. Their clubbing movements of tentacles are observed as early signs of exposure to toxic agents, before reproductive changes or death.

Impact of Metal Nanoparticles on Earthworms

Mc Shane *et al.* (2010) recorded the exposure of 10,000mg TiO_2/kg soil not significantly affected the biomass of *Eisenia spp*. In contrast, Hecknann *et al.* (2008) reported a 49% reduction in the number of juveniles produced by *E. fetida* following 28d of exposure to 1,000 mg TiO_2/kg soil. Recent nano-ecotoxicological studies indicates so far that increasing dose provides an increase in response, although this may not follow traditional mass based dose-respose relationships (Oberdorster *et al.* 2005). Ni-NP had a much lower toxicity to *E. fetida* than $NiCl_2$ possibly due to a strong agglomeration of the Ni particles combined with little or no oxidation of the NP-Ni particle (Besenbacher and Norskov, 1993). There was also a tendency of increased biomass was reported by Gurr *et al.* (2005) for Cu-NP, and Ni-NP exposed worms. Very limited information is available for toxicity tests of nanoparticles (TiO_2-NP; Ag NO_3, $Al2O_3$, SiO_2, TiO_2, ZnO_2) against earthworms except few including Hu *et al.* (2010); Petersen *et al.* (2008). Oberdorster *et al.* (2004) demonstrated NPs may affect soil ecosystem *via:* 1) direct effect; 2) changes in the bioavailability of toxins or nutrients; 3) indirect effects resulting from their interaction with natural organic compounds; 4) interaction with toxic organic compounds which may amplify or alleviate their toxicity. It was also observed earthworms are able to differentiate particles through unknown mode of action (McShane *et al*., 2006). Gomes *et al*. (2012) demonstrated NPs altered mRNA levels for specific genes mainly involved in metabolism, transcription and translation or in the stress response and generates oxidative stress conditions. The reduction in growth and development, damaged cuticle with underlying pathologies of epidermis, muscles and gut barrier on exposure of fullerene NPs (C60) to *Lumbricus rubellus* was reported by Vander Ploeg *et al*.,(2013). Li *et al*. (2011) observed *Eisenia fetida* shown neither response of antioxidant enzyme expression or activity nor acute toxicity in C60 spiked soil. We studied effect of ZnO-NPs (Gupta *et al*., 2014). on *E. fetida* in terms of reproductive behavior, antioxidant enzyme activities and accumulation of Zn^{++} remote from portal of entry. It may concluded that interaction of nanoparticles with earthworm is unpredictable that may result in ecologically significant effects on behavior at environmentally relevant concentrations.

Biological Uptake and Mechanisms of toxicity

Published quantitative research on uptake and accumulation of NPs by whole

organisms is very limited. It is known that organisms living in NPs containing environments incorporate them within their bodies, mainly *via* the gut (Baun *et al.* 2008; Roberts *et al.* 2007) with a possibility of translocation within the body. Most of the initial work, in this area was undertaken on standard animal models (daphnids) used in ecotoxicology. Feng *et al.* (2005) demonstrated the uptake of fluorescent carboxylated NPs by *Daphnia magna* and translocation from the gut to reserve fat droplets. Some NPs such as quantum dots and CNTs(carbon nanotubes) are intentionally designed to interact with proteins, nucleic acids or cell membranes for labeling or drug delivery purposes (Gao *et al.* 2008; Medintz *et al.* 2008). The toxicity mechanisms have not yet been completely elucidated for nanoparticles. Possible mechanisms includes disruption of membranes or membrane potential, oxidation of proteins, genotoxicity, and interruption of energy transduction, formation of ROS and release of toxic constituents.. Meng *et al* (2007) reported NPs first, adhered to the surface alter the membrane properties, therefore affecting the permeability and the respiration of the cell, they can penetrate inside cell and caused DNA damage and they can also release toxic Ag+ ions. Degradation of lipo-polysaccharide molecules forms pits in the membrane that changes membrane permeability due to silver nanoparticles as reported by Moore *et al.* (2006). Gold nanoparticles (AuNP) can easily enter into cells (Connor *et al.* 2005) and bind strongly to amine and thiol groups of cells that enabled their surface modification with amino acids and proteins (Oberdorster 2001;Ville *et al.*,1995).

Impact of NPs in biological system

Damage to membrane integrity

The imaging applications of NPs are mainly targeted on cell membrane. NPs attached to cell surface and compromise with the integrity and functions of the cell membrane. Silicon (Si) NPs and fullerene derivatives can embed themselves in the membranes (Zang *et al.* 2015). Carboxyfullerene puncture bacterial cell membrane in a gram positive bacterial strain and causes cell death (Huang *et al.*,2001). Ingle *et al.* (2008) reported Au-NPs weaken cell membranes and causes heat shock responses in *Escherichia coli*. NPs can also indirectly causes membrane damage through the generation of ROS which can oxidize double bonds on fatty acid tails of membrane phospholipids in a process known as lipid peroxidation.This increases membrane permeability and fluidity, making cell more susceptible to osmotic stress or hindering nutrient uptake (Cabiscol *et al.*, 2000). Peroxidized fatty acids may trigger reactions that generate other free radicals leading to more cell membrane and DNA damage (Valembois *et al.*,1994).

Protein destabilization and oxidation

NPs-protein interactions have been optimized for a variety of biomedical applications. As quantum dots are used to target and fluorescently label proteins for imaging (Jaiswal *et al.* 2003; Medintz *et al.* 2005). Oberdorster *et al.* (2004) observed ROS can also lead to the formation of disulfide bonds between sulfur containing amino acids thus disturbing the structure and function of the protein.

Nucleic Acid damage

Interactions of NPs with nucleic acids have applications in DNA labeling or DNA cleavage. The tagged nucleotides with NPs especially quantum dots commonly used as labeling agents for bioimaging applications (Dyadyusha *et al.*, 2005;Dubertret *et al.*, 2002). Cabiscol *et al.* (2000) observed some

NPs indirectly damage DNA because of ROS production, which breaks DNA strand, cross linking and adducts of bases or sugars. Mson *et al.* (2006) stated TiO2NPs generates oxygen radicals that can nick supercoiled DNA. Scientific committee on Emerging and Newly Identified Health Risks, (2007) shown possibility of tumor formation through DNA damage and increase in cell proliferation associated with inflammation.

Cell disruption due to ROS

NPs induces ROS that may damage every cell component, and this interaction tends to trigger further radical formation.

Interruption of Energy transfer

Electron transport phosphosylation and energy transduction processes may be disrupted if membrane integrity is compromised or if a sensitive NPs contacts membrane bound electron carriers and withdraws electrons from the transport chain. Fullerene derivatives have been reported to inhibit *E. coli* respiration of glucose by Mc Shane and Ritter 2010).

Release of toxic components from NPs

Certain NPs causes toxicity to bacterial cells by releasing harmful components, such as heavy metals or ions. Quantum dots are semiconductor nano-crystals that contain noble or transition metals such as CdSe, CdTe, CdSeTe, Zn Se. In As or PbSe in their Core, CdS or ZnS in their shell, and an organic coating (Dubertret 2002*)*.. Release of silver ion has been implicated in toxicity of silver NPs. It is believed that silver ions interact with thiol groups of proteins, resulting in inactivation of vital enzymes (Matsumura *et al*., 1996). Silver ions have also been shown to prevent DNA replication and affect the structure and permeability of the cell (Feng *et a*l. 2000).

Conclusions

We discussed the toxicity studies of engineered nanoparticles in biological systems. Researchers observed toxicity of nanoparticles at different levels and it depends especially, on size and composition of nanoparticles. However, the mechanism of their action in biological system is not very clear. It is essential to decrease toxicity of engineered nanoparticles at non-significant level to ensure safe application of nanoparticles to organisms and to the environment. Further, understanding of human health implications and ecological consequences in environment at exposure of unintended engineered nanoparticles is essential before the commercial benefits of these nanomaterials fully realized.

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References

Baun, A., Hartmann, N.B., Grieger, K., Kusk, K.O., 2008. Ecotoxicity of engineered nanoparticles to aquatic invertebrates: a brief review and recommendations for future toxicity testing. *Ecotoxicology* 17: 387–395.

Besenbacher F. and Nørskov J.K., 1993. Oxygen chemisorption on metal surfaces: General trends for Cu, Ni and Ag Prog. *Surf. Sci*., 44 : 5–25.

Blaise C. and Kusui T., 1997. Acute toxicity assessment of industrial effluents with a microplate-based Hydra attenuata

assay. *Environ. Toxicol. Water. Qual.* 12: 53-60.

Borgmann U., Couillard Y., Doyle P. and Dixon D.G., 2005. Toxicity of sixty-three metals and metalloids to Hyalella azteca at two levels of water hardness. *Environmental Toxicology and Chemistry* 24(3): 641–652.

Bury N. R. and Wood C. M., 1999. Mechanism of branchial apical silver uptake by rainbow trout is via the proton-coupled Na+ channel. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. 277: R1385–R1391.

Cabiscol E., Tamarit J. and Ros J., 2000.Oxidative stress in bacteria and protein damage by reactive oxygen species. *Int. Micobiol*. 3:2-8.

Cai Weibo , Gao Ting , Hong Hao, and Jiangtao Sun, 2008. Applications of gold nanoparticles in cancer nanotechnology. *Nanotechnol Sci Appl.* 1: 10.2147/NSA.S3788.

Choi O K and Hu Z Q., 2009. Nitrification inhibition by silver nanoparticles. *Water Science & Technology* 59 (9): 1699-1702; DOI: 10.2166/wst.2009.205.

Connor E E, Mwamuka J, Gole A, Murphy C J and Wyatt M D., 2005. Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. *Small* 1(3):325-7.

Dabbousi B. O., Rodriguez-Viej O. J. and Bawendi M. G., 1997. (CdSe)ZnS core-shell Qdots: synthesis and characterization of a size series of highly luminescent nanocrystallites. *J. Phys. Chem.* B 101, 9463–9475 10.1021/jp971091y.

Dani R K, Kang M, Kalita M, Smith P E, Bossmann S H and Chikan V., 2008. MspA porin-gold nanoparticle assemblies: enhanced binding through a controlled cysteine mutation. *Nano Lett*. 8:1229–1236.

Dubertret B, Skourides P, Norris DJ, Noireaux V, Brivanlou A H and Libchaber A., 2002. *In vivo* imaging of quantum dots encapsulated in phospholipid micelles. *Science*. Nov 29;298(5599):1759.

Dyadyusha L, Yin H, Jaiswal S, Brown T, Baumberg J J, Booy F P, 2005. Quenching of CdSe quantum dot emission, a new approach for biosensing. *Chem. Commun.* 3201–3203 10.1039/B500664C.

Federici G, Shaw B J and Handy R D, 2007. Toxicity of titanium dioxide nanoparticles to rainbow trout (*Oncorhynchus mykiss*): Gill injury, oxidative stress, and other physiological effects. *Aquatic Toxicology* 84: 415–430.

Feng Q L, Wu J, Chen G Q, Cui F Z, Kim T N, Kim J O, 2000. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *J. Biomed. Mater. Res*. 52:662–668.

Gao X L, Chen J, Cahenjy hu B X, Chen Hz, Jiang X G., 2008.Quantum dots bearing lectin- functionalized nanoparticles as a platform for *in vivo* brain imaging. *Bioconjugate Cem* 19 :2189-2195.

Gogoi S K, Gopinath P, Paul A, Ramesh A, Ghosh S S, Chattopadhyay A., 2006. Green fluorescent protein-expressing *Escherichia coli* as a model system for investigating the antimicrobial activities of silver nanoparticles. *Langmuir* 22: 22-26.

Gomes João, Fernando Pereira, Paula Cristina Silva Albuquerque Rosa Maria Mendes Miranda d and Maria Teresa Freire Vieira., 2012 Determination of Airborne Nanoparticles from Welding Operations. Langmuir 22: 12-22.

Griffitt R J, Luo J, Gao J, Bonzango J C, and Barber D. S., 2008. Effects of particle composition and species on toxicity of metallic nanoparticles in aquatic organisms. *Environ. Toxicol. Chem.* 27: 234–237.

Gurr J R, Wang A S, Chen C H and Jan K Y., 2005. Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. *Toxicology* 213:66–73.

Heckmann D, Meyer A, Laufer B, Zahn G, Stragies R, and Kessler H., 2008. Rational design of highly active and selective ligands for the alpha-5 beta-1 integrin receptor. *Chembiochem* 9: 1397–1407.

Heinlaan M, Ivask A, Blinova I, Dubourguier H C and Kahru A., 2008. Toxicity of nanosized and bulk ZnO, CuO and TiO2 to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus. Chemosphere* 71: 1308–1316.

Holbrook R, David Karen E, Murphy Jayne, Morrow B and Ken D., 2008. Cole Trophic transfer of nanoparticles in a simplified invertebrate food web Nature Nanotechnology *3, 352 – 355. doi:10.1038/nnano.2008.110*

Holway, D. A., 2005 *Hydra* population reproduction toxicity test method. In: Blaise C. & Ferarad, J. (eds.) Small-Scale Freshwater Toxicity Investigations. Netherlands: Springer.

Hu CW, Li M, Cui Y B, Li D S Chen J and Yang L Y., 2009. Toxicological effects of TiO_2 and ZnO nanoparticles in soil on earthworm *Eisenia foetida. Soil Biol. Biochem.* 42:586–591.

Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X.,2007. Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomum camphora leaf. *Nanotechnology* 18:105104.1–105104.11

Ingle A P, Gade A K, Pierrat S, S €onnichsen C, Rai M K., 2008. Mycosynthesis of silver nanoparticles using the fungus *Fusarium acuminatum* and its activity against some human pathogenic bacteria. *Curr Nanosci* 4:141–144

Köhler J., Abahmane L., Albert J., Mayer G., 2008. Preparation of metal nanoparticles with varied composition for catalytical applications in microreactors. *Chem. Eng. Sci*., 63 : 5048

Jaiswal J K, Mattoussi H, Mauro J M and Siamon S M., 2003. Long term multiple color imaging of live cells using quantum dot bicojugates. *Nature Biotechnology* 21:47-51.

Johansen A., 2008. Effects of C60 fullerene nanoparticles on soil bacteria and protozoans. *Environ. Toxicol. Chem.* 27:1895–1903.

Joncxyk E and Gilron G., 2005.Acute and chronic toxicity testing on *Daphnia* sp. In: Blaise C, Férard J-F (eds), Small-scale freshwater toxicity investigations, Vol. 1: Toxicity tests methods, Springer Verlag, Dordrecht, NL, pp 337-394.

Li X-B, Xie Q S, Shi S S, Duan Y S and Ou-Yang, Y B.,2011. Antibacterial effect of silver nanoparticles on *Staphylococcus aureus. Biometals* 24: 135–141

Martens D W and Servizi J A., 1993. Suspended sediment particles inside gills and spleens of juvenile pacific salmon (*Oncorhynchus* spp.). *Can. J. Fisheries Aquat. Sci.* 50: 586–590

Mason T G, Graves S M, Wilking J N and Lin M Y., 2006. Extreme emulsification: Formation and structure of nanoemulsions.

Condensed Matter Physics 9(1): 193-199.

Matsumura Y and Maeda H., 1986. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Res*. 46 (12): 6387–6392.

Mazon A F, Monteiro E A, Pinheiro G H, and Fernandes M N., 2002. Hematological and physiological changes induced by short-term exposure to copper in the freshwater fish, *Prochilodus scrofa*. *Braz. J. Biol*. 62, 621–631.

McShane M. and Ritter D., 2010. Microcapsules as optical biosensors. *J. Mat. Chem*. 20: 8189-8193.

McShane M J, 2002. Potential for glucose monitoring with nanoengineered fluorescent biosensors. *Diab. Technol. Ther*. 4: 533-538.

McShane M J, 2006. Microcapsules as "smart tattoo" glucose sensors: engineering systems with enzymes and glucose-binding sensing elements. In Geddes C. D. and Lakowicz J. R. (Eds.), Vol. 11, Springer, New York.

Medintz I L, Mattoussi H and Clapp A R., 2008. Potential clinical applications of quantum dots. *Int. J. Nanomed*. 3, 151.

Medintz I L, Uyeda H., Goldman E R and Mattoussi H., 2005. Quantum dot bioconjugates for imaging, labelling and sensing. *Nat. Mater*. 4: 435-446.

Meng H, Chen Z, Xing G, Yuan H, Chen C, Zhao F, Zhang C, and Zhao Y., 2007. Ultrahigh reactivity provokes nanotoxicity: Explanation of oral toxicity of nano-copper particles. *Toxicol. Lett.* 175: 102–110.

Moore M. N., 2006. Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? *Environ. Int.* 32: 967–976.

Morgan I J, Henry R P. and Wood C M., 1997. The mechanism of acute silver nitrate toxicity in freshwater rainbow trout (*Oncorhynchus mykiss*). doi: 10.1016/j.soilbio..12.007

Morgan T P, Grosell M, Playle R C, and Wood C M., 2004. The time course of silver accumulation in rainbow trout during static exposure to silver nitrate: Physiological regulation or an artifact of the exposure conditions? *Aquat. Toxicol*. 66: 55.

.Nel A, Xia T, Mädler L, Li N., 2006. Toxic potential of materials at the nanolevel. *Science* 311:622–627

Oberdörster E., 2004. Manufactured Nanomaterials (Fullerenes, C60) Induce Oxidative Stress in the Brain of Juvenile Largemouth Bass. *Environmental Health Perspectives* 112 (10): 1058-1062.

Oberdörster G., 2005. Inhaled Nano-sized Particles: Potential effects and mechanisms. Proceedings of the First International Symposium on Occupational Health Implications of Nanomaterials, 12-14 October 2004, Buxton, Great-Britain. Edited by the Health and Safety Executive, Great-Britain and the National Institute for Occupational Safety and Health, United States, July 2005, http://www.hsl.gov.uk/capabilities/nanosymrep_final.pdf.

Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W and Cox C., 2004. Translocation of inhaled ultrafine particles to the brain. *Inhal Toxicol* 16 (6-7) : 437-45.

Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Lunts A, Kreyling W, Cox C, 2002. Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. *J Toxicol Environ Health* 65 (20) : 1531-43.

Oberdorster G. 2001. Pulmonary effects of inhaled ultrafine particles. *Int Arch Occup Environ Health* 74 : 1–8.

Petersen E J, Huang Q G and Weber W.J.. 2008. Bioaccumulation of radio-labeled carbon nanotubes by *Eisenia foetida*. *Environ. Sci. Technol*. 42:3090–3095

Pickering K D and Wiesner M R., 2005.Fullerol-sensitized production of reactive oxygen species in aqueous solution. *Environ. Sci. Technol*. 39:1359-1365.

Reeves J F, Davies S J, Dodd N J F and Jha AN., 2008.Hydroxyl radicals (OH) are associated with titanium dioxide (TiO2) nanoparticle-induced cytotoxicity and oxidative DNA damage in fish cells. *Mutat. Res. Fundam. Mol. Mech. Mutagen*. 640:113-122.

Roberts, A. P. and Mount A. S., 2007. *In vivo* biomodification of bipid-boated barbon nanotubes by *Daphnia magna*. *Environmental Science and Technology* 41: 3025-3029.

Van der Ploeg M J, Handy R.D, Heckmann L H, Van der Hout, Nico W and Van Den Brink, 2013.C60 exposure induced tissue damage and gene expression alterations in the earthworm *Lumbricus rubellus*. *Nanotoxicology* 7(4):432-440.

Van der Ploeg MJC,Baveco Hans,Van der Hout Annemarlet, Bakker, R.,Rietjens Ivonne and Van den Brink W. Nico, 2010.Effects of C60 nanoparticle exposure on earthworms (*Lumbricus ruellus*) and implications for population dynamics. *Environmental Pollution* 159(1):198-203.

Ville P, Roch P, Cooper E, Masson P and Narrbomnne J,1995. PCBs increase molecular-related activities (lysosome, antibacterial, hemolysis,proteases) but inhibit macrophage-related functions (phagocytosis, wound healing) in earthworms. *J.Invertebr.Pathol.* 65:217-224.

Zeng Zhiyang, Patel Jiten, Lee Shih-Hui, McCallum Monica, Tyagi Anuradha, Yan Mingdi and Shea Kenneth J.,2015.Synthetic polymer nanoparticle-polysaccharide interactions: A systematic study. *J.Am.Chem.Soc*.134:2681-2690.