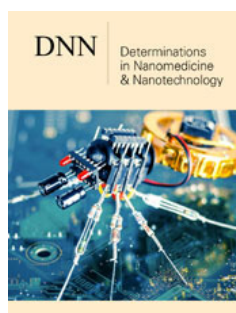


# Risks of Nanomaterials and Nanoparticles in the Aquatic Environment

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## Abstract

In the last decades Nanomaterials (NMs) have attracted a great deal of attention due to their many technologically interesting properties. Nanotechnology applications advanced very quickly while very little has been done to measure and assess the risks of Nanoparticles (NPs) to biological systems and to the ecosystems. The small size of nanoparticles and their properties can become easily a vehicle for binding and transport of toxic chemical pollutants. There have been a number of studies showing that NPs and nanotubes can be released in the environment and cause harmful effects to humans and/or living organisms [1]. Studies showed that NPs can associate to naturally occurring colloids and particles affecting bioavailability and uptake into cells and organisms. Uptakes by endocytotic routes are identified as probable major mechanisms of entry cellular compartments leading to various types of toxic cell injury through free radical reactions. This report aims to address the possible hazards associated with the potential release of NMs and NPs into the aquatic environment and review their harmful effects to aquatic organisms.

**Keywords:** Nanomaterials; Nanoparticles; Risk assessment; Environmental pollution; Toxicology

**Abbreviations:** NMs: Nanomaterials; PM: Particulate Matter; ROS: Reactive Oxygen Species; DNA: Deoxyribonucleic Acid

## Introduction

### Nanotechnology applications in science and technology and environmental implications

Nanotechnologies are a relatively new field of science and technologies with a global market estimated in the range of \$14-16 billion in 2008-2010 [2,3]. Nano-scale materials constitute a diverse range of products and applications in electronics, optics, textiles, medical devices, catalysts, biosensors cosmetics, food packaging, water treatment technologies, telecommunications, pharmaceuticals, fuel cells, etc. In the last few years there is a wide debate about the benefits of nanotechnologies for humans and the technological society from the diverse applications of new products, but also about the risks of many manufactured Nanomaterials (NMs) and consumer products to human health and the environment. One of the consequences of the rapid progress now being made in nanotechnologies and their variety of application in everyday life is that we are becoming inescapably enmeshed in a host of very tricky ethical issues. There are concerns among scientists and environmentalists that manufactured Nanoparticles (NPs) will be released in the environment from these products over their life use through their erosion. Also, these products could generate wastes containing nanomaterials (domestic or industrial waste). There is an uncertainty whether or not the sewage treatment works could remove the released nanomaterials from the effluents. At present there are not many studies on the environmental problems, risks to human health and ecotoxicological issues of nanomaterials. Nanomaterials (NMs) are defined as materials with at least one dimension between about 1nm and 100nm. These materials can be nanofilms, nanowires and nanotubes or nanoparticles. Although there are not yet

international definitions of NMs and their sizes, but aggregates with overall dimension in the  $\mu\text{m}$  range, but made of primary particles of  $<100\text{nm}$  would be regarded as a NM. From the toxicological point of view, particles with diameter  $10\text{-}2.5\mu\text{m}$  are defined as coarse particles, for diameter of  $2.5\mu\text{m}$  or less are fine particles, and for less than  $0.1\mu\text{m}$  ( $<0.1\mu\text{m}$ ) as ultrafine particles [4].

## Nanomaterials and Risks to Biological Systems

A recent report by the Royal Society and the Royal Academy of Engineering in the United Kingdom of nanoscience and nanomaterials highlighted the possible toxicological and pathological risks to human health and the environment that may be presented by novel nano-products, especially the development of nanotechnology-based drug delivery systems. Manufactured NPs represent an intermediate supramolecular state of matter between bulk and molecular material. NPs are particles with sizes between  $0.1\text{-}100\text{nm}$  (diameter) but provide a very large surface to volume ratio. The surface properties of NPs and their biocompatibility depend on the charges carried by the particle and its chemical reactivity. It has been shown that polycationic macromolecules have a strong interaction with cell membranes *in vitro*. Also, the interaction of NPs with the surface lining layers of biological tissues is determined by their surface chemistry and reactivity. It has been shown that modification of  $\text{C}_{60}$ -fullerenes to reduce their lipophilicity results in reduced toxicity. Nanofibers and nanotubes may present special problems with respect to their retention in cells and tissues of biological systems. There is a threshold for the length of a nanofiber that is critical for induction of adverse biological effects. So, biodegradability of nanomaterials into smaller sizes may be a factor for increasing harmful biological effects. Fine and ultrafine particles of natural origin (sea salt, volcanic dust and combustion products of forest fires) are known for their ability to penetrate the respiratory system and cause adverse effects [5]. In recent decades the problem of Particulate Matter (PM) of very small size from the industrial and automobile combustion products has been investigated. PM in the range of  $2.5\mu\text{m}$  or smaller can enter the respiratory system by inhalation and retained in the alveoli. It is known that PM can act through the generation of free radicals and Reactive Oxygen Species (ROS) in the epithelial cells of the lining causing lipid peroxidation, oxidative stress and oxidative damage to enzymes and the cellular DNA.

## Biological Interactions of Nanoparticles and Uptake by Living Systems

Scientists are concerned for the effects of the NPs on biological systems because of their novel appearance in the environment and the lack of protective mechanisms in the course of biological evolution in living organisms. The very large surface area of NPs can have a high affinity for toxic metals and organic chemicals thus affecting their toxicological properties [6]. A typical example of concern for scientists are the new nanoparticle-mediated drug delivery systems that are using encapsulated drugs into nanoscale particles in order to target at appropriate concentration sites of oxidative damage in neurodegenerative diseases (Alzheimer's, Parkinson's). Also, nanoparticle-based medicines are used for

treatment and diagnosis of cancer, because NPs facilitate entry and intracellular targeting at sites of malignant growths of cancer patients. It is inevitable that scientists to worry about environmental pollution by nano-scale manufactured materials since it looks that in the near future nanotechnology and nanoscience's will produce a great variety of NMs with widespread use. Inhalation or ingestion are likely to be major routes for the uptake of NPs in terrestrial animals and humans. But in the aquatic environment the routes of entry of NPs can be through the gills and other external surface epithelia. At the cellular level, most internalization of NPs will occur via endocytosis. Endocytotic pathways into cells of living organisms can either lead to the endosomal and lysosomal compartments or via cell-surface lipid raft associated domains known as caveolae which avoids the degradative fate of material entering the endosomal/lysosomal system [7].

This endocytotic pathway is a route exploited by many viral pathogens. Scientists in the drug-delivery systems have designed nanoparticle properties and their sizes so that they can enter their target cells by these same routes. Pathways of endocytosis are exploited by nanotechnology specialists. It is obvious that from one hand there substantial benefits for targeted delivery of pharmaceuticals and new therapeutic and diagnostic advances in medicine, but also there are possible toxicological risk to humans and the environment from the release of these nanomaterials.

## Release of Nanoparticles into the Aquatic Environment

Environmental release of NPs into aquatic environment poses new environmental problems that need to be studied in the near future. Scientists have already formulated the areas of research which need further investigation: The hydrodynamic behaviour of small particles, the NPs association with larger sediments and colloid particulates, the binding of lipophilic organic compounds and metals, synergistic effects with other chemical pollutants that will enhance their toxicity, routes of NPs uptake into living organisms, the significance of size and surface properties, and the effects of NPs on the aquatic ecosystems [8]. It is a well-known fact that most of the industrial waste and all urban water sewage end up in waterways (rivers, lakes, coastal waters, etc). It is inevitable that industrial nanoscale products and by-products to enter the aquatic environment. Studies showed that in various aquatic environments suspended sediment particles play an important role in sequestering and transporting chemical pollutants over significant distances and the hydrodynamic and morphological characteristics of rivers and coastal zones will determine the distribution of bound NPs. In the marine and coastal aquatic environments the sea-surface microlayer of water, containing sugars, proteins and lipids, play an important role in the behaviour of NPs. Especially, the lipid moiety is going to be a major factor in providing a medium to partition into of lipophilic NPs (fullerenes and carbon nanofibers are such NPs) [9]. This situation undoubtedly influences the NPs behaviour and bioavailability in the sub-surface ecosystems. The sub-surface marine or coastal ecosystems are largely microbial (bacteria and protists) but also include pelagic eggs and larvae of

many invertebrate and fish species. Potential routes for the uptake of NPs by aquatic organisms include direct ingestion, entry through gills, olfactory organs and body wall (through the skin). Eukaryotic organisms, such as protists and metazoans, have highly developed processes for the cellular internalization of nanoscale (<100nm) and microscale (100nm-100.000nm) particles which are called endocytosis and phagocytosis. In invertebrate animals the cellular immune system, gut epithelium and hepatopancreas is likely to be targeted by ingested nanoparticles. In the case of fish, the liver is a probable target of NPs following endocytic transport across the intestinal epithelium into the hepatic portal blood system followed by endocytosis into hepatocytes.

### Studies for Potential Toxic Effects of Nanoparticles

Toxicological studies *in vivo* with NPs are very limited. Fullerenes were used in many initial studies since they are well known nanostructures with defined size and physicochemical characteristics. Studies showed that fullerene mixed with cultured mammalian cells caused oxidative damage and that their cytotoxicity is related to their lipophilicity. Modification of the surface of fullerenes to reduce their lipophilicity, by introducing aliphatic and hydroxyl groups, showed subsequently that reduced their toxicity. Most of the knowledge on NPs and NMs toxicity until recently was from mammalian studies (rats, mice, guinea pigs), especially respiratory toxicity, with exposure to carbon nanotubes, ultrafine TiO<sub>2</sub>, ultrafine cadmium particles, metal oxides particles, etc). Most studies showed that NPs cause significant lung damage when exposed intra-tracheal doses, inflammatory and fibrotic responses. But in recent years and taking into account the rapid increase of the manufactured nanomaterials and nanotechnology products, the lethal and sub-lethal toxicity of NPs was applied to wildlife (fish, invertebrates, aquatic organisms, mussels, etc). Studies with blue mussels (*Mytilus edulis*) and cockles showed that nanoparticles enter the digestive glands of mussels by endocytosis. It was demonstrated that nano-size particles (sucrose polyester, a zero-calorie food additive) was isolated in the hepatopancreas of whole mussels from seawater. Uptake was increased substantially as well as the cellular toxicity of PAH anthracene. Studies on fish and invertebrates showed that C60 fullerenes are toxic in the mg/L range, but the LC50 (lethal concentration 50%) values obtained are dependent on the method of preparation of nanomaterials and the addition of dispersants. These studies showed that NPs were present in the gut, and the gills (showed the greatest increase) of fishes when the delivery route was via aqueous exposure. The liver of trout appeared to be an important target organ by NPs. Biochemical changes were observed also in the liver [10].

Studies with carbon-based NMs showed that the brain is a potential target organ. There is some logic to this effect given that several biomedical studies have attempted to exploit the permeability of the blood-brain barrier to natural lipid micelles and particulates (by endocytosis) to develop nano-drug delivery techniques for the brain. Exposure of rainbow trout to nanoparticles of TiO<sub>2</sub> did not show any brain injury, although gills showed oxidative stress. It seems likely that brain injury depends on the nature of the particles. Toxicological studies until now are very limited and there are no

data on many organ systems (skeletal muscle, spleen, kidney, bone function). There is a need for more studies on absorption, distribution, localization of NMs in the body, metabolism and excretion in aquatic organisms. Also, there is a need for more studies on oxidative stress through the generation of free radicals and ROS from NPs, disturbances to trace elements metabolism, vascular injury, changes in antioxidant defenses in the epithelial cells, and injury in gills during aqueous exposure. Studies showed that NPs caused oxidative stress in aquatic organisms, especially in DNA which is known for its sensitivity to oxidizing agents *in vitro* experiments with buffers containing soluble proteins and exposure to hydrophobic NPs (CeO, carbon-nanotubes, polymer-coated quantum dots) show an increased rate of protein fibril nucleation (at pH2, lower than the physiological). There are few reports on the effects of NPs to invertebrates. *Daphnia magna* (waterflea) is a standard organism for ecotoxicology tests. Several studies used *Daphnia* to estimate LC50 values. Chronic effects on *Daphnia magna*, exposed for 21 days to up to 5mg/L on C60 fullerenes showed a mortality rate of 40% for the highest concentration. Other studies with *D. magna* found changes in locomotor behaviour. Ecotoxicological studies with NPs in earthworms, bacteria, algae (*Desmodesmus subspicatus*) and plants (marine nanoplankton, plant cells) have been published. There are also suggestions by some scientists that NPs transport by air can be a risk to farm animals and the human food chain.

### Conclusion

Scientists agree that the spread of nano-scale products will be beneficial to science, medicine and technology, but their release into the environment and especially in the aquatic environment might cause adverse health effects to humans, living organisms and sensitive ecosystems. The development of an effective working relationship between industry, government and an independent environmental science community will facilitate the development of an appropriate ecotoxicological studies and the identification of environmental hazards.

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