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The toxicity, ways of exposure and effects of Cu nanoparticles and Cu bulk salts on different organisms

Aasma Noureen, Farhat Jabeen*

Department of Zoology, Wildlife & Fisheries GC University Faisalabad, Pakistan

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Abstract

Nanotoxicology is projected as an emerging branch of toxicology to address the gaps in knowledge and adverse health effects likely to be caused by nanomaterials. This branch of nanotoxicology would make an important contribution to the development of a sustainable nanotechnology and covers the physicochemical factors, routes of exposure, biodistribution, molecular factors, genotoxicity and regulatory aspects. It is also involved in proposing reliable, healthy, and safe test protocols for nanomaterials in human and environmental risk assessment. Some nanoparticles such as copper is harmful to aquatic life, a small fraction of its nanoparticles can dissolve in water and produce toxic impacts on fish and other aquatic life. Copper nanoparticles are widely used as antimicrobial (antiviral, antibacterial, antifouling, antifungal), antibiotic treatment alternatives, nanocomposite coating, catalyst, lubricants, inks, for filtration of air and liquid. Fish are vulnerable to copper nanoparticles because it can induce gill injury and acute lethality. They are also emitted as particulates from smelters, metal foundries, and as pollutant from asphalt and rubber tires. Their long-term biological consequences on aquatic species are still unclear so it is pertinent to assess the toxicity of Cu nanoparticles and Cu salt in aquatic life. In view of these facts the following review article presents the toxicity, ways of exposure and effects of Cu nanoparticles on different organisms in vivo and vitro.

* **Corresponding Author:** Farhat Jabeen ✉ farjabeen2004@yahoo.co.in

Introduction

The research in nanotechnology has gained a significant priority worldwide. Many engineered nano size materials have been extensively used in consumer products but the adverse effects of these nanoparticles on the environment and organisms have recently drawn much attention. Rapid growth of nanotechnology and its common application in electronics, as alternative source of energy, in cosmetics and medicine (therapeutic, diagnostic or drug delivery devices) proves that it supports our life but at the same time there are growing concerns about human exposure to these nano materials which may have adverse health effects (Yousefian and Payam, 2012).

Nanotechnology has the potential to change and develop many sectors from consumer products to health, transportation, energy and agriculture. In addition to these shared benefits, nanotechnology presents new opportunities to improve how we measure, monitor, manage, and minimize toxins in the environment, At the same time as an increasing interest in, and rapid development of, a wide range of materials and products containing nanoparticles and engineered nanoscale materials, awareness has grown that the longer term potential toxic effects of such materials and their potential environmental impact are poorly understood. Existing methods have been assessed and new methods sought by which such materials could be analysed on a routine basis during development and manufacture. Nanotoxicology would make an important contribution to the development of a sustainable nanotechnology. Nanotoxicology covers the physicochemical factors, routes of exposure, biodistribution, molecular factors, genotoxicity, and regulatory aspects (Fig. 1). It is also involved in proposing reliable, healthy, and safe test protocols for nanomaterials in human and environmental risk assessment (Arora *et al.*, 2011).

Some nanoparticles such as copper are harmful to aquatic life. A small fraction of its nanoparticles can dissolve in water and produce toxic impacts on fish and other aquatic life (Ates *et al.*, 2014). Copper nanoparticles are widely used as antimicrobial (antiviral, antibacterial, and antifouling, antifungal), antibiotic treatment alternatives, nanocomposite coating, catalyst, lubricants, inks, filler materials for enhanced conductivity and wear resistance (Brownheim, 2011). Fish are vulnerable to copper nanoparticles because it can induce gill injury and acute lethality. Nano size copper particles are extensively used as a bactericide, additives in inks, lubricants, metallic coatings of circuits and batteries, for filtration of air and liquid. They are also emitted as particulates from smelters, metal foundries, and as pollutant from asphalt and rubber tires (Midander *et al.*, 2009). Their long-term biological consequences on aquatic species are still unclear so it is pertinent to assess the toxicity of Cu nanoparticles (Cu-NPs) and Cu salt in aquatic life. In view of these facts the following review article presents the toxicity, ways of exposure and effects of Cu nanoparticles on different organisms in vivo and vitro.

Ways of Copper Nanoparticles Utilization

Concurrently, copper nanoparticles have been investigated for their use in biotechnological applications that may one day fight with some of these disease conditions. For example, researchers have combined copper nanoparticles with a polymer material to make a composite capable of releasing metal species in a controlled manner in order to inhibit the growth of fungi and other pathogenic microorganisms (Cioffi *et al.*, 2005).

The germicidal properties of copper have been well recognized in both its particulate and soluble forms. Copper and copper oxide dispersions have been used in maritime industries since the 19th century in antifouling coatings. The downside to this application is the eventual mass release of copper nanoparticles into the aquatic system. However, more recently, Cioffi *et al.* (2005) proposed a similar strategy as a way to control the growth of *Saccharomyces cerevisiae* yeast and molds through the use of nanocomposites with spinning coatings that are equipped to release copper. They conclude that this nanostructured coating seems to be very promising as

there is the capability to control the metal release Cioffi *et al.* (2005). Another area of interest is the incorporation of low-copper-loading materials into packaging materials for food. Furthermore, copper oxide nanopowder has also been suggested as an anti-microbial preservative for use in wood and food products (Grocholl, 2005b). In addition to controlling the growth of yeasts and molds, copper nanoparticles (Cu-NPs) have also been shown to be effective in controlling the growth of bacteria such as *Escherichia coli* and *Bacillius subtilis*. The theorized mechanism of Cu-NPs acting as an antibacterial agent is "due to the interactions with the SH groups leading to protein denaturation" (Schrand *et al.*, 2010).

Manufactured nano size copper particles have also been used as additives in lubricants, inks, polymers and plastics, as well as metallic coatings. They provide excellent lubrication in oil due to mending effects and as an additive they reduce friction as well as wear and tear on surfaces (Liu *et al.*, 2004). In lithium ion batteries, nano size copper particles are deposited evenly on to graphite surfaces as an anode material (Guo *et al.*, 2002). Copper nanoparticles have also been used in various skin products to enhance healing and prevention of infection (Midander *et al.*, 2009).

Factors which increase Cu nanoparticles toxicity

Along with chemical composition, dose, and exposure route of nanoparticles the nanosize, nanosurface, dissolution and aggregation also increases its toxicity (Chang *et al.*, 2012**)**.

Size and surface area

The novel properties of nanomaterials are related to their size and surface area. Several studies have reported that nano size particles always exhibit more serious toxicity than bulks and suggested that their size was one of the key factors influencing the toxic effect of NPs. The size of nanoparticle is directly linked with surface area, solubility and chemical reactivity. For example, decreasing size results in increasing nanoparticles surface area. This promotes not only the accumulation of nanoparticles, but also an increase of reactivity. Because of their tiny size, nanoparticles can cross the small intestine and further distribute into the blood, brain, kidney and liver (Zhao *et al.*, 2007).

Dissolution

Another key factor of toxicity is the dissolution of Cu nanoparticles. Solubility is an important property that explains the reasons of toxic effects on many organisms. Rapid aggregations of copper nanoparticles occurred after suspension in water, resulting in 50–60% of added mass leaving the water column. While dissolution of particulate copper occurred, it was insufficient to explain the mortality in nanocopper exposures because nano size particles have larger surface areas to interact with solvent molecules than bulk ones with the same weight and NPs show faster dissolution (Griffitt *et al.,* 2007).

Importance of Exposure Routes

Cu nanoparticles can cause toxicity via pulmonary, oral, nasal, skin or other routes. In the environment the Cu and CuO nanoparticles are dissolved into metal ions, which can freely penetrate cells via ion channels and biological pumps. Once the bearable range is exceeded, toxic effects may occur. It is well known that dissolved Cu-NPs can inhibit Na^{+}/K^{+}-ATPase activity, resulting in ion regulatory toxicity (Li *et al.*, 1998). The toxicities of Cu-NPs to gastric tissues and kidney may be caused by increasing H^{+} and massive production of $HCO3^{-}$ Chen *et al.*, 2006. For metal oxide NPs, blood capillaries and lymphatic tissue play important roles in absorbability. Nanoparticles entering blood capillaries or lymphatic tissue may be carried to other organs where they accumulate. A test of inflammation induction in vascular endothelial cells indicated that nanoparticles entering vessels can lead to considerable cell toxicity in addition to a pronounced inflammatory response.

Copper Nanoparticles Toxicity

As a naturally occurring metallic compound, copper may exist by itself or may be associated with other elements. Copper particulates released into the air may be formed both naturally and by anthropogenic sources. Volcanic eruptions are the main natural

source of windblown copper particulates in dust. Copper smelters or other processing facilities are sources of copper particulates from manufacturing processes. Many times copper particles are removed from the atmosphere by precipitation, but they can be easily resuspended in the form of copper dust. In the U.S. the mean ranges of copper in the ambient air can vary widely from 5 to 200 ng/ m^3. Copper nanoparticles are released into the waterways usually via anthropogenic sources such as the effluent of industries or sewage treatment plants. Copper nanoparticles concentrations in drinking water may vary depending on pH and hardness of the local water.

Copper is a necessary trace element in the body. Certain mechanisms have evolved for the use, transport, and excretion of copper from the body. Copper is an essential nutrient because it is incorporated as one of many metallic enzymes necessary for proper hemoglobin formation, carbohydrate metabolism, collagen, elastin and hair keratin, antioxidant defense etc. Normal copper absorption occurs in the stomach and small intestine. Complex molecules have been known to exist in blood, but researchers have only been able to discover cellular copper transporters in the last decade. In organisms, Cu is one of the indispensable elements for maintaining homeostasis. Cu ions may cause toxicity once they exceed the physiological tolerance range *in vivo*. Therefore, the possible health effects and toxicology of Cu-NPs have caused great concerns in both the public and scientific researchers. Toxicity assessment studies have primarily focused on investigating the effects of different exposure routes such as the respiratory or gastrointestinal tracts.

Environmental research into Cu-NPs' toxicity has mostly focused the effects on organisms, especially those in aqueous environments. The most common experiment models zebra fish, tilapia and rainbow trout whose growth and toxicity are treated as environmental relevance indicators. Results showed that the toxicities of bulk and nano size Cu were largely influenced by soluble Cu ions (Griffitt *et al.*, 2007), these publications proved that the soluble Cu forms were highly toxic to fish. The results indicated that Cu-NPs toxicity is three to four times higher than that of ionic Cu because of the larger uptake of NPs. Griffitt *et al.* (2007) compared the responses of fish exposed to nano Cu solution and soluble Cu and reported that the effects of gill morphology and transcription were not solely due to the dissolution of Cu-NPs. Cu-NPs induced toxic effects by triggering ROS production and DNA damage in bacteria. Table 1 shows the possible toxicological effects of nanoparticles. Fig. 2 represents the overview of the different pathways inducing cellular toxicity by Cu-NPs.

Table 1. Possible toxicological effects of Cu nanoparticals.

Effects	Physiological outcomes
ROS generation	Protein, DNA and membrane injury
Oxidative stress	Phase II enzyme induction, inflammation
Mitochondrial perturbation	Inner membrane damage, permeability transition (PT) pore opening, energy failure, apoptosis, apo-necrosis,
Inflammation	Tissue infiltration with inflammatory cells, fibrosis, granulomas, atherogenesis, acute phase protein expression (e.g., C-reactive protein)
DNA damage	Mutagenesis, metaplasia, carcinogenesis

Ways of Exposure of Cu Nanoparticles to Organisms

Anthropogenic inputs

Cu-NPs are widely used as anti-microbial in commercial products such as deodorants and sticky plasters hence they are often the most modeled. The release of NPs to domestic wastepipes inevitably leads to wastewater treatment plants which drain to the oceans. It is worth examining their efficiency in dealing with NPs. Studies suggests wastewater treatment could remove 60-70% of NPs, whilst water

purification plants could remove up to 96%, depending on the method employed (Ganesh *et al.* 2010).

Natural organic matter

River systems contain a large amount of natural organic matter (NOM) and this has been shown to affect metal nanoparticles (Me-NPs). It becomes clear that NPs are unlikely to remain as single particles or even homo-aggregates. Instead both the particles and any dissolved metal ions may be highly complex by NOM and remain in suspension as they move toward the ocean.

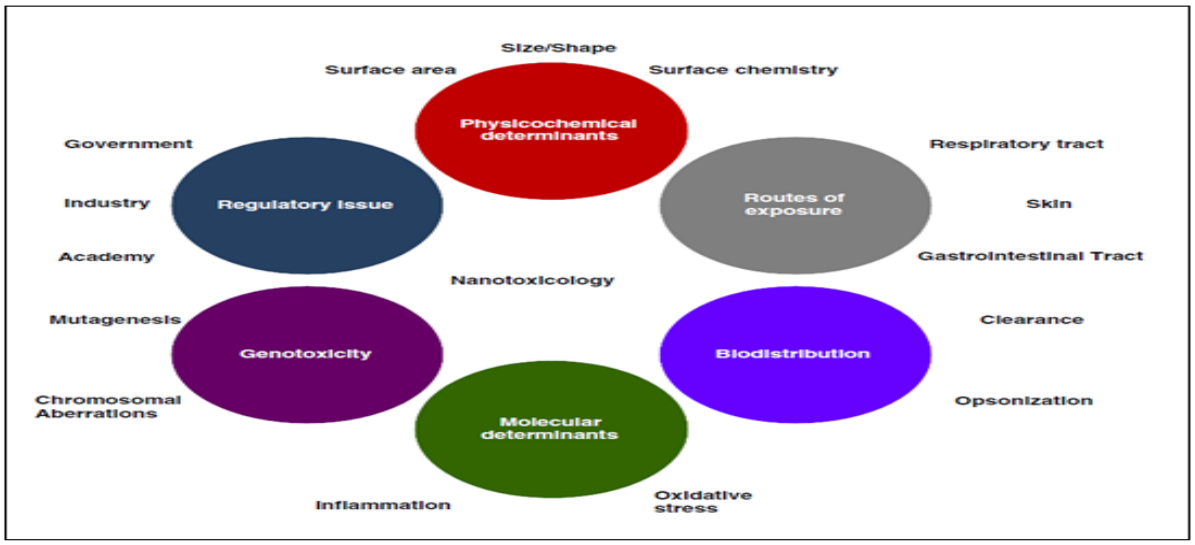

Fig. 1. Different aspects of Nanotoxicology (Arora *et al.*, 2012).

The study of Cu Toxicity

The toxicity of Cu nanoparticles was evaluated in following organisms

Daphnia magna

Fan *et al.*, 2010 investigated that the toxicity of copper in natural water to *Daphnia magna* is enhanced by nano-TiO_2.The study demonstrated that at a concentration generally considered to be safe in the environment, nano-TiO_2 remarkably enhanced the toxicity of copper to Daphnia magna by increasing the copper bioaccumulation. The copper was found to be adsorbed on to the nano-TiO_2 and ingested and accumulated in the animals, thereby causing toxic injury. The nano-TiO_2 may compete for free copper ions with sulfhydryl groups, causing the inhibition of the detoxification by metallothioneins.

Nile tilapia

Harabawya *et al.*, 2013 investigated the role of vitamins A, C, E and selenium as antioxidants against genotoxicity and cytotoxicity of cadmium, copper, lead and zinc on erythrocytes of Nile tilapia (*Oreochromis niloticus*). Metals induced genotoxic and cytotoxic alterations in erythrocytes of Nile tilapia. The addition of vitamin E alone and in combination of Se with vitamins A, C and E in the diet appeared to modulate the genotoxic effects of metals.

Japanese medaka

Barjhoux *et al.*, 2011 worked on the effects of copper and cadmium spiked-sediments on embryonic development of Japanese medaka (*Oryzias latipes*). Observations on control embryos demonstrated that the sediment-contact exposure protocol performed in this study provides acceptable conditions for medaka embryo development. Results obtained in contaminated groups showed obvious sublethal effects of both Cu and Cd on medaka development. Developmental defects mainly included spinal deformities, yolk-sac mal-absorption and cardio-vascular injuries. Moreover, genotoxic effects were induced by both metals in 2-day-old larvae. Consequently, the percentage of deformed larvae and

tail DNA measurements appeared as relevant markers in sediment toxicity assessment. This study demonstrates the importance of performing both chemical analyses and pertinent toxicity tests to evaluate hazards of pollutants accumulated in sediments since low concentrations of heavy metals could result in non-negligible deleterious effects on early developmental stages of various fish species.

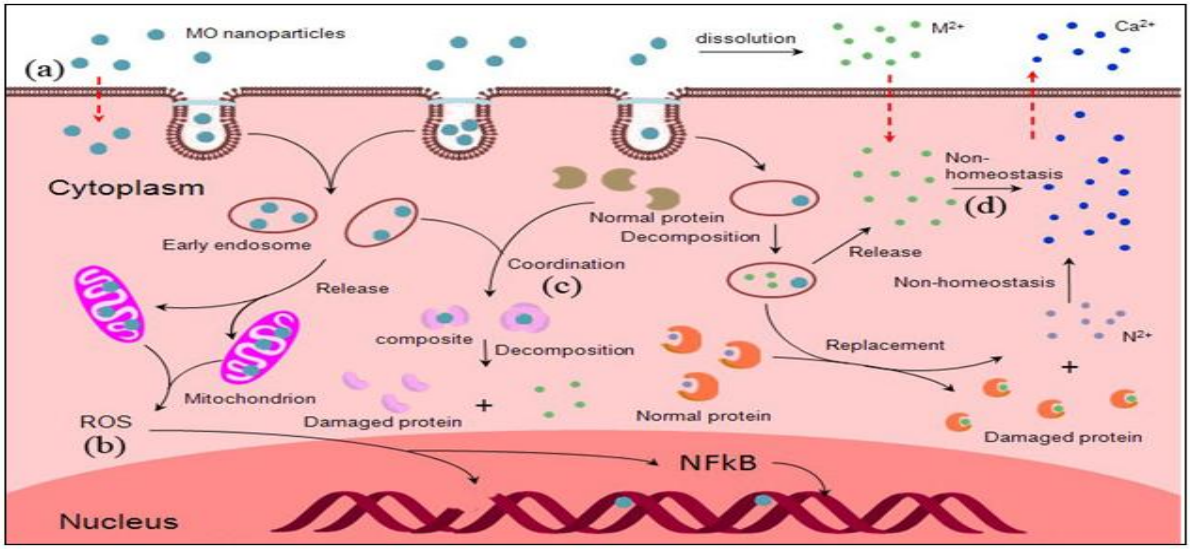

Fig. 2. Schematic overview of the different pathways inducing cellular toxicity by Cu NPs. (a) Potential mechanisms of Cu NPs' entry into cells; (b) The ROS effect of intracellular Cu NPs; (c) The coordination effect of Cu2+released from NPs in cell; (d) The non-homeostasis effect disrupted by Cu^{2+} (Chang *et al.,* 2012).

Rainbow trout

Shaw *et al.*, 2012 investigated the effects of waterborne copper nanoparticles and copper sulphate on rainbow trout (*Oncorhynchus mykiss*) and its physiology and accumulation. Emerging data suggested that some types of nanoparticles (NPs) are toxic to fish, and produce well-known toxicity of dissolved metals; there are also concerns about whether metal-containing NPs present a similar or different hazard to metal salts. Copper accumulation was seen in the gills of fish in both $CuSO_4$ treatments at day 4 and all Cu treatments at day 10 compared to controls. There were some transient changes in haematology and depletion of plasma Na^+ that was treatment related. Overall, Cu-NPs have similar types of toxic effects to $CuSO_4$, which can occur at lower tissue Cu concentrations than expected for the dissolved metal.

Zebra fish

Griffitt *et al.*, 2007 investigated that copper nanoparticles causes gill injury and acute lethality in zebra fish (*Danio rerio*). They demonstrated that nanocopper is acutely toxic to zebra fish, with a 48h LC50 concentration of 1.5 mg/L. Histological and biochemical analysis revealed that the gill was the primary target organ for nanocopper. To further investigate the effects of nanocopper on the gill, zebra fish were exposed to 100 μg/L of nanocopper or to the concentration of soluble copper matching that present due to dissolution of the particles. Under these conditions, nanocopper produced different morphological effects and global gene expression patterns in the gill than soluble copper, clearly demonstrating the effects of nanocopper on gill are not mediated solely by dissolution.

Podocytes (glomerular cells)

Xu *et al.*, 2012 assessed nanocopper induced apoptosis in podocytes via increasing oxidative stress. Nano size copper particles (nano-Cu), one of the representative metal nanomaterials, were used in several domains. In order to investigate the cytotoxicity induced by nano-Cu in podocytes, which was the key player of the glomerular filtration barrier, podocytes were treated with different concentrations

of nano-Cu. Results showed that nano-Cu affected the oxidant-antioxidant balance and had cytotoxicity in podocytes, resulting in the enhanced generation of ROS and MDA. Results suggested that the increased oxidative stress was a key mechanism in the podocyte apoptosis induced by nano-Cu, which could provide evidence for further research on the toxicity of nano-Cu.

Mice

Chen *et al.*, 2006 studied acute toxicological effects of copper nanoparticles *in vivo*. To assess the toxicity of copper nanoparticles (23.5 nm) in vivo, LD50, morphological changes, pathological examinations and blood biochemical indexes of experimental mice were studied comparatively with micro-copper particles (17 μm) and cupric ions ($CuCl_2 \cdot 2H_2O$). Kidney, liver and spleen were found to be target organs of nano-copper particles. Nanoparticles induce gravely toxicological effects and heavy injuries on kidney, liver and spleen of experimental mice but micro-copper particles do not on mass basis. Results indicated a gender dependent feature of nanotoxicity.

Mammalian and Piscine cell lines

Song *et al.*, 2014 assessed the species-specific toxicity of copper nanoparticles among mammalian and piscine cell lines. The four copper nanoparticles (Cu-NPs) with the size of 25, 50,78 and 100 nm and one type of micron-sized particles (MPs) (~500 nm) were exposed to two mammalian (H4IIE and HepG2) and two piscine (PLHC-1 and RTH-149) cell lines to test the species-specific toxicities of Cu-NPs. The results showed that the morphologies, ion release and size of the particles all played an important role when investigating the toxicity. . This study revealed that the morphologies, ion release rate of NPs as well as the species-specific vulnerabilities of cells should all be considered when explaining toxicity test results among particles and among species.

Methods Used for the Assessment of Cu Nanotoxicity

As with any other man-made materials, both *in vitro* and *in vivo* studies on biological effects of nanoparticles need to be performed. *In vitro* model systems provide a rapid and effective means to assess nanoparticles for a number of toxicological endpoints. These assays are suited for high-throughput screening of an ever increasing number of new engineered nanomaterials obviating the need for *in vivo* testing of individual materials. They also serve as well-defined systems for studying the structure–activity relationships involving nanomaterials.

Some of the distinct advantages of *in vitro* systems using various cell lines include:

(1) Revelation of primary effects of target cells in the absence of secondary effects caused by inflammation

(2) Identification of primary mechanisms of toxicity in the absence of the physiological and compensatory factors that confound the interpretation of whole animal studies

(3) Efficiency, rapidity and cost-effectiveness

(4) Scope for improvements in design of subsequent expensive whole animal studies

Genotoxicity assays

Several genotoxicity assays can be carried out in order to assess the genotoxicity.

Micronucleus (MN) assay

Several types of nanomaterials were shown to induce a significant increase of MN frequencies. Based on the micronucleus test (MN) data on 21nanomaterials, it was proposed that the *in vitro* MN test is quite appropriate to screen nanoparticles for potential genotoxicity. However it was recommended that protocols should be formulated to as to achieve maximum sensitivity and avoid false negatives. Determination of the cellular dose, cytochalasin-B treatment, and time of exposure, serum levels and choice of cytotoxicity assay was advised for a better interpretation of MN frequency results.

Comet assay

Comet assay is widely used *in vitro* assay in fundamental research for DNA damage and repair, in genotoxicity testing of novel chemicals and pharmaceuticals, environmental biomonitoring and human population monitoring. It has been employed

for toxicity assessment of nanoparticles. The comet assay use to investigate the toxicity of manufactured nanoparticles. Findings indicate that majority of the nanoparticles exhibited high reactivity and cause DNA strand breaks or oxidative DNA lesions. Considering the sensitivity of the assay it can enable the assessment of their relative potency. However, the author also states that, additional methods to measure DNA damage should be employed and more studies investigating mutagenicity would prove valuable.

Ames test

(or Bacterial Reversion Mutation Test) is yet another in vitro assay used to assess the genotoxic potential of nanomaterials. The test employs histidine dependent (auxotrophic) mutant strains of *Salmonella typhimurium*. This test is usually employed as an adjunct technique because it is difficult to interpret the data generated in a prokaryotic system to a eukaryotic genotoxicity testing. Furthermore results could be ambiguous in some instances when certain nanomaterials are not able to cross the bacterial wall or in situations where the nanomaterials are bactericidal.

Conclusion

Nanoparticle toxicology in aquatic systems is complex. In the first instance particle size, shape, chemistry and capping agents will all play a role regarding the stability and thus bioavailability of the NP within any media. Release kinetics then become a factor as NPs are complexes of hetero-aggregates along the course of a river, with mobility and aggregation determined by the molecular weight of natural organic matter in the water. As ionic strength increases in the pathway to estuaries and oceans, so zeta potential decreases, causing aggregation and promoting rapid sedimentation.

The four major ways in which nanoparticles may interact with an organism are:

1) Adsorption to the surface (cell, organ or body),

2) Cellular internalization,

3) Dissolution of ions from the NP

4) Mechanistic nano-effects

The oceans are a vast resource containing innumerate food webs. Current data indicates that Cu NPs can have highly adverse effects on key organisms such as primary producers, damage to which would have far-reaching impacts not only on nutrient production and food availability in marine trophic webs, but also on carbon and silicon cycling. The aggregation properties of Cu-NPs makes them directly available to larger organisms, whilst their adsorption properties also allows micro-organisms to become vectors for delivery to higher organisms.

Current anthropogenic discharge of Cu-NPs is not of a volume that chronic exposure would be expected to be harmful to marine organisms. Acute exposures may have an extremely localized impact, but the diffuse nature of the oceans means effects will not be widespread. A fuller understanding of the long term consequences of these particles in the marine environment is warranted and, importantly, also a need to relate the effects and release of these NPs to biomarkers in ecologically relevant species that can be used to inform on potential impacts on aquatic systems.

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