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Nanotoxicology: A Review

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Abstract

Nanotoxicology represents a new and growing research area in toxicology. It deals with the assessment of the toxicological properties of nanoparticles (NPs) with the intention of determining whether (and to what extent) they pose an environmental or societal threat. Inherent properties of NPs (including size, shape, surface area, surface charge, crystal structure, coating, and solubility/dissolution) as well as environmental factors (such as temperature, pH, ionic strength, salinity, and organic matter) collectively influence NP behavior, fate and transport, and ultimately toxicity. The mechanisms underlying the toxicity of nanomaterials (NMs) have recently been studied extensively. Reactive oxygen species (ROS) toxicity represents one such mechanism. An overproduction of ROS induces oxidative stress, resulting in inability of the cells to maintain normal physiological redox-regulated functions. In the context of this book, this chapter includes topics pertaining to chemical and physical properties of NMs and characterization for proper toxicological evaluation, exposure, and environmental fate and transport, and ecological and genotoxic effects. This chapter reviews the available research pertaining specifically to NMs in the aquatic environment (in plants, aquatic invertebrates, and fish) and their use in biomarker studies.

Keywords: nanomaterials, characterization techniques, nanotoxicology, biomarkers, analytical methods, in vitro studies, in vivo studies

1. Introduction

Nanotoxicology is regarded as the assessment of the toxicological properties of nanoparticles (NPs) with the intention of determining whether (and to what extent) they may pose an environmental or societal threat. Nanotechnology has advanced exponentially over the past
decade, with nanoscale materials being exploited in several applications and in several disciplines (including industry, science, pharmacy, medicine, electronics, and communication products). Vance et al. [1] reported a 30-fold increase in nano-based products between 2011 and 2015 (Figure 1) and an estimated global market of over $1 trillion in 2015 [2]. Metal NPs (specifically, carbon and silver NPs) represent the largest and fastest growing group of NPs (Figure 2). Hence, human and environmental exposure is already occurring and is predicted to increase dramatically. This growth in nanotechnology has not advanced without concerns regarding their potential adverse environmental impacts. Several reviews have reported on the toxicity of various NPs [3, 4]. However, much is still unknown.

Figure 1. Nanomaterial growth trend 2010–2015 [1].

Figure 2. Composition of nanomaterials (adapted from Vance et al. [1]).

Nanomaterials (NMs) are generally defined as a substance having particles with at least one dimension of 1–100 nm in length. Their novel physical and chemical characteristics have made them useful in several applications; however, these very properties can be potentially toxic.
Once introduced into aquatic ecosystems, the fate and toxicity of NPs and its uptake by aquatic organisms depend on several factors. Both the properties of NP (such as size, shape, and coatings) and water chemistry (such as dissolved organic carbon, ionic strength, pH, temperature) will largely influence the extent to which NPs will either remain in suspension, partition to dissolved organic carbon in the water column, form aggregates, or adsorb to suspended particles. In aquatic organisms, the accumulation of NPs is dependent on both the uptake and the elimination of the NP out of the organism. These processes also regulate the bioaccumulation (and bioavailability) of NPs. The availability of appropriate methodologies is needed to address key issues in nanotoxicology and to gain a better understanding of nanoparticle toxicity mechanisms (including oxidative stress, cytotoxicity, genotoxicity, and inflammatory responses). Exposure to NMs is largely through ingestion and adsorption to surface epithelia such as the gills.

Aquatic ecosystems are progressively coming under pressure due to the presence of emerging anthropogenic contaminants, including NMs, posing health hazards to inhabitant organisms. In recent years, increasing data demonstrated that NPs could induce toxicity and genotoxicity under a variety of exposure scenarios. An accepted mechanism by which NPs may induce cytotoxicity is considered to be through the induction of reactive oxygen species (ROS) which can induce oxidative stress which in turn may lead to cytotoxicity, DNA damage, and other effects.

Although research on the environmental impacts of NMs has grown dramatically over the past decade, studies investigating the environmental fate, transport, and toxicity of a variety of nanomaterials are still lacking. This chapter will review the available research pertaining specifically to NMs in the aquatic environment (in plants, aquatic invertebrates, and fish) and their use in biomarkers studies.

2. Physical and chemical properties of NMs influencing their toxicity

The behavior of NPs in various environmental matrices is complex and involves several processes. Properties of NMs are unique and different from conventional materials. Properties such as (1) particle size, (2) surface area and charge, (3) shape/structure, (4) solubility, and (5) surface coatings are known to affect NP toxicity.

Small owing to their small size, NMs have unique physical and chemical characteristics such as magnetic, optical, thermal, mechanical, electrical properties which make them suitable in several applications including in medicine, electronics, and energy production, and in several consumer products. However, these very properties have the potential to affect humans and the environment adversely. NPs can easily penetrate cell membranes and other biological barriers into living organisms causing cell damage. Studies reporting increased toxicity of NPs when compared to their larger bulk particles have led to a generally assumed hypothesis that NPs are more potent in causing damage. Lankvel et al. [5] reported the significance of particle size of AgNPs, reporting size-specific tissue distribution and size-specific toxicity. Scown et al. [6] reported the lowest aggregation potential for the smallest AgNPs (i.e., 10 nm vs. 35 and...
600–1600 nm) and was most highly concentrated in the gills and liver. Gaiser et al. [7] studied the acute and chronic toxicities of nano- and bulk Ag and CeO₂. Reported mortality rates for Ag and AgNP were as follows: micro-Ag at 0.1 mg/L was 13% and at 1 mg/L was 80%, while for AgNP at 0.1 mg/L was 57% and at 1 mg/L was 100%.

Although NPs size can be the most distinguishing characteristic when compared to conventional particles, shape and morphology also represent important factors when considering NP toxicity. Morphology (particles, spheres, rods, cubes, truncated triangles, wires, films, and coatings) affects NP kinetics and their transport in the environment. NP shape is also of importance as triangular nanoplates was reported to have greater inhibition of *Escherichia coli* than spherical- or rod-shaped AgNPs or Ag⁺ which can be attributed to the increased reactive, high atom density of the triangular nanoplates [8, 9].

When NPs are discarded, they can enter the aquatic environment as aggregates and soluble ions, which can be highly toxic to aquatic organisms. Aggregation and dissolution are key processes governing NP behavior and toxicity in the aquatic environment. These processes are largely driven by size and surface properties of NMs, as well as by the stability of natural colloids (such as dissolved organic matter). Colloidal stability is affected by several factors including the type environmental conditions such pH, temperature, and ionic strength. Romer et al. [10], investigating the stability of AgNPs, reported rapid aggregation in media with high ionic strength. Similarly, Walters et al. [11] reported higher toxicity due to the formation of smaller aggregates at elevated temperatures. These and other studies reported changes in organism exposure levels and consequent toxicity due to levels of aggregation [12]. Dissolution of NPs is also a significant process determining NPs effects in the aquatic environment. Most NPs do not dissolve in solution, but form colloid dispersions which will either remain dispersed or aggregate. As such, interactions with other colloid materials will affect the rate at which particles aggregate in an aqueous environment.

In the natural environment, NPs are not present in isolation. As such, it is important to consider the presence of other environmental stressors. For example, Walters et al. [13] reported that higher temperatures resulted in higher toxicity due to the formation of smaller aggregates at elevated temperatures and that AgNP dissolution and sedimentation contributed to a higher availability and toxicity of AgNP (and Ag⁺) to *Potamonautes perlatus*. Similarly, Liu and Hurt [14] reported higher dissolution rates of AgNPs with increased temperature. In the presence of dissolved oxygen (DO), AgNPs tend to aggregate and release Agions which in turns induce aggregation and oxidation [12].

Surface charge is a major factor in determining the particle dispersion characteristics and also will influence the adsorption of ions and biomolecules [15]. Baalousha [16] reported disaggregation of FeO NPs due to enhanced surface charge. Similarly, El Badawy et al. [17] reported surface charge-dependent toxicity of AgNPs. In addition, surface coating is indirectly related to aggregation and dissolution, as it is reported to increase the surface charge.

These unique physical and chemical properties of NPs raise concern as the conventional assumptions of chemical reactivity and behavior may not necessarily apply with regard to NPs.
3. Characterization

Nanomaterial behavior and toxicity are influenced by their physical and chemical properties. As such, characterization of NMs is essential in order to understand how their physical and chemical properties correlate with chemical, ecological, or biological responses. Full characterization of NPs includes determining the bulk (shape, size, phase, electronic structure, and crystallinity) and surface (surface area, arrangement of surface atoms, surface electronic structure, surface composition, and functionality) properties of the NM. In addition, environmental factors (such as temperature, pH, ionic strength, salinity, organic matter) may also affect NP behavior and toxicity.

Standardized tests established by the Organization for Economic Co-operation and Development (OECD) summarized some of the analytical methods commonly used in the characterization of NMs (OECD ENV/JM/MONO (2016)2. The morphology of NMs is frequently characterized using scanning electron microscopy (SEM) or transmission electron microscopy (TEM). Energy-dispersive X-ray (EDX) spectrometry coupled to SEM is a common method to characterize elemental analysis of NMs. Particle size in aqueous phase may also be determined indirectly by dynamic light scattering (DLS) which measures the Brownian movement of the NPs, or by electrophoretic light scattering spectroscopy (ELS) which uses oscillating electric field. X-ray powder diffraction (XRD) is employed to measure particle size in the dry state and which applies the Scherrer method. Murdock et al. [18] investigated the use of DLS to characterize NM dispersion.

Brunauer-Emmet-Teller (BET) is used to measure surface area. Zeta potential measures surface charge in particles in the aqueous phase and is a fundamental parameters known to affect stability. Atomic force microscopy (AFM) and scanning tunneling microscopy (STM) allow three-dimensional imaging of nanometer scale surfaces and the measurement of forces between surfaces at the pico newton scale. UV-vis and Fourier transform infrared spectroscopy (FTIR) are spectroscopic techniques used in the characterization of fullerenes in solution [19, 20]. Surface enhanced Raman spectroscopy (SERS) is a surface-sensitive technique that enhances Raman scattering by nanostructures allowing the detection of single molecules. Chemical characterization techniques include Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), and Energy-dispersive X-ray spectroscopy (EDS) used to investigate the elemental composition of NMs.

4. Routes of exposure in the aquatic environment

Due to the surge in nanotechnology, there have been significant increases in the number of various NPs released into the aquatic environment. Figure 3 provides a summary of the possible routes in a typical aquatic environment to nanoparticles, potential interactions, and the possible clearance routes. Aquatic ecosystems are susceptible to environmental contamination since they are at the receiving end of contaminants, particularly from runoff sources.
Identified sources of NPs in the aquatic environment include production facilities, production processes, wastewater treatment plants, and accidents during the transport. In addition, aquatic ecosystems are known to sequester and transport contaminants, including NMs. Baun et al. showed that NPs may adhere to algae which may then be consumed by filter-feeders and transfer to higher trophic levels. In the aquatic environment, NPs may aggregate thus reducing the NPs available for direct uptake in the aqueous phase by aquatic organisms. However, aggregated NPs may settle into sediment thereby posing a threat to benthic organisms. In the aquatic environment, NMs are generally associated with sediments [21]. Sediments and soil represent porous environmental matrices which typically have large specific surface areas.

Figure 3. Possible pathways of nanoparticles in the aquatic environment.

Uptake of nanoparticles into the aquatic biota is a major concern. Nanomaterials are able to cross biological barriers, gaining entering due to their small size. In aquatic organisms, the major routes of entry are via ingestion or direct passage across the gill and other external surface epithelia. In invertebrates, the cellular immune system, gut epithelium, and hepatopancreas are likely to be targeted [22]. Recent studies with Mozambique tilapia (Oreochromis mossambicus) have indicated that nickel NPs may be internalized via these routes [23]. In addition, NPs that are taken up via ingestion through the digestive tract may accumulate in the hepatopancreas [24]. The hepatopancreas is responsible for metabolism and detoxification.
and is known for intracellular lysosomal digestion of food via internalization by endocytosis [22]. Nanosize particles have also been demonstrated to enter the liver of fish.

Endocytosis (<100 nm) and phagocytosis (100–100,000 nm) represent the two processes by which NPs might be absorbed into eukaryotic cells. At the cellular level, internalizations of NPs occur via endocytosis. Iron oxide NP internalization via endocytosis has been reported by Auffan et al. [26].

NPs released into the environment are affected by environmental factors such as pH, temperature, and presence of organic matter. The pH affects NP surface charge and consequently also aggregation. This has been reported by Gilbert et al. [27] who reported a pH-driven aggregation and disaggregation with larger aggregate radius at higher pH. Furthermore, Adams and Kramer [28] reported increased mobility under increased acidification. Temperature is also known to affect aggregation. Walters et al. [11] reported that formation of smaller aggregates at higher temperatures suggests higher toxicity. Liu and Hurt [14] reported higher dissolution rates of AgNPs with increased temperature. NPs can be immobilized as a result of sorption or binding to particles such as organic matter. These effects have been reported. For example, Chen and Elimelech [29] reported that, in the presence of humic acid, the adsorbed humic acid on the fullerene NPs led to steric repulsion, stabilization of the NP suspension, and reduced aggregation.

5. Mechanisms of NP toxicity

Many studies have attempted to elucidate the mechanisms of NP toxicity and distinguish between their bulk counterparts. Nanomaterials differ from their bulk counterparts in several ways, including high surface/volume ratio. Other factors such as dissolution, size, shape, aggregation state, surface coatings, and solution chemistry also influence the toxicity of NPs.

The toxicity of various NMs AgNP [5–7, 30–32]), CuO NP [19, 33], TiO₂ NP [34], and Ni NP [23] has been studied in various aquatic species, such as *Daphnia magna* [32, 33], fish [6, 35], algae [36], and marine [37] and freshwater [13] crabs. Silver, carbon, and titanium NMs are among the most widely used types NMs used as additives in cosmetics and pharmaceuticals. Also, different NMs exhibit different properties had hence have different toxicity potencies. For example, Heinlaan et al. [38] compared the toxicities of three nanometal oxides: ZnO NPs, CuO NPs, and TiO₂ NPs. ZnO NPs was determined to be the most toxic; whereas Zhu et al. [39] reported CuO NP the most potent to cytotoxicity and genotoxicity.

The assessment of NP toxicity has largely been assessed in vitro, reporting inducing various negative effects at different levels of cellular organization. Typical end points measured include end points examined which include mortality, as well as sublethal effects such as development, growth, respiration, malformation, oxidative stress, and gene expression. Generation of reactive oxygen species (ROS) and free radicals causes oxidative stress (activation or inhibition of the antioxidant defense system), lipid peroxidation, and DNA damage. Toxicity of NPs will be discussed further in the following sections.
5.1. Oxidative stress

Oxidative stress is referred to as an imbalance between the production of reactive oxygen species (ROS) and the cells’ ability to reduce ROS, which may be as a result an increased ROS production, a decrease in the cell’s defense mechanisms, or a combination of both [40]. An overproduction of ROS may induce oxidative stress, resulting in cells failing to maintain normal physiological redox-regulated functions further resulting in oxidative modification of proteins to generate protein radicals [41], initiation of lipid peroxidation [42], DNA strand breaks and modification to nucleic acids [43], modulation of gene expression [44], thereby leading to cell death and genotoxic effects [45]. To minimize the effects of ROS-oxidative damage to cellular components, biological systems have developed a complex antioxidant system, comprised of both enzymatic and non-enzymatic defense mechanisms. Figure 4 summarizes the redox cycle including ROS generation by NPs and the antioxidant defense system.

![Figure 4](image.png)

**Figure 4.** ROS production and defense mechanisms (adapted from Unfired et al. [46]).

The antioxidant defense system has evolved to provide a balance between the production and removal of ROS. These are catalyzed by a number of different enzymes including Phase I and Phase II enzymes. Phase I enzymes, such as cytochrome P450, initiate the detoxification process by introducing a polar moiety which renders a lipophilic contaminant more hydrophilic. Activity of Phase I enzymes typically leads to an increase in ROS production. Phase II enzymes are involved in conjugating metabolized xenobiotics to endogenous molecules. Phase III involves further modification and excretion.
The potential role of oxidative stress as a mechanism of toxicity of AgNPs was evaluated by several authors. Walters et al. [13] studied oxidative stress, viz. antioxidant enzyme activity following a 7-day exposure to AgNPs (100 nm) at 10 and 100 μg/mL. The levels of ROS and oxidative stress were concentration-dependant, with an x-fold increase compared to control levels. Federici et al. [47] measured ROS generation following 14 days of exposure to TiO$_2$ NPs (average particle size = 21 nm at 0.1, 0.5, or 1.0 mg/mL). The level of ROS (i.e., thiobarbituric acid-reactive substances (TBARS)) in the gills, intestine, and brain of the rainbow trout (Oncorhynchus mykiss) increased in a concentration-dependent manner. Statistically significant increases (twofold or more) in the gills, intestine, and brain vs. the control group were also reported. In line with these findings, Oberholster et al. [48] investigated the generation of superoxide (O$_2^-$) and consequent stimulation of antioxidant defense mechanisms following a 10-day exposure to spiked sediment with various concentrations of NMs. These authors also reported concentration-dependant increases in enzyme activities. In another study with AgNPs, Gomes et al. [49] reported oxidative damage for higher doses (0, 100, 300, 600, 1000, 1500 mg/kg) and exposure times (4 days vs. 28 days) in Eisenia fetida. Induction of oxidative stress and antioxidant enzymes by titanium oxide NP (TiO$_2$ NP) in Daphnia magna was studied by Kim et al. [34]. The authors’ results indicated that the levels in activities of catalase (CAT), glutathione peroxidase (GPX), and glutathione S-transferase (GST) increase with increasing TiO$_2$ NP concentration. The levels of activities of superoxide dismutase (SOD) were reduced in the tissues with increasing TiO$_2$ NP concentration. In another study, Tedesco et al. [50] found signs of oxidative stress in the form of lysosomal instability. In line with these findings, Moore [4] reported decreased lysosomal stability resulting from apoptosis induction by AgNP.

5.2. Ecotoxicity

The potential ecotoxicity of NPs has currently provoked public and scientific dialogues due to debates around the risks and benefits of these materials. As such, studies on the ecotoxicological fate and effects of NMs have increased in recent years. There has been extensive research investigating the toxicity of NPs to aquatic organisms with several recent reviews reporting on ecotoxicology of NPs [3, 4]. Data on the biological effects of NPs indicate that NPs can be toxic to bacteria, algae, invertebrates, fish, and mammals. Nonetheless, nano-ecotoxicology studies remain poorly and unevenly distributed as most research undertaken has largely been restricted to a narrow range of test species. Most of the current ecotoxicological data pertaining to NMs have been done on Daphnia magna. These crustaceans represent the food and energy link between algae and fish [51]; therefore, these studies are particularly relevant. Park and Choi [32] studied the ecotoxicity effects of AgNPs to D. magna and reported increased mortality. Asghari et al. reported abnormal swimming in D. magna following exposure to AgNPs, while Heilinla et al. [33] reported ultrastructural changes in the midgut of D. magna upon exposure to CuO NPs.

Nanoparticles are able to penetrate the semipermeable membranes of some aquatic organisms, thereby forming aggregates around the exoskeleton of aquatic organisms [52]. Uptake of various NPs by aquatic organisms crustacean Daphnia magna [33, 53], the polychaete Nereis diversicolor [30], and the freshwater algae Ochromonas danica [31] has been reported (Figure 5).
Uptake of NPs generally occurs across the gills and other epithelial surfaces [6]. Scown et al. [6] reported size-dependant uptake of AgNPs (10–35 nm) and associated oxidative stress in the gills of Danio rerio, while Maria et al. [54] reported reduced LPO levels in gills and hepatopancreas of female Carcinus maenas. In crustaceans, toxicants are largely sequestered in the hepatopancreas and gills [55]. Walters et al. [13] reported higher levels on enzymatic activities in the hepatopancreas when compared to the gills suggesting that the hepatopancreas might be a more sensitive organ [56] to AgNP exposure and also implies a lower ability to scavenge O$_2^-$ [37].

Studies showing enhanced ecotoxicity of NPs when compared to their bulk counterparts have led to the assumption that NPs generally represent a more potent threat. Once such study investigated the toxicity of various NPs with bulk counterparts [57], the authors reported significantly differences in toxicity (24-h LC$_{50}$) between Al$_2$O$_3$ NPs (82 mg/L) and bulk Al$_2$O$_3$ (153 mg/L) and between TiO$_2$ NPs (80 mg/L) and bulk TiO$_2$(136 mg/L).

Studies reporting on reproduction and developmental end points are common. Wu et al. [58] recently showed that AgNPs induced a variety of morphological malformations such as edema, spinal abnormalities, fin fold abnormalities, heart malformations, and eye defects in Japanese...
medaka (*Oryzias latipes*). Wiench et al. [59] performed a 21-day chronic Daphnia reproduction study using coated TiO$_2$ NPs and reported that the NOEC for adult mortality was 30 mg/L, while the NOEC for offspring production was 3 mg/L. The 21-day EC10 and EC50 values for reproductive effects were 5 mg/L and 26.6 mg/L, respectively.

5.3. Genotoxicity

An important issue relating to the toxicity of NPs in biological media is the ability to cause damage to the genetic material, particularly since NPs have the capacity to cross cell membranes. In the section below, evidence of NP-induced genotoxicity is reviewed. DNA is a significant cellular component highly susceptible to oxidative damage. As such, there has been increasing interest in the analysis of the potential nanoparticle genotoxicity to aquatic organisms.

Genotoxic assessments of various NPs have largely been reported on in *vitro* studies. Reported abilities of NPs include chromosomal fragmentation, DNA strand breakages, point mutations, oxidative DNA adducts, and alterations in gene expression profiles and consequently may initiate and promote mutagenesis and carcinogenesis. Primary genotoxicity stemming from the direct interactions of NP with DNA following NP internalization has been reported [60]. Genotoxicity mediated by the generation of excess ROS, referred to as secondary genotoxicity, has been reported. Oberholster et al. [48], using DNA strand breakage as an indicator of genotoxicity, reported concentration-dependent effects to several NPs (α-alumina, β-alumina, precipitated silica; silica fume, calcined silica fume, colloidal antimony pentoxide, and superfine amorphous ferric oxide). DNA cleavage, an indicator of irreversible completion of apoptosis, occurred in organisms exposed to 5000 μg/kg of precipitated silica, amorphous ferric oxide, and colloidal antimony pentoxide NMs. The inter-nucleosomal DNA ladderbands occurred at 500 μg/kg of γ-alumina and α-alumina.

As with NP toxicity, NPs are also known to have more adverse genotoxic effects than their bulk counterparts. For example, Park and Choi [32] studied the genotoxicity of AgNPs on the freshwater crustacean *Daphnia magna*. Their results reported a higher degree of DNA damage in the form of DNA strand breaks in AgNPs when compared to Ag ions. Similarly, NP size is also known to affect its genotoxicity potential, inducing significant DNA and chromosomal damages compared to the larger NPs. This size effect was confirmed: the authors showed that smaller sized TiO$_2$ NPs (10 nm) have significant chromosomal damage when compared to the larger TiO$_2$NP (>200 nm) [61].

As such, there is a general consensus that smaller sized NPs produce higher reactivity and thus higher genotoxicity [62, 63]. However, particle size is not the only factor that determines particle (geno-)toxicity. Nanoparticle surface coating has also been reported to promote genotoxicity. Surface coating modifies the particle surface, and therefore, they may also alter the particle’s genotoxicity. For instance, Hong et al. [64] reported positively charged coatings of iron oxide NPs which consequently resulted in increased DNA strand breaks, while the impact of genotoxicity of negatively charged coatings was insignificant. Similarly, Lui et al. [65] reported various genotoxic responses of iron oxide NPs depending on the type of coating.
polyethylene glycol (PEG) coating exhibited mutagenic activity, while solid electrolyte interphase (SEI) exhibited no genotoxicity.

As with ecotoxicological effects, genotoxicity effects of coated NPs have also been investigated. Ahamed et al. [66] reported that coated AgNPs resulted in more upregulation of these proteins, suggesting that coated Ag NPs causes greater genotoxicity than uncoated AgNP. In similar study, AshaRani et al. [35] investigated the genotoxicity of coated silver NPs vs. uncoated silver NPs in the zebrafish embryos (*Danio rerio*). The authors reported that NPs which penetrated the nucleus cells able to take alterations and breakings to DNA.

### 6. Conclusions

The use of NMs in consumer products and their potential environmental and human health risks is of increasing concern. As nanotechnologies and products increase, nanoproducts entering the aquatic ecosystems and other water sources too will increase, thereby increasing the potential threat to aquatic organisms. This chapter provides a review of nanotoxicology—an emerging multidisciplinary field of science—with special focus on the effects of metal-NMs on aquatic invertebrates. NMs, depending on the size, shape, elemental materials, and the surface functional groups, induce oxidative stress thus leading to (nano)toxicity and genotoxicity. The risks associated with NMs (i.e., its fate, behavior, and toxicity in the environment) are largely unknown and difficult to predict. As the ultimate sink for conventional contaminants, the aquatic ecosystem is therefore predisposed to the potential effects of NPs.

Although our knowledge on the toxicity of various NMs in the aquatic environment has increased over the past few years, there is still a lack of knowledge regarding exposure concentrations, bioaccumulation in tissues, as well as environmental factors which could potentially affect its toxicity or bioaccumulation. Exposure to NPs is inevitable since NPs become more widely used, but there remains much more to be understood regarding their safety.

Although current toxicity testing protocols is generally applicable to identify deleterious effects associated with NPs, the mechanisms of action that govern toxicity of NMs are the subject of ongoing research. Research into new analytical methods is also required to address the special properties of NMs. The outcomes will thus enable researchers to predict the toxicological effects of AgNPs with the intent of guiding its development, application, and regulation. This will be important when considering measures for exposure control and environmental remediation of AgNPs.

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