Chapter 6
Environmental Bioeffects and Safety Assessment of Silver Nanoparticles

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Abstract The large production and expanding application of silver nanoparticles (AgNPs) in consumer market would inevitably bring additional sources of AgNPs in the natural environment, and the long-term and incremental exposure to both biota and human is also expected, which prompts scientists to consider more comprehensively the impacts of AgNPs on ecosystem health and safety in light of their toxicity as shown in Chap. 5. As the number of literature focused on the ecological effects of AgNPs gradually increase, it is possible to describe preliminarily the current knowledge of environmental bioeffects and safety assessment of AgNPs. In this chapter, we try to summarize and discuss the works that has been done so far to follow the environmental bioeffects and risk assessment of AgNPs.

6.1 Environmental Bioeffects

6.1.1 Bioavailability and Uptake

There are extensive evidences that the environmental bioeffects of nanoparticles (NPs) are strongly dependent on their bioavailability in the environment [1, 2]. In general, given the high reactivity of silver nanoparticles (AgNPs) in natural environments, organisms as we above-mentioned in Chap. 5 could be exposed to various forms of silver, including free silver ions, dissolved organic and inorganic silver

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complexes, and discrete single and/or aggregated and/or agglomerated AgNPs, which could show observed differences in their bioavailability to the organisms [3, 4]. Normally, compared to AgNPs and dissolved organic and inorganic silver complexes, free silver ions are more available for organisms.

The intrinsic properties of AgNPs such as structure, size, shape, surface coating, and composition, would largely affect their uptake and bioaccumulation to organisms. George et al. [5] investigated and compared the bioavailability of nanosized Ag spheres, plates, and wires in Holtfreter's medium, finding that the bioavailability of Ag is significantly lower for Ag-nanoplate relative to Ag-nanosphere. Size dependent uptake of AgNPs in *Daphnia magna* was also reported [6]. Commercial AgNPs with three nominal sizes (20, 50, 100 nm) and two surface coatings (citrate and tannic acid) were used in the study to investigate the size effect on the influx rate in a model organism, *D. magna*. For both types of surface coatings and different AgNP concentrations, the uptake rates always followed the sequence of 20 > 50 > 100 nm (Fig. 6.1), suggesting that the size of AgNPs played a key role in the bioavailability of AgNPs. After AgNPs enter the natural environment, their properties would be gradually altered, which would in turn lead to the changes in their bioavailability to organisms [7]. A previous work investigated the effect of different coatings on the silver ions release from AgNPs in natural water under artificial light condition, and found that bare- and citrate-coated AgNPs aggregated quickly in natural waters, while sterically dispersed AgNPs coated with Tween 80 were stable for a longer time and released silver ions much quicker, which indicated a higher bioavailability and toxicity toward aquatic organisms [8].

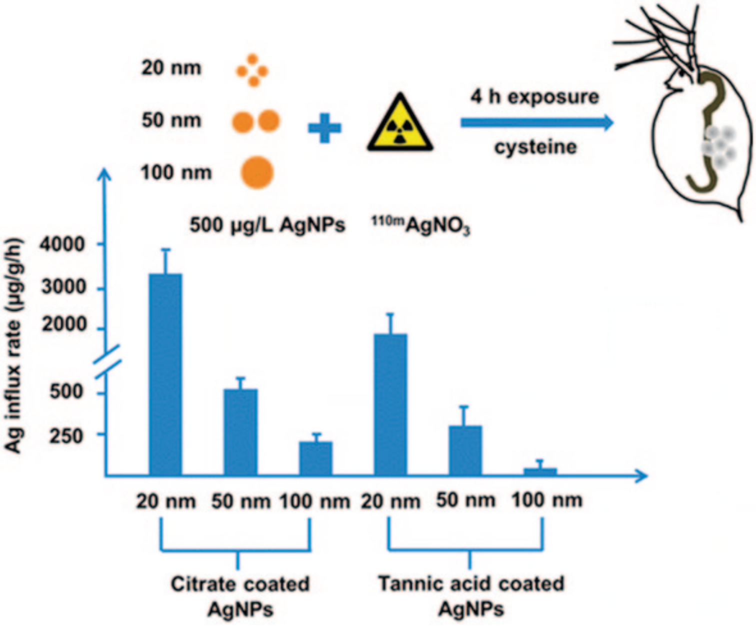

Fig. 6.1 Size-dependent uptake of silver nanoparticles in *Daphnia magna*. (Reprinted with the permission from ref. [6], Copyright 2012 American Chemical Society)

In addition, previous studies also reported that various factors like the pH, ionic strength/composition, and natural organic matter (NOM) could affect the silver speciation in the environment (Fig. 6.2), which in turn altered the bioavailability and uptake of AgNPs [1, 9, 10]. In particular, NOM has been reported to have the ability to mitigate the toxicity of AgNPs to biofilm due to the marked reduction in their bioavailability [11]. Another study also reported that the toxicity of AgNPs to *Ceriodaphnia dubia* decreased greatly in the presence of NOM, probably because the DOC coating inhibited silver ion release or made AgNPs less available for *C. dubia* [12]. Wang and coworker [13] investigated the effects of salinity on the bioavailability of citrate-coated AgNPs in the presence or absence of a nonionic surfactant (Tween 20) to marine medaka (*Oryzias melastigma*), and limited bioavailability of citrated-coated AgNPs was observed at high salinity (e.g., 30 psu). Additionally, they also found that well dispersion is of great importance for the bioavailability of AgNPs in a highly ionic environment. Clearly, the bioavailability of AgNPs is strongly dependent on environmental conditions.

Bioavailability has been reported to be one of the most important factors controlling uptake and bioaccumulation of metal-containing nanoparticles in the organisms. The uptake efficiency is dependent on bioavailability of AgNPs [14]. Without bioavailability, the dose dependent ecotoxicological effects of AgNPs could not be fully evaluated. Wang [13] found that the calculated uptake rate constant of citrate-coated AgNPs in marine medaka (*O. melastigma*) was 2.1 L/kg/d at a salinity of 15 psu, while no uptake was determined at salinity of 30 psu due to the limited bioavailability of AgNPs under this condition. Moreover, Aerle et al. [15] found that toxicity in exposed zebrafish embryos (*Danio rerio*) caused by AgNPs is principally associated with bioavailable silver ions in the water.

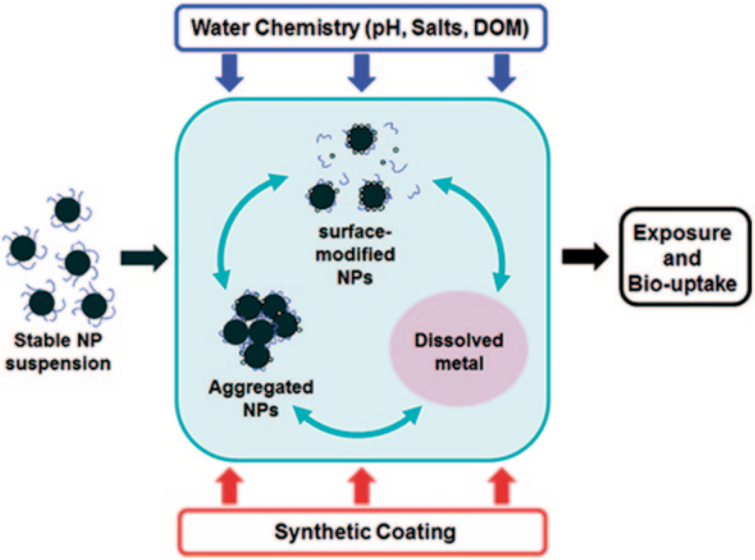

Fig. 6.2 Forms and bioavailability of silver in the environment. (Reprinted with the permission from ref. [1], Copyright 2012 American Chemical Society)

Till date, many works focused on variation of bioavailability and toxicity of AgNPs resulted from chemical transformations have been reported [9, 16, 17]. Among the various transformations, sulfidation of AgNPs has recently attracted much attention, since Kim et al. discovered the presence of Ag_2S-NPs in sewage sludge due to the AgNP transformation during wastewater treatment processes [18]. AgNPs easily react with inorganic sulfide to transform into Ag_2S, and the degree of sulfidation is closely related with the HS^-/Ag ratio, the polydispersity, and aggregation state of AgNPs [17]. The sulfidation has also been proved to be one of the best ways to control the silver ion release of AgNPs, which would in turn lead to great reduction in *Escherichia coli* growth inhibition [19]. Generally, the higher the $Ag_2S:Ag^0$ ratio was in the sulfidized AgNPs, the less growth inhibition of *E. coli* was observed (Fig. 6.3). Moreover, Levard and coworkers [16] reported the sulfidation could significantly reduce toxicity of AgNPs to four different types of eukaryotic organisms, including zebrafish, killifish, nematode worm, and least

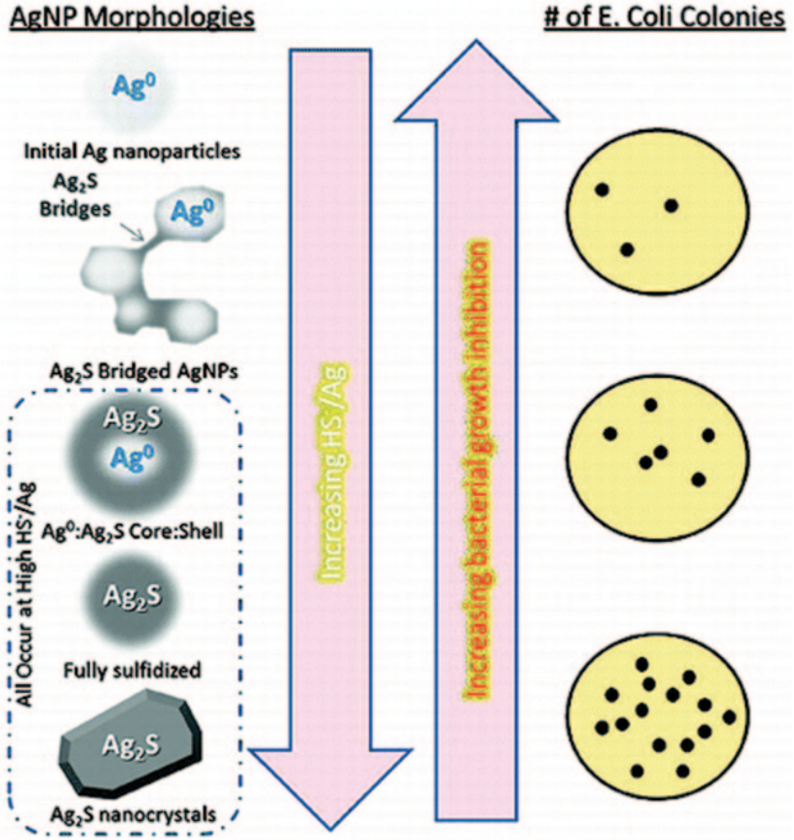

Fig. 6.3 Sulfidation of silver nanoparticles decreases *Escherichia coli* growth inhibition. (Reprinted with the permission from ref. [19], Copyright 2012 American Chemical Society)

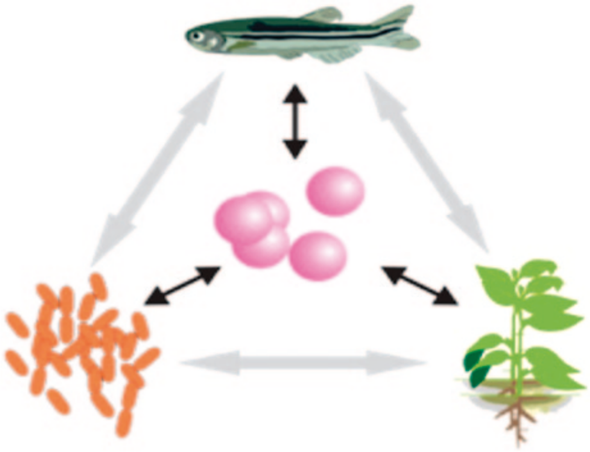

Fig. 6.4 Ecotoxicological effects of silver nanoparticles (*AgNPs*) in the natural environment. (Reprinted with the permission from ref. [2], Copyright 2013 American Chemical Society)

duckweed. The toxicity decrease was significant for killifish and duckweed, even for a very low degree of sulfidation (about 2 mol % S). This was attributed to the lower solubility of Ag_2S compared to AgNPs, which resulted in a dramatic decrease in Ag^+release. In addition to sulfidation, chloridation has also been proved to play an important role in mitigating the toxicity of AgNPs [9]. In the presence of chloride with Cl/Ag ratios ≤ 2675, AgCl particles can be directly formed and precipitated on the surface of AgNPs, which slowed down the oxidation and dissolution rate of AgNPs. The decreased amount of soluble Ag resulted in much lower growth inhibition toward *E. coli*. In total, the bioavailability of AgNPs could decrease greatly in the presence of sulfide or chloride in the aquatic environment [9, 20].

6.1.2 Ecotoxicological Effects

Over the past decade, AgNPs have been extremely popular in biological and medical fields due to their antimicrobial activity toward a broad spectrum of microorganisms. It is obvious that most, if not all, of the usage would create wastes, increase potential for their widespread release into the water body, and hence raise concerns on the ecotoxicological effects of AgNPs. Accordingly, much work on the ecotoxicological effects of AgNPs has been investigated using a suite of in vitro and in vivo tests. Hence, we would summarize the current body of literature focused on the ecotoxicological effects of AgNPs.

As Fig. 6.4 cited from Maurer-Jones et al. [2] points out, for the various components of the food web, namely bacteria, plants, and multicellular aquatic and terrestrial organisms, most literature concentrated on these organisms. In general, bacteria, acting as the components of the base of food web, play a very important role in the natural environment. Till date, there has been a large number of literature on effects of AgNPs on bacteria [9, 21]. Fabrega et al. [22] investigated the impact of humic acid on the toxicity of AgNPs to *Pseudomonas fluorescens* over a 24 h exposure at pH 6–9, which demonstrated that humic acid could mitigate toxicity of AgNPs to the bacterial at pH 9, while the toxicological response could not be explained by the dose of dissolved silver. Normally, the toxicological effect is strongly dependent on the concentration of AgNPs or released silver ions; bacterial (bacterioplankton) production could be significantly inhibited once the concentration of AgNPs is higher than 1 mg/L [23]. However, in treatments with low concentrations of AgNPs (10 and 20 μg Ag/L) after 48 h, the production of bacterial community did not alter much but with a reduction of alkaline phosphatase. In light of this finding, the authors declared that AgNP concentrations in the ng/L range in natural aquatic environments are unlikely to negatively impact aquatic biogeochemical cycles [23].

The effect of AgNPs of different sizes (5, 10, and 25 nm) and coatings (poly ethylene glycol and carbon) on the plants like poplars (*populus deltoides nigra*) and *Arabidopsis thaliana* were investigated at a wide range of concentrations (0.01–100 mg/L) by Wang et al. [24]. They demonstrated that all forms of silver used in their study were phytotoxic to these plants once AgNPs was above a specific concentration. More importantly, a stimulatory effect was found on root elongation, fresh weight, and evapotranspiration of both plants even at a narrow range of sublethal concentrations of AgNPs. Dimkpa et al. also reported that AgNPs affect the growth and metabolism of wheat in a sand growth matrix, and obvious reduction in the length of shoots and roots of wheat was observed in a dose-dependent manner (Fig. 6.5) [25]. Moreover, transmission electron microscopy (TEM) images clearly showed that AgNPs were present in both the shoots and roots of the exposed wheat, implying the uptake and transport of AgNPs in the sand. The possible accumulation of AgNPs in food crops may pose a potential threat to the humans. AgNPs could also trigger the alteration in gene expression in *A. thaliana*. *A. thaliana* exposed to 5 mg/L polyvinylpyrrolidone (PVP)-coated AgNPs (20 nm) showed upregulation

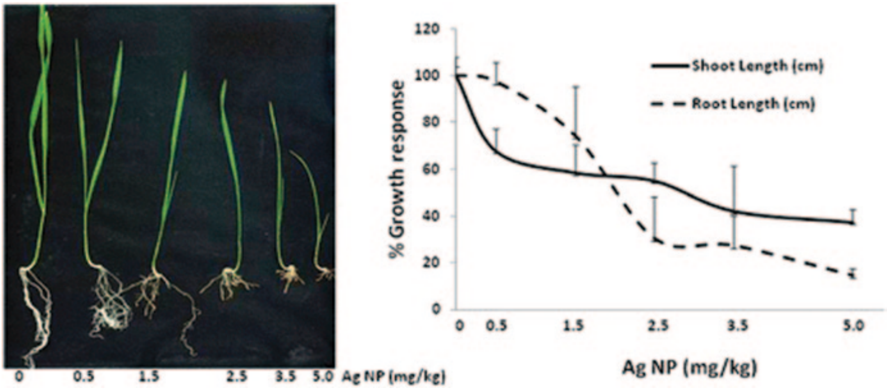

Fig. 6.5 Dose-dependent growth response of wheat to challenge with AgNPs for 14 days in a sand matrix. (Reprinted with the permission from ref. [25], Copyright 2013 American Chemical Society)

of 286 genes and downregulation of 81 genes compared to nonexposed plants [26]. Further study revealed that upregulated genes were primarily related to the response to metals and oxidative stress, while downregulated genes were more related to response to pathogens and hormonal stimuli. In another study conducted by Patlolla et al. [27], broad bean plant seedlings were exposed to AgNPs (60 nm) in a range of 12.5–100 mg/L, and the frequency of chromosomal aberrations and micronuclei induction increased in a AgNP dose-dependent manner. Together, all these works suggest that AgNPs could greatly impact plant development. Additionally, AgNPs have the potential to impact the eukaryotic organism development. More than 50 % of *Drosophila melanogaster* exposed to 20 mg/L AgNPs were unable to leave their pupae, and they could not even finish their development cycle under this condition (Fig. 6.6) [28]. Another study also reported that AgNPs could induce heat shock stress, oxidative stress, DNA damage, and apoptosis in *D. melanogaster* [29], implying that more careful assessments of AgNP risks are warranted.

In addition to the bacteria and plants, AgNPs also have been found to be toxic to multicellular aquatic and terrestrial organisms. Medaka and zebrafish, two of the most frequently used fish models, have been used to study ecotoxicological effects of AgNPs in the aquatic environment. Kwok et al. [30] reported that gum arabic-coated AgNPs showed the highest toxicity to Japanese medaka (*Oryzias latipes*), while PVP-coated and citrate-coated AgNPs exhibited similar and lower toxicity. Bar-Ilan et al. [31] found that silver ions, released from AgNPs, could surely exert significant toxicity to zebrafish embryos. The alteration of zebrafish developments and larval behaviors caused by AgNPs was also reported [32]. Moreover, it is likely that AgNPs would affect the organisms along with food chains, though information on this topic is sorely lacking right now.

Although it is very important to begin with classical biological models like medaka and zebrafish, there has been some work focused on the impact of AgNPs on another susceptible fish to fully understand the ecotoxicological effects. Recently, Jang and coworkers [33] investigated the toxicokinetics of total silver in common

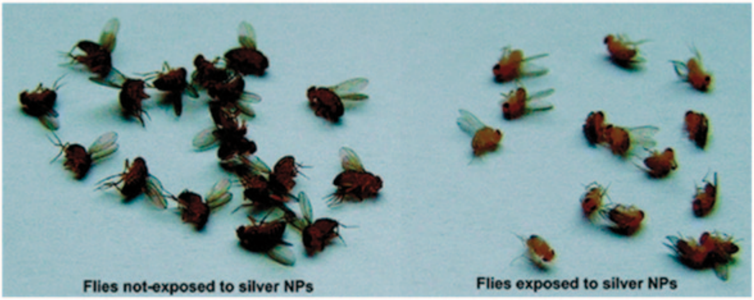

Fig. 6.6 Comparison of body pigmentation and body proportion of flies hatched on culture medium without AgNPs (on the *left*) and with AgNPs at a concentration of 20 mg/L of silver (on the *right*). (Reprinted with the permission from ref. [28], Copyright 2011 American Chemical Society)

carp (*Cayprinus carpio*). After carp was exposed to AgNPs at 0.62 mg/L for 7 days and followed by a 2 week depuration period, they found that liver contained the highest concentration of silver (5.61 mg/kg), and its concentration still kept a high-level (4.22 mg/kg) even after depuration for 14 days. Thus, the function of liver would be affected by this level of silver.

Earthworm is one of the classical models to investigate the effect of AgNPs in soil on the terrestrial organisms, since it comprises a critical component of the ecosystem. Till date, a handful of experiments have been conducted on earthworm response to AgNPs in soils. Heckmann et al. [34] investigated the effect of high dose of AgNPs (1000 mg/kg) on the earthworms, and 100% reproductive failure was observed, which was also reported by Shoults-Wilson et al. [35]. In another study, after the earthworm *Eisenia fetida* was exposed to AgNPs (10 and 80 nm) for 14 days, the activities of acid phosphatase and Na^+, K^+-ATPase were inhibited significantly in a dose-dependent manner, and the toxicity of 10 nm AgNPs was higher than the larger ones [36].

Taken all together, these findings demonstrate that AgNPs could impose toxicological effects on organisms in the environment, which should raise much more concerns in the future.

6.1.3 Combined Toxicity with Various Environmental Pollutants

Normally, organisms are exposed to more than one kind of pollutants in the natural environment, such as organic pollutants like persistent organic pollutants (POPs) and antibiotics, and inorganic pollutants like metal ions and other NPs. The coexistence of AgNPs and other environmental pollutants would mutually impact their fate, transport, and transformation, and further lead to changes in their toxicity to organisms. In this regard, some studies have raised the question about the possible interaction between AgNPs and other pollutants in the environment and about the mutual influence on their combined toxicity. However, understandings of the environmental fate and in situ toxicity of AgNPs with various other environmental pollutants on biota and ecosystems is sorely lacking right now. Ghosh and coworkers [37] investigated synergistic action of cinnamaldehyde with AgNPs against spore-forming bacteria, and found that the antibacterial activity of AgNPs was greatly enhanced in the presence of cinnamaldehyde. In addition to that, combination of AgNPs and antibiotic like amoxicillin could exhibit a greater bactericidal efficiency on *E. coli* cells compared to AgNPs or amoxicillin alone [38], as also reported in another study conducted by Hwang et al. [39]. Torre-Roche et al. [40] demonstrated that exposure of *Cucurbita pepo* (zucchini) and *Glycine max* (soybean) to AgNPs led to a significant reduction in the uptake of p,p'-Dichlorodiphenyldichloroethylene (p'-DDE). In the presence of 500 mg/L bulk of Ag or AgNPs, DDE uptake by *C. pepo* can be suppressed by 21–29%. The interaction of AgNPs with cocontaminants was greatly influenced by the Ag particle size (bulk vs NP) and plant species. Ultravoilet (UV)-induced toxicity reduction of citrate-AgNPs in the presence of perfluorocarboxylic acids was also reported recently [41]. Perfluorocarboxylic

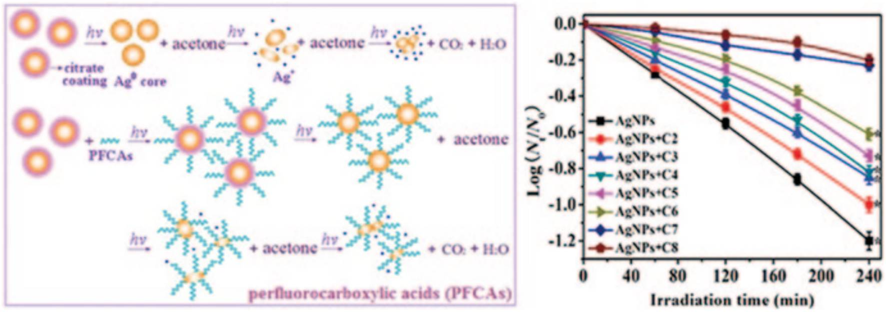

Fig. 6.7 Kinetics of *E. Coli* inactivation by AgNPs with or without perfluorinated carboxylic acids (*PFCAs*) under ultravoilet (UV) irradiation. (Reprinted with the permission from ref. [41], Copyright 2014 American Chemical Society)

acids with carbon chain length C2–C8 adsorbed on the surface of AgNPs would suppress Ag^{+}dissolution, enhance the AgNP stability, and decline the reactive oxygen species (ROS) production. UV-induced toxicity of AgNPs toward *E. coli* cells was decreased largely as well, and longer chain length of perfluorocarboxylic acids resulted in less toxicity (Fig. 6.7), which was probably due to the lower Ag^{+}dissolution. These studies highlight that when assessing the risks posed by AgNPs, both their inherent toxicity and their possible interactions with various organic or inorganic pollutions should be considered.

In total, these existing findings on the synergistic effects of AgNPs and other various environmental pollutants have already pointed out the scientific chances in the future. In particular, although the enhanced toxicity of combined AgNPs and other environmental pollutants has been found, the potential mechanism of the enhancement is still not clear.

6.2 Exposure and Risk Assessment

AgNPs have been one of the highest developed nanomaterials around the world, and it is estimated that about 500 t/a AgNPs were produced worldwide [42]. Moreover, as surveyed on October 2013, among the 1628 consumer products that containing nanomaterials, 383 were clamed to contain AgNPs [43], and the number is still growing. The rapid production and application of AgNPs have led to increased exposure and substantial release of AgNPs into the natural system. Once reached the environment, there are a variety of ways that AgNPs transported to water bodies, plants, and environmental organisms, which would ultimately get recycled back to humans. Commonly, the primary human exposure of AgNPs would occur via ingestion (food and water), inhalation (AgNP powders and dusts), and skin contact (various consumer products). And among the myriad of products containing AgNPs, humans are exposed mostly to three major categories that are food, consumer products, and medical products [44].

AgNPs are commonly added in water filters for household use to kill bacteria in drinking water; however, the inevitable release of AgNPs in water would result in the direct ingestion of AgNPs in everyone's life. Moreover, as it was reported, nanotechnologies are being involved throughout all stages of food production chain. In order to prolong the shelf life of food, AgNPs are used as additives and directly included into food. In the processing process, AgNPs are also reported to be incorporated into food preparation equipments. To decrease food decay and prevent bacterial growth during conservation, AgNPs containing packaging materials and food storage devices are frequently used. In general, the exposure risks largely depend on the ways that AgNPs were incorporated into the products. For example, for AgNPs that are bound in the packaging materials or coated on the surface of food storage devices or bags, though the direct contact may result in the release of AgNPs, the amount is relatively small, and a low exposure is expected. However, for AgNPs that are included as additives in food, they may be eaten directly and the consumer exposure may be high [44].

AgNPs are incorporated into a myriad of consumer products in our everyday life, such as nanosilver toothpastes, towels, soaps, cosmetics, antiodor sprays, disinfectants, functional textiles, socks, washing machines, and so on. Undoubtedly, the frequent contact with these products would increase the dermal exposure to AgNPs. Kulthong et al. [45] examined the release of AgNPs from nanosilver treated fabrics in artificial sweat to mimic the behavior of Ag nanotextiles during wearing. They found that silver was released from different textiles to varying degrees, ranging from 0 mg/kg to about 322 mg/kg of fabric weight. The amount of silver released was dependent on the quantity of silver coating, the fabric quality, and the artificial sweat formulations. This study indicated that sweat might promote the transfer of AgNPs from treated fabrics to the skin surface to enhance the dermal exposure during wearing. Moreover, the daily use of nanosilver toothpastes, towels, soaps, cosmetics would also result in the chronic exposure to AgNPs. Nanosilver spray, which is used to kill bacteria in the air, is also an important source for human exposure. Quadros and Marr [46] determined and characterized the emissions of airborne particles from consumer spray products containing AgNPs, and further assessed the potential for inhalation exposure to silver during product use. Surprisingly, they found that the normal use of silver-containing spray products carried the potential for inhalation of silver-containing aerosols, and up to 70 ng of silver might deposit in the respiratory tract during product use. The direct use of spray can also result in Ag deposition on skins or on surfaces of the walls, tables, floors, and other household products. For children (e.g., toddlers), touching these surfaces, handling sprayed products and then mouthing their hands, or mouthing of objects (e.g., toys) that have been sprayed with AgNPs are also possible routes for oral exposures [47].

Silver is well known for its excellent antimicrobial properties for decades, and AgNPs have long been used in medical areas. Nanosilver dressings and band-aid are widely applied to avoid infections after surgeries; however, there is a risk that AgNPs released would enter the body directly through broken skins. AgNPs are also used as additives in bone cements, coatings of implants for joint replacement, and intramedullary nails for bone fractures. The implant of AgNPs can also lead to the slow release of Ag, which may pose a potential exposure to human bodies.

Though a large variety of products containing AgNPs are appeared on the market, only little information about the products is available. In most cases, the manufactures only declare that their products possess superior antibacterial ability in the presence of AgNPs; however, information on the concentration, size, shape of AgNPs is missing. To estimate the risk of exposure, more information of the products is needed such as the types of incorporation, the concentration and forms of AgNPs, the size, surface coatings, and the dispersion state [44].

Frequent washing, abrasion, and disposal can lead to AgNPs release into the environment [48]. In spite of the role of wastewater treatment plant in removal of AgNPs from waste streams, tens or hundreds ng/L level of nanosized silver was still determined in the effluent of wastewater treatment plants. Accordingly, the concentration of AgNPs in the aquatic environment was predicted to reach ng/L level [49]. Since AgNPs are being widely used all over the world, the amounts of AgNPs and their transformation products in aquatic environments are expected to gradually increase over time. Recently, Comfort et al. [50] evaluated the cell response to long-term exposure of low-dose AgNPs, and found that chronic exposure of HaCaT cells to AgNPs in the ng/L range could activate sustained stress and signaling responses. However, up to now, there is no direct evidence that AgNPs at ng/L levels in the aquatic environment could harm organisms. Thus, scientists should raise more concerns about the potential risks of low levels of AgNPs in aquatic systems.

As previous studies reported, majority of AgNPs in water stream will be removed in wastewater treatment processes, causing a large amount of nanosized silver accumulated in the sludge. However, sewage sludge is used as fertilizers to modify the soils in some countries, which would inevitably impose risks of AgNPs on the bacteria, plants, and animals in terrestrial systems.

In natural system, AgNPs would undergo different pathways during transport, and the related environmental risks may be gradually mitigated by modification, aggregation, or complexing. The potential hazards are largely dependent on the species of silver; however, studies on the transformation of AgNPs in the natural system are still limited. Thus, in order to accurately assess the risks of AgNP exposure, much more information about the transformation of AgNPs and factors affecting the silver species and toxicity in the environment is also needed.

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