Bay Area Clean Water Agencies

Leading the Way to Protect Our Bay

A Joint Powers Public Agency

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March 19, 2009

Nathanael R. Martin
U.S. Environmental Protection Agency (U.S. EPA)
Office of Pesticide Programs (OPP)
Regulatory Public Docket (7502P)
1200 Pennsylvania Ave., NW.
Washington, DC 20460–0001

RE: Petition for Rulemaking Requesting U.S. EPA Regulate Nanoscale Silver Products as Pesticides (Docket Number EPA–HQ–OPP–2008–0650) - Support

Dear Mr. Martin:

The Bay Area Clean Water Agencies (BACWA) agrees with the petitioners that nanosilver products should be regulated as pesticides. Products designed with nanoscale silver use the silver—or ions released from the silver—as a biocide. BACWA's member agencies are very concerned about the water quality impacts from the discharge of silver ions from these products into our municipal wastewater systems. These concerns have been expressed in previous letters to U.S. EPA from our colleagues at Tri-TAC and the National Association of Clean Water Agencies. We respectfully request that U.S. EPA register as pesticides all consumer products that by design contain substances that function as pesticides that can end up in our sewer systems and waterways.

BACWA is a joint public powers authority representing 55 public utilities that collect and treat municipal wastewater. Our membership includes large metropolitan facilities such as East Bay Municipal Utility District, the City and County of San Francisco, Central Contra Costa Sanitary District, East Bay Dischargers Authority, and the City of San Jose. Our members come from the nine counties that surround the San Francisco Bay. Many of our member agencies also manage potable water treatment, distribution systems, and biosolids residual programs.

In November 2008, EPA opened a public review and comment period on a petition for rulemaking to require formal registration of all products containing nanoscale silver under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). We agree with

the petitioners—nanosilver products should be regulated as pesticides. There is no technical reason for U.S. EPA to decline to use its FIFRA authorities "to prevent unreasonable adverse effects on the environment" from use of these products. Regulating nanosilver as a pesticide will require specific tests and data that will better inform the U.S. EPA as to whether these products should be on the market.

We appreciated U.S. EPA's decision to regulate silver ion-generating products like the Samsung "Silver Wash" washing machine. This decision recognized our concerns about the potential water quality impacts of residential pesticide uses—and affirmed U.S. EPA's responsibility to regulate releases of biocidal silver into the environment.

Silver is highly toxic to aquatic life at low concentrations, is persistent, and can bioaccumulate in some aquatic organisms, such as clams. Due to concerns about bioaccumulation and the placing of strict silver effluent limits in discharge permits, publicly-owned wastewater treatment works (POTWs) have implemented pollution prevention programs to identify and reduce silver discharges to sanitary sewer systems. These programs have been very successful in reducing POTW influent and effluent silver concentrations. These programs have also reduced silver concentrations in biosolids, ensuring that silver will not limit options for biosolids reuse.

Ordinary use of nanosilver products can result in silver releases to municipal wastewater treatment systems. For example, releases occur when silver-impregnated fabrics are laundered, when silver-containing plastics are washed, and when silver-containing personal care products are washed off. When Benn and Westerhoff (2008) measured silver releases from washing nanosilver-impregnated socks, they found that some products lost silver so quickly most of the nanosilver in these products would be washed into the municipal wastewater system during the products' lifetimes.[1] Widespread use of household products that release silver ions into sanitary sewer systems could increase silver concentrations in POTW influents and effluents, potentially leading to adverse effects on the nation's waterways.

A well-respected San Francisco Bay Area scientist, Dr. Samuel Luoma, recently reviewed the environmental risks from nanosilver products.[2] Dr. Luoma spent most of his career at the U.S. Geological Survey's (USGS's) Menlo Park office, where he oversaw a long-term research study that demonstrated adverse impacts of POTW silver discharges on clams in San Francisco Bay. In the 1980s and 1990s, our member agencies made substantial (multi-million dollar) investments in process improvements and programs to control commercial and industrial silver discharges. Past silver

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[1] Benn, T. M. and P. Westerhoff (2008). "Nanoparticle silver released into water from commercially available sock fabrics." *Environmental Science & Technology* **42**(11): 4133-9.

[2] Luoma, Samuel N. (2008). *Silver Nanotechnologies and the Environment: Old Problems or New Challenges?* Woodrow Wilson International Center for Scholars, Project on Emerging Nanotechnologies. Publication PEN 15. September.

discharges to POTWs came primarily from developing photographs and X-ray films. Our members have controlled these discharges, which are now phasing out as photographers and medical offices transition to digital technologies. Dr. Luoma's USGS research team documented the dramatic recovery of South San Francisco Bay clam populations that occurred as a result of the silver discharge reductions we achieved.[3]

We have enclosed a copy of Dr. Luoma's review, which we request U.S. EPA carefully consider. Highlights of Dr. Luoma's findings include (from the report's Executive Summary):

- "Aside from releasing silver, the toxicity, bioaccumulative potential and persistence of nanosilver materials are just beginning to be known. But enough is known to be certain that risks must be investigated."

- "Nearly one-third of nanosilver products on the market in September 2007 had the potential to disperse silver or silver nanoparticles into the environment."

- "The mass of silver dispersed to the environment from new products could be substantial if use of one product, or a combination of such products, becomes widespread. Traditional photography established a precedent for how a silver-based technology that was used by millions of people could constitute an environmental risk. Release of silver to waste streams when photographs were developed was the primary cause of silver contamination in water bodies receiving wastes from human activities, and of adverse ecological effects where studies were conducted."

- "Risk assessments will require information about mass loadings to the environment. Such information is not currently available. Neither government reporting requirements nor product information is sufficient to construct reliable estimates of mass discharges from these new nanosilver technologies, but the potential exists for releases comparable to or greater than those from consumer usage of traditional photography."

Using available information (which is acknowledged to be limited), Blaser et al. (2008) attempted a rough estimate of the environmental releases that are currently occurring in Europe from selected nanosilver products.[4] Luoma (2008) completed a similar rough

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[3] Hornberger, M. I., S. N. Luoma, et al. (2000). "Linkage of bioaccumulation and biological effects to changes in pollutant loads in South San Francisco Bay." *Environmental Science and Technology.* **34**: 2401–2409; Brown, C. L., F. Parchaso, et al. (2003). "Assessing toxicant effects in a complex estuary: a case study of effects of silver on reproduction in the bivalve, Potamocorbula amurensis, in San Francisco Bay." *International Journal of Human and Ecological Risk Assessment.* **9:** 96–119.

[4] Blaser, S. A., M. Scheringer et al. (2008). "Estimation of cumulative aquatic exposure and risk due to silver: Contribution of nano-functionalized plastics and textiles." *Science of the Total Environment* **390** (2-3): 396-409.

estimate, using the South San Francisco Bay as an example (copies of both papers are enclosed).[5] Both of these estimates employed Blaser et al.'s estimates of the efficiency of silver removal by POTWs (both assumed that 80% of silver-containing wastewaters are treated sufficiently to remove 90% of the silver).[6] On the basis of our POTW management experience, we recommend that U.S. EPA consider the removal efficiency estimate of Blaser et al. as more realistic than the estimate employed by Benn and Westerhoff, who made the unrealistic assumption that 99.8% of silver is removed by wastewater treatment and that all silver-containing wastewaters flow to a wastewater treatment facility.

Wastewater treatment processes at POTWs commonly employ nitrifying bacteria to oxidize ammonium ions to nitrites and nitrates. This "nitrification" is critical to biological nutrient removal. In two related studies, Okkyoung Choi and colleagues found that nanosilver particles less than 5 nanometers in diameter are uniquely toxic to nitrifying bacteria.[7] (Copies of both papers are enclosed). These studies emphasize the importance of careful management of nanosilver products to ensure that discharges do not interfere with POTW treatment processes.

Taken together, this scientific information suggests that if nanosilver product use becomes common, wastewater discharges could reach levels not seen in the last two decades—and could have adverse impacts on our wastewater treatment process as well as on the quality of our effluent and biosolids. POTWs are subject to National Pollutant Discharge Elimination System (NPDES) permits under the Clean Water Act. These permits include toxicity limits and may also include quantitative effluent limitations for silver. Exceeding these limitations has serious consequences, including monetary fines and penalties and the risk of citizen lawsuits. Under California law, our members are liable for daily Mandatory Minimum Penalties should violations of their discharge permits occur.

It is distressing to POTWs to observe the increasing prevalence of household products that use silver nanoparticles and other toxic chemicals for general antimicrobial purposes. POTWs are proud of our history of taking effective actions that reduce discharges of toxic pollutants to the environment. While POTWs have the authority to regulate industrial and commercial sources of silver and other toxic pollutants, we have

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[5] Luoma, Samuel N. (2008). *Silver Nanotechnologies and the Environment: Old Problems or New Challenges?* Woodrow Wilson International Center for Scholars, Project on Emerging Nanotechnologies. Publication PEN 15. September.

[6] This estimate recognizes that some silver-containing wastewaters (e.g., swimming pool water) are discharged to storm drains, where the water flows directly to surface waters without any type of treatment.

[7] Choi, O., K. K. Deng, et al. (2008). "The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth." *Water Research* **42**: 2066-2074; Choi, O. and Z. Hu (2008). "Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria." *Environmental Science & Technology* **42**(12): 4583-8.

little or no control over the discharge of pollutants from the thousands of households we serve. Silver is a toxic metal that cannot degrade in the environment and registered for use as a pesticide in numerous products. To allow the unrestricted usage of products that intentionally release silver into the environment would be an irresponsible neglect of the principles of environmental sustainability that should strongly influence such decisions.

In summary, BACWA recommends that U.S. EPA require the registration of all products in which silver nanoparticles function as biocides. We also ask that during the registration process, U.S. EPA obtain data on the quantities of silver ions and nanosilver particles released to the sewer system from ordinary use—including washing—of nanosilver-containing products. These data should be used to impose necessary restrictions to ensure that water quality standards are not exceeded and that discharged nanosilver does not interfere with biological wastewater treatment processes. Since nanosilver products may contain relatively high silver concentrations, measures to ensure proper disposal of treated items at end of life should be considered.[8] We request that U.S. EPA consult with the Food and Drug Administration regarding similar products that may not be regulated by U.S. EPA and consider these products in environmental risk assessments and risk management decisions. Efficacy claims for all products should also be carefully evaluated. In addition, ongoing monitoring and reporting of unit sales and silver releases should be required to determine whether registration should be continued or canceled.

Thank you for your consideration of our comments on the petition for rulemaking requesting that U.S. EPA regulate nanoscale silver products as pesticides. If you have any questions, please contact me at 510 547-1174 or mpla-cleanwater@comcast.net.

Sincerely,

Michele Pla
Executive Director
Bay Area Clean Water Agencies

Enclosures

1. Luoma, Samuel N. (2008). *Silver Nanotechnologies and the Environment: Old Problems or New Challenges?* Woodrow Wilson International Center for Scholars, Project on Emerging Nanotechnologies. Publication PEN 15. September.

[8] Product silver concentrations can exceed 1,000 parts per million (ppm) (see Benn and Westerhoff, 2008, enclosed), which is twice California's hazardous waste standard for total silver content (500 ppm, see California Code of Regulations, Title 22, Chapter 11, Article 3).

2. Benn, T. M. and P. Westerhoff (2008). "Nanoparticle silver released into water from commercially available sock fabrics." *Environmental Science & Technology* **42**(11): 4133-9.
3. Blaser, S. A., M. Scheringer, et al. (2008). "Estimation of cumulative aquatic exposure and risk due to silver: Contribution of nano-functionalized plastics and textiles." *Science of the Total Environment* **390** (2-3): 396-409.
4. Choi, O. and Z. Hu (2008). "Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria." *Environmental Science & Technology* **42**(12): 4583-8.
5. Choi, O., K. K. Deng, et al. (2008). "The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth." *Water Research* **42**: 2066-2074.

Note: Enclosures 2 through 5 are copyrighted materials that cannot be posted in the public docket. These have been submitted via email to Nathanael R. Martin. We request that U.S. EPA provide these materials to its staff that are reviewing these comments.

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Woodrow Wilson International Center for Scholars
Project on Emerging Nanotechnologies

THE PEW CHARITABLE TRUSTS

SILVER NANOTECHNOLOGIES AND THE ENVIRONMENT:

OLD PROBLEMS OR NEW CHALLENGES?

Samuel N. Luoma

Project on Emerging Nanotechnologies is supported by THE PEW CHARITABLE TRUSTS

PEN 15
SEPTEMBER 2008

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SILVER NANOTECHNOLOGIES AND THE ENVIRONMENT:
OLD PROBLEMS OR NEW CHALLENGES?

Samuel N. Luoma

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FOREWORD

Dr. Samuel Luoma has given us an excellent description and analysis of the science of silver and nanosilver. His paper raises many questions for policy makers. Its subtitle, "Old Problems or New Challenges," is appropriate, because the subject of the paper is both. Metals are among the oldest of environmental problems. Lead, silver and mercury have posed health hazards for thousands of years, and they are as persistent in the environmental policy world as they are in the environment. Nanotechnology is a new challenge, but the scope of the policy issues it presents is as broad and difficult as the technology itself.

As the paper makes clear, there is much we do not know about the environmental pathways of nanosilver, its environmental effects and its impact on human health. However, as Luoma notes, ionic silver, a form of nanosilver, when tested in the laboratory, is one of the most toxic metals to aquatic organisms. Ionic silver is being used now in washing machines and other products. The need for research is urgent. The major experiment being conducted now is to put nanosilver products on the market, expose large numbers of people and broad areas of the environment and then wait and hope that nothing bad happens. This is a dangerous way to proceed. The experiments need to come before the marketing so that damage can be avoided rather than regretted.

Dr. Luoma employs a useful environmental framework, starting with sources of nanosilver, then dealing with its pathways in the environment and ending with receptors and impact. Policy makers use the same model, only in reverse. They start with the question of whether there is an impact, then analyze the environmental pathways and finally deal with whether and how to control the sources.

The impacts are the policy starting point, so the fact that less than 5 percent of the money being spent on nanotechnology by the U.S. government is being spent to study health and environmental impacts demonstrates a questionable sense of priorities. That is the major policy issue. However, there is also a need for surveillance and reporting. Workers, consumers, lakes and streams are being exposed to nanosilver and, while the experimentation is unfortunate, society should at least learn from it. People working with nano need to be monitored, and key aspects of the environment exposed to nanosilver should be investigated. Some of this will be done by scientific institutions, public and private. However, some of it, for example, medical monitoring of workers, may require government regulation.

There is another connection between regulation and impacts, one that is less well recognized. As Luoma notes, "the formulation and form of a nanoparticle has great influence on the risks that it poses." Silver in different nanoproducts can be in the form of silver ions, silver colloid solutions or silver nanoparticles. The nanosilver can come in different shapes, have different electrical charges and be combined with other materials and coated in different ways. Each of these factors, as well as others, affects toxicity and environmental behavior. If we are to discover how these different factors impact nanosilver's toxicity and environmental behavior, it will only be by testing a large number of specific products that have different characteristics. This is not the kind

of testing that will be done by universities or government laboratories. The only way that these data are likely to be collected is by requiring manufacturers to test their nanosilver products. Although it would be neater and more efficient to mandate testing of nanoproducts only after we knew how particular product characteristics influence toxicity, in reality the only way we are going to gain this knowledge is by first mandating that manufacturers test their nanoproducts for health and environmental effects.

As Dr. Luoma describes, little is known about the environmental pathways of nanosilver. The policy challenge that emerges from his description is how to match the antiquated air-water-land basis of existing laws with the inherently cross-media nature of the problem. Nanosilver can go from a manufacturing plant to a waste-treatment plant to sludge to crops to the human-food chain. It is considered primarily a water problem in the environment but primarily an air problem in the workplace. Like climate change, acid rain and genetically modified crops, nanosilver is a problem that fits poorly into the old boxes of the existing regulatory system.

One reason a cross-media approach is necessary is that it allows a policy maker to consider which sources of pollution or exposure are most important and which can be most efficiently and effectively addressed. Current efforts to address nanosilver are using the few cross-media tools the United States has—specifically, the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and the Toxic Substances Control Act (TSCA). The two acts are quite different in several ways. TSCA is broad and potentially could cover most nanomaterials. FIFRA, by contrast, is limited to pesticides, which are defined to include antimicrobials. However, since nanosilver is used primarily as an antimicrobial, most nanosilver products may come under FIFRA. The acts also differ in the degree of public protection and product oversight they offer. FIFRA is quite stringent and puts the burden of proof for safety on the manufacturer. TSCA is riddled with loopholes and puts the burden of proof on the U.S. Environmental Protection Agency (USEPA) to show that a substance is harmful.

The extent to which USEPA will use FIFRA to regulate nanosilver products is uncertain. The agency has reversed a previous decision and decided that the Samsung Silver Wash washing machine, which emits silver ions into every wash load, must be registered as a pesticide. However, that decision was drawn in the narrowest possible terms, making it clear that the agency has not decided to require registration for the numerous other commercial products that are using nanosilver as an antimicrobial. Several environmental groups have joined to petition the agency to require registration for the other products, but the agency has not yet responded. Meanwhile, USEPA's San Francisco regional office has imposed a fine on a company selling computer keyboards and mouses coated with nanosilver on the grounds that the products should have been registered under FIFRA. However, it is not clear that this represents a general policy, either in Region IX or for USEPA as a whole. It seems more likely that this is a one-time case, perhaps intended as a signal to discourage widespread use of nanosilver coatings.

There is no legal or technical reason why FIFRA could not be used to regulate most nanosilver products. However, an initiative to do so would require dollars and personnel, and both are in short supply within USEPA. More important, it is not clear that the agency would

want to launch a major regulatory initiative in the waning days of a fervently antiregulatory administration. The Bush administration has significantly reduced USEPA's budget, and the current USEPA administrator seems willing to be guided by White House directives when it comes to major decisions.

Dr. Luoma, while conceding that little is known about the quantities or concentrations of nanosilver releases from various sources, states that "industrial releases associated with manufacturing the nanosilver that goes into the consumer products or production of the products themselves is likely to be greater than consumer releases." If this is so, it will be necessary to look to the Clean Water Act (CWA) and the Clean Air Act (CAA) to control nanoreleases. This is unfortunate, because at present there are major technical obstacles to using these acts. Practical methods for monitoring nanosilver in air and water and methods for controlling releases to air and water are lacking.

The monitoring problem is especially difficult because it is not clear what should be monitored. Simple measures of quantity, mass or concentration that are used for other pollutants are probably not adequate for monitoring nanomaterials. As noted above, there are more than a dozen characteristics of nanosilver that are relevant to its health and environmental impact. There is no technique for ambient monitoring all these characteristics, nor is it clear how they can be narrowed to a manageable number for monitoring. Without the ability to monitor, it is difficult to regulate using the CAA or CWA, although some version of "good management practices" might be used until monitoring methods are developed.

Silver is an old problem, and nanosilver is a new challenge. The scope of the new challenge is not yet clear because it is unclear how much nanosilver will be used as an antimicrobial and because new uses are likely to be discovered. Regardless of the scope of the nanosilver problem, it underscores the need for new approaches to oversight to deal with the new technologies and problems of the new century. Laws and institutions shaped in the mid-20th century are not likely to succeed in addressing 21st-century problems. Developing a new approach to oversight and regulation may be the biggest challenge of all.

—J. Clarence Davies
Senior Advisor, Project on Emerging Nanotechnologies
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ABOUT THE AUTHOR

Dr. Samuel N. Luoma leads science policy coordination for the John Muir Institute of the Environment at the University of California, Davis. He is also editor-in-chief of *San Francisco Estuary & Watershed Science* and is a scientific associate with The Natural History Museum in London, United Kingdom (UK). Prior to this, he was a senior research hydrologist with the U.S. Geological Survey. He served as the first lead scientist for the CALFED Bay-Delta program, an innovative program of environmental restoration of over 40 percent of California's watershed, and water management issues for 60 percent of California's water supply. His specific research interests are studying the bioavailability and effects of pollutants in aquatic environments and developing better ways to merge environmental science and policy. He is an author on more than 200 peer-reviewed publications. He wrote *Introduction to Environmental Issues*, published in 1984 by Macmillan Press, and, with coauthor Philip Rainbow, recently finished *Metal Contamination in Aquatic Environments: Science and Lateral Management*, which will be released by Cambridge University Press in October 2008. He is an editorial advisor for the highly respected *Marine Ecology Progress Series*, and on the editorial board of *Oceanologia.* He was a W. J. Fulbright Distinguished Scholar in the UK in 2004 and is a Fellow of the American Association for the Advancement of Science. His awards include the President's Rank Award for career accomplishments as a senior civil servant, the U.S. Department of Interior's Distinguished Service Award and the University of California at Davis Wendell Kilgore Award for environmental toxicology. He has served nationally and internationally as a scientific expert or advisor on issues at the interface of science and environmental management, including sediment quality criteria (U.S. Environmental Protection Agency SAB Subcommittee), Bioavailability of Contaminants in Soils and Sediments (Canadian National Research Council, 1987, U.S. National Research Council subcommittee, 2000–2002), mining issues (United Nations Educational, Scientific and Cultural Organization; Global Mining Initiative), selenium issues, environmental monitoring and metal effects.

EXECUTIVE SUMMARY

Nanomaterials with silver as an ingredient raise new challenges for environmental managers. Potentially great benefits are accompanied by a potential for environmental risks, posed both by the physical and chemical traits of the materials. We need not assume that because nano is new, we have no scientific basis for managing risks, however. Our existing knowledge of silver in the environment provides a starting point for some assessments, and points toward some of the new questions raised by the unique properties of nanoparticles. Starting from what we know about silver itself, this report identifies 12 lessons for managing environmental risks from nanosilver. These lessons help set the stage for both the research strategy and the risk management strategy.

- Silver itself is classified as an environmental hazard because it is toxic, persistent and bioaccumulative under at least some circumstances. Aside from releasing silver, the toxicity, bioaccumulative potential and persistence of nanosilver materials are just beginning to be known. But enough is known to be certain that risks must be investigated.

- Nearly one-third of nanosilver products on the market in September 2007 had the potential to disperse silver or silver nanoparticles into the environment. The silver content of these materials appears to vary widely. Reports on the form of the silver in these products are generally inconsistent and do not follow scientific definitions. Guidelines for concentrations and formulations of reduced toxicity might offer opportunities for regulation.

- The mass of silver dispersed to the environment from new products could be substantial if use of one product, or a combination of such products, becomes widespread. Traditional photography established a precedent for how a silver-based technology that was used by millions of people could constitute an environmental risk. Release of silver to waste streams when photographs were developed was the primary cause of silver contamination in water bodies receiving wastes from human activities, and of adverse ecological effects where studies were conducted.

- Risk assessment(s) will ultimately be necessary for at least some products employing silver nanomaterials. Risk assessments will require information about mass loadings to the environment. Such information is not currently available. Neither government reporting requirements nor product information is sufficient to construct reliable estimates of mass discharges from these new nanosilver technologies, but the potential exists for releases comparable to or greater than those from consumer usage of traditional photography.

- There are no examples of adverse effects from nanosilver technologies occurring in the environment at the present. But environmental surveillance is a critical requirement for a future

risk management strategy, because silver nanoproducts are rapidly proliferating through the consumer marketplace. Few if any methodologies exist for routine environmental surveillance of nanomaterials, including nanosilver. Monitoring silver itself, in water, sediment or biomonitors, could be a viable interim approach until methods specific to the nanomaterial are developed.

- Silver concentrations in natural waters, even those contaminated by human activities, range from 0.03 to 500 nanograms/liter (ng/L). Even substantial proliferation of silver nanotechnologies is unlikely to produce pollutant concentrations in excess of the ng/L range. Environmental surveillance methodologies must be capable of detecting changes in concentrations within this range.

- Toxicity testing should focus on realistic exposure conditions and exposures in the ng/L range, and not on short-term acute toxicity. Sensitive toxicity tests and environmental case studies have shown that silver metal is toxic at concentrations equal to or greater than 50 ng/L. One well-designed study on nanosilver has shown toxicity at even lower concentrations to the development of fish embryos. Even though the potential concentrations in contaminated waters may seem low, environmental risks cannot be discounted.

- The environmental risks from silver itself can be mitigated by a tendency of the silver ion to form strong complexes that are apparently of very low bioavailability and toxicity. In particular, complexes with sulfides strongly reduce bioavailability under some circumstances. It is not yet clear to what extent such speciation reactions will affect the toxicity of nanosilver. If organic/sulfide coatings, or complexation, in natural waters similarly reduce bioavailability of nanosilver particles, the risks to natural waters will be reduced. But it is also possible that nanoparticles shield silver ions from such interactions, delivering free silver ions to the membranes of organisms or into cells (a "Trojan horse" mechanism). In that case, an accentuation of environmental risks would be expected beyond that associated with a similar mass of silver itself. The Trojan horse mechanism is an important area for future research, especially for nanosilver.

- The environmental fate of nanosilver will depend upon the nature of the nanoparticle. Nanoparticles that aggregate and/or associate with dissolved or particulate materials in nature will likely end up deposited in sediments or soils. The bioavailability of these materials will be determined by their uptake when ingested by organisms. Some types of silver nanoparticles are engineered to remain dispersed in water, however. The persistence of these particles, on timescales of environmental relevance (days to years), is not known.

- Silver is highly toxic to bacteria, and that toxicity seems to be accentuated when silver is delivered by a nanoparticle. Dose response with different delivery systems and in different delivery environments has not been systematically studied.

- When the ionic form is bioavailable, silver is more toxic to aquatic organisms than any other metal except mercury. But no comparable body of information is available for nanosilver. Uptake of nanomaterials by endocytosis appears to explain toxicity in higher organisms (marine invertebrates). Other portals for uptake across the membrane (e.g., protein transporters or pores) also appear to exist. Risk of toxicity may be accentuated if endocytosis delivers a bundle of potential silver ions, in the form of a nanosilver particle, to the interior of cells, where it can release silver ions in the proximity of cell machinery. Signs of silver stress in such circumstances should include lysosomal destabilization and generation of reduced oxygen species. Nanosilver may also affect development of embryos and other aspects of reproduction at environmentally realistic concentrations. All these mechanisms deserve further investigation.

- Silver is not known as a systemic toxin to humans except at extreme doses. Silver itself is taken into the body but seems to largely deposit in innocuous forms in basement membranes, away from intracellular machinery, where it could cause damage. Whether nanosilver particles have a similar fate in human tissues is unknown. One study showed that once inside cells, silver nanoparticles are more toxic than particles composed of more innocuous materials such as iron, titanium or molybdenum. There is controversy about whether silver treatment of wounds might slow growth of healthy cells, at least in some circumstances. Indirect effects have not been adequately investigated. Examples of areas needing further research include toxicity to bacteria on the skin from chronic silver exposure (as in silver-laden clothing or bedding materials) and effects to or in the gut from chronic or "colloidal silver," which contains dispersed nanoparticles.

Thus, existing knowledge provides a powerful baseline from which to identify research priorities and to begin making scientifically defensible policy decisions about nanosilver. Adequate resources for research, interdisciplinary collaboration, new ways to integrate interests of diverse institutions and linkage between research and decision making are necessary if we are to fully exploit the potential benefits, and limit the unnecessary risks, of this rapidly proliferating technology.

I. INTRODUCTION

Silver has been known since antiquity for its many properties useful to humans. It is, however, an element of many faces. It is used as a precious commodity in currencies, ornaments and jewelry. It has the highest electrical conductivity of any element, a property useful in electrical contacts and conductors. Its chemical traits allow uses ranging from dental alloys to explosives. The way it reacts to light (photochemistry) was manipulated to develop traditional photography. Claims of medicinal properties have followed silver since the time of Hippocrates, the father of medicine. Most important, silver has long been used as a disinfectant; for example, in treating wounds and burns, because of its broad-spectrum toxicity to bacteria and, perhaps, to fungus and viruses, as well as its reputation of limited toxicity to humans.

On the other hand, silver is designated by the U.S. Environmental Protection Agency (USEPA) as a priority pollutant in natural waters. The inclusion of silver on the 1977 priority pollutant list[1] (still in effect) means it is one of 136 chemicals whose discharge to the aquatic environment must be regulated. This designation is based upon silver's persistence in the environment and its high toxicity to some life forms when released to natural waters from photographic facilities, smelters, mines or urban wastes. The dichotomies in the long history of human contact with silver, its use as a biocide and its designation as an environmental toxin stem from the complexities of silver's behavior in the environment. Notably, silver has not been studied in depth compared to other heavy metal pollutants.

The environmental implications of silver are of increasing interest because new technologies are rapidly emerging that carry with them elements of silver's complex nature and history. Recent advances in nanoscience have uncovered novel properties in materials at the nanoscale (materials typically smaller than 100 nanometers [nm] in one critical dimension). Nanotechnologies use this knowledge to synthesize, modify and manipulate nanomaterials. The resulting products have unique physical, chemical and biological characteristics[2] (Text box 1).

Commercial products that generate silver ions or contain nanosilver are one of the most rapidly growing classes of nanoproducts. Most of the emerging products exploit silver's effectiveness in killing a wide range of bacteria (thus the term *broad-spectrum biocide*), including some of the strains that have proven resistant to modern antibiotics. What is new is that advances in nanotechnology allow heretofore unavailable methods of manipulating silver so that it can be readily incorporated into plastics, fabrics and onto surfaces (Henig, 2007). Perhaps most important, nanosilver particles deliver toxic silver ions in large doses directly to sites where they most effectively attack microbes. And the technology appears to be cost-effective.

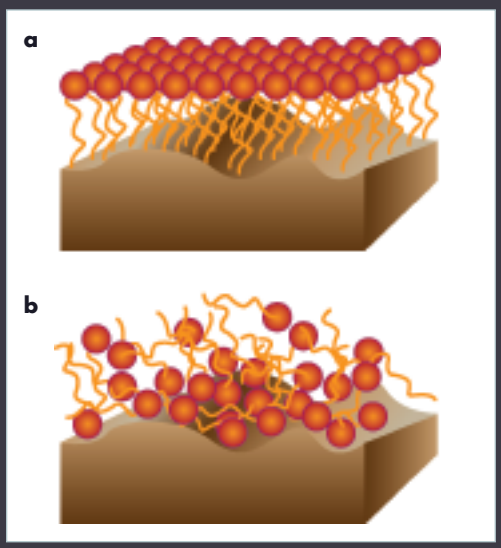
To date, silver is used in more manufacturer-identified consumer products than any other nanomaterial.[3] Hundreds of nanosilver products are currently on the market, and their number is growing rapidly. Searching Google for "nanosilver" yielded 3.5 million hits in October 2007, more than half of which were for nanosilver products. But most of the data on products

TEXT BOX 1. Nanoparticles, nanomaterials and nanotechnology

Nanoscience is defined by the Royal Society and Royal Academy of Engineering, United Kingdom (2004) as the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at a larger scale. The academy defines ***nanotechnologies*** as the design, characterization, production and application of structures, devices and systems by controlling shape and size at the nanometer scale. Terms such as *nanoparticle* and *nanomaterial* are used inconsistently and/or interchangeably in commercial, and even scientific, literature. The official standards organization of the United Kingdom, the British Standards Institution (BSI), has recently provided some formal definitions. The BSI defines the ***nanoscale*** as between 1–100 nm. A ***nanomaterial*** is defined by BSI as having one or more external dimension in the nanoscale (BSI, 2007). A ***nanoobject*** is a discrete piece of material with one or more external dimensions in the nanoscale. A ***nanoparticle*** is a nanoobject with all three external dimensions in the nanoscale. A ***manufactured nanoparticle*** is a solid entity with size from approximately 1 nm to 100 nm in at least two dimensions that has been produced by a manufacturing process. ***Nanoproducts*** are those to which nanoparticles "are intentionally added, mixed, attached, embedded or suspended."

Nanomaterials are of interest because they have novel properties and functions attributable to their small size. First, they have greater surface area when compared to the same mass of material in larger particles (Royal Society and Royal Academy of Engineering, 2004). Larger surface area per unit mass can make materials more chemically reactive. Some materials, such as gold, are inert in their larger particles but are reactive as nanoparticles. Second, quantum effects can begin to dominate the behavior of matter at the nanoscale, particularly the smaller nanomaterials. The result is development of unique optical, electrical and magnetic behaviors. Materials can be produced that are nanoscale in one dimension (very thin surface coatings), in two dimensions (nanowires and nanotubes) or in all three dimensions (nanoparticles). The feature common to the diverse activities characterized as "nanotechnology" is the tiny dimensions on which they operate. The ability to systematically control the distribution of particles or to manipulate matter on this scale is what has driven new advances in nanotechnology (see Figure 1).

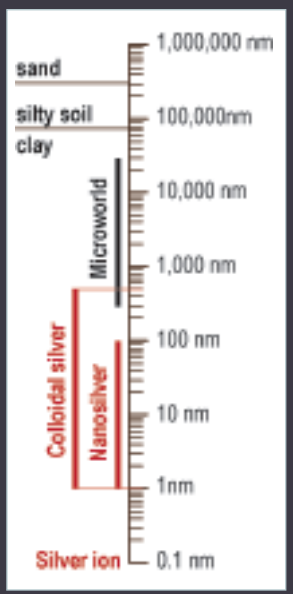
FIGURE 1



Nanotechnology deals with nanoparticles aligned in an ordered manner as subunits in a functional system. (a) An example of nanoparticles systematically aligned on a surface, as they might be when used electronic communications. (b) An example of unorganized nanoparticles on a surface. Even though they are of appropriate size, they will not be functional if they lack order. In that case, the term nanotechnology does not apply. (*Wired* magazine, December 2005. Available at http://www.wired.com/science/discoveries/news/2005/12/69772)

In this report, s*ilver* refers to any specified form of the element silver or to the mixture of forms that occur in that particular environmental setting. The ***silver ion*** is the most fundamental entity of silver. It is an atom in which the number of electrons is one less than the number of protons, creating a positively charged cation (thus written Ag^+). The ionic radius of a silver ion is ~0.1 nm (Figure 2). A silver ion is not usually considered a particle, and its surface area is irrelevant in the context we are considering here. But ions are highly reactive because they

FIGURE 2



A comparison of different scales: ion, 0.1 nm; nano, 1–100 nm; micro, 1000–100,000 nm; colloidal, 1–1000 nm. Clay, silt and sand are classifications of the size of particles in soils.

are charged. An ion can associate with other ions, but the ion itself is inherently persistent and cannot be destroyed. Complex interactions blur precise boundaries among macromolecules, nanoparticles, colloids and particles (Lead and Wilkinson, 2007). But here we refer to ***silver nanomaterial*** or ***nanoparticles*** as made up of many atoms of silver in the form of silver ions— clusters of metallic silver atoms and/or silver compounds (e.g., Balogh et al., 2001) engineered into a particle of nanoscale size. High surface area is a particularly important property for nanosilver, because it increases the rate at which silver ions are released. A ***nanosilver particle,*** in contrast to an ion, is not necessarily persistent. Particles can dissolve or disaggregate, for example, which means they fundamentally transform and will not necessarily re-form, losing the properties of a particle. Thus, silver ions and silver nanoparticles are fundamentally different. The term *colloid* is often also applied to silver. A ***colloid*** (Figure 2) is defined as a particle anywhere in the wide range between 1 nm and 1,000 nm. That is, a colloid may or may not be a nanoparticle. Aquatic colloids can also be defined by their physical behavior. Colloids are held in suspension in natural waters, aiding transport of any material associated with them (colloid-facilitated transport). Particles are larger and tend to settle to the bottom if undisturbed. In this report, ***nanosilver*** and ***silver nanoparticle*** refer to a nanoparticle or a nanocoating comprised of many atoms of silver engineered for a specified use. Silver nanoparticles are usually engineered to release silver ions, which are the source of antibacterial activity.

using or containing nanosilver are anecdotal. There are no reporting requirements or official government registries for such products. A recent survey used the Internet in an attempt to identify products that employed the emerging silver technologies (Fauss, 2008). The 240 products that were identified in this survey, which concluded in September 2007 were limited to those that advertised their use of nanosilver. Nevertheless, the range of products and proposals is impressive.[4]

A number of products use nanosilver in medicine and water purification. Because of their potential to address long-standing and difficult problems, such uses are expected to grow rapidly. For example, a number of new uses of nanosilver coatings on medical devices seem to reduce infection rates (Gibbons and Warner, 2005). Highly organized microbial communities called biofilms are the leading culprit in many life-threatening infections and are particularly difficult to eliminate once established within the human body. Nonliving surfaces that penetrate the body or are implanted within the body are prone to supporting growth of microbial biofilms. Nanosilver coatings on the surfaces of artificial joints, pacemakers, artificial heart valves and Teflon sleeves for the repair of blood vessels and catheters, among other devices, have great potential to prevent these deadly microbial growths. A number of companies are now marketing urinary, dialysis and other catheters with such coatings. Silver-impregnated bandages and dressings are the treatment of choice for serious burns and are now available over-the-counter for the local treatment of wounds and elimination of pathogenic bacteria (Vermuelen et al., 2007). Ceramic filters that incorporate a coating of nanosilver for water purification are proposed as a small-scale solution to the drinking water purification prob-

lems of billions of people (Lubick, 2008).

The greatest growth, however, is in consumer products utilizing nanosilver to fight bacterial growth in circumstances where the benefits are less clear. The Wilson Center website[3] shows that nanosilver can be found in tableware, chopsticks, food preparation equipment and food storage containers. Colloidal silver was apparently sprayed on surfaces of the Hong Kong underground transport system as a public health measure, a move that is also being considered by the city of London.[5] Silver ion generators are commercially available that disperse the ion into the waters of machines used to wash clothes and dishes, and nanosilver is appearing in appliances like refrigerators, vacuums, air-filtration devices and computer keyboards. Nanosilver is being spun into thread, incorporated into plastics, impregnated into filters and painted onto product surfaces. Products that can be purchased with nanosilver ingredients include slippers, socks, shoe liners and women's undergarments; outerwear and sportswear; and bedding materials like comforters, sheets and mattress covers. There's even a nanosilver baby mug and pacifier. Nanosilver can be found in personal-grooming kits, female-hygiene products, beauty soaps, cleansers and fabric softeners. It is used as a preservative in cosmetics, where it is combined with nanoparticles of titanium dioxide. Nanosilver sprays or mists can be purchased on the Internet to disinfect and deodorize surfaces in kitchens, bathrooms and baby clothes. Claims of general health benefits from drinking silver solutions also are heard. One company's website recommends ingesting a teaspoon of silver colloid per day "to help maintain health," and one tablespoon four times per day to "help fortify the immune system." Another website[6] claims that "the number of people using colloidal silver as a dietary supplement on a daily basis is measured in the millions."

FIGURE 3

A "business black sock" impregnated with nanosilver as shown by the Wilson Center's Project on Emerging Nanotechnologies. The manufacturer states that "the nano particles of silver will help maintain healthy, bacteria-free feet even when you have been at the office all day." And "one nanomaterial that is having an early impact in health care products is nano-silver. Silver has been used for the treatment of medical ailments for over 100 years due to its natural antibacterial and anti fungal properties. The nano-silver particles typically measure 25 nm which means that a relatively small volume of silver gives an extremely large relative surface area, increasing the particles contact with bacteria or fungi, and vastly improving its bactericidal and fungicidal effectiveness." Available at http://www.nanotechproject.org/inventories/consumer/browse/products/5430/

Risks, efficacy or even necessity are not always obvious for many of the consumer products. Many of these products bring nanosilver directly into contact with the human body (Henig, 2007). Others have the potential to disperse (nano) silver to the environment during and after their use.

No known cases exist of people or the environment being harmed specifically by nanomaterials or nanosilver. The absence of cases could reflect limited experience with nanomaterials or lack of knowledge about what effects to expect. For this reason, unease over poor understanding of the potential health and environmental risks from nanomaterials is growing. Such concerns were expressed by the Royal Society and the Royal Academy of Engineering in the United Kingdom (2004), the European Commission's Action Plan for Nanotechnology (2005), USEPA's Nanotechnology White Paper (USEPA, 2007) and a growing number of editorials in trade and popular publications. Recent scientific analyses identify the grand challenges in understanding risks from nanomaterials (Maynard et al., 2006). Other articles suggest strategies for developing the necessary knowledge about risks (Owen and Handy, 2007; Oberdörster et al., 2005) and address managing risks within existing legal frameworks (Davies, 2007). All these analyses cite the almost complete lack of scientifically based knowledge about risks from materials with the unique physical properties that accompany particles this small and emphasize the importance of balancing risks and benefits.

The purpose of this review is to address environmental risks from nanomaterials containing or composed of silver, including those that intentionally release silver ions. The central question involves a trade-off between *unknown* risks and *established* benefits for society (Colvin, 2003). For nanosilver, that situation is complicated by limited understanding of both benefits and environmental implications. In addition, the rapid growth of emerging silver technologies has created an atmosphere of confusion about the science that unnecessarily adds to the incoherence of the dialogue.

Understanding of implications of silver metal in the environment provides an important context for understanding the implications of nanosilver. At least part of the risk from nanosilver will stem from release of silver ions (Blaser et al., 2008). The existing knowledge about the metal provides a place to begin a systematic analysis of the potential environmental risks from the nanomaterials, and can at the least be used to highlight important investigative needs. Therefore we will first address the environmental effects of silver metal. Implications of increasing silver metal releases to the environment are the first order of risks emerging from silver nanotechnology. Implications of releasing silver in nanoparticle form could add to (or subtract from) the risks from silver metal contamination. Nanosilver implications could differ from silver metal implications in some ways, but the concepts that guide assessment of those risks should have many areas of similarity. While there are uncertainties about implications, there is enough evidence from laboratory tests with both silver metal and nanosilver to be certain that potential adverse effects from silver nanotechnologies must be investigated (Davies, 2007).

Human society has repeatedly faced challenges with chemicals whose immediate benefits were clear and whose potential risks were unknown. In some cases, commercial applications moved forward in a "grand experiment" with nature. Substantial and ongoing environmental or human-health damage were the result in examples that include asbestos, long-lived pesticides like DDT, persistent chemicals like dioxin and polychlorinated biphenyls and the climatic changes now attributable to combustion of fossil fuels. Such mistakes have contributed both to degradation of the environment and to an erosion of public trust in the traditional institutions assigned to protect the environment (Löfsted, 2005). The social atmosphere is now one where uncertainty about risks from a new technology can "affect the trajectory of commercialization" (Colvin, 2003). If unanticipated adverse effects are discovered, or the perception of such effects grows, opportunities could be lost for substantial benefits to society from even those aspects of the technologies that are relatively benign (Davies, 2007). It is imperative that the scientific community begin to aggressively address the issue of risks from new technologies, such as the emerging silver technologies and the other nanotechnologies of which they are a part (Maynard et al., 2006), in order to "strike the balance between the harm that could be done by proceeding with an innovation and the harm that could be done by not proceeding" (Davies quoted in Henig, 2007).

Our knowledge is not adequate to conduct a full risk assessment for nanosilver. But the risk assessment paradigm (Suter, 2006) provides a structure within which to analyze potential for nanorisks. The next section of this report addresses what is known about silver metal. **Section III** addresses the unique implications of using and releasing silver in nanoparticle form. The report concludes with recommendations for next steps.

II. FATE AND EFFECTS OF SILVER IN THE ENVIRONMENT

HISTORY OF SILVER TOXICITY

One of the important uncertainties about nanosilver technologies is the contradiction between the long history of intimate human use of silver and its classification as a persistent and toxic pollutant. Silver (Ag) is a chemical element with an atomic weight of 47. It is rare (67th in abundance among the elements) and thus a precious metal that has long been handled as currency and worn as jewelry. Silver implements have long been associated with eating and drinking. It is used in the highest-quality cutlery ("silverware") and was used in storage vessels for water and wine in civilizations dating back to the Phoenicians (lead was also used in this way by the Romans). Many such uses are thought to reflect its powers to prevent decay of foodstuffs. The long history of human contact with bulk silver includes no obvious negative side effects on human health, an argument sometimes used to imply that the likelihood that significant environmental impacts will occur from the new silver technologies is low.

Silver's use in medicine also has a long history. Around 1884, the German obstetrician C. S. F. Crede introduced l% silver nitrate as an eye solution to prevent infections in babies born of mothers with gonorrhea (Eisler, 1996). Silver nitrate eye drops are still a legal requirement for newborn infants in some jurisdictions (Chen and Schleusner, 2007). Silver compounds were used extensively to prevent wound infection in World War I, and silver was found in caustics, germicides, antiseptics and astringents, presumably as a disinfectant. With the advent of more selective antibiotics like penicillin and cephalosporin, most medicinal uses of silver declined. A mixture of silver and sulfa drugs (e.g., silver sulfadiazine cream) remains the standard antibacterial treatment for serious burn wounds.

A cursory historical analysis seems to point toward silver as a benign disinfectant; however, complexities appear upon more careful examination and as uses in medicine grow. Hollinger (1996) predicted that "as the intentional utilization of silver in pharmaceutical preparations and devices increases, subtle toxic effects of silver may be predictable and expected." He cited delayed wound healing, absorption into systemic circulation and localized toxicity to cells as areas needing investigation.

Episodes of environmental toxicity resulting from silver pollution are rare (Rodgers et al., 1997); however, a more careful examination shows evidence of potential ecological significance. Ionic silver is one of the most toxic metals known to aquatic organisms in laboratory testing (e.g., Eisler, 1996). Silver persists and accumulates to elevated concentrations in water, sediments, soils and organisms where human wastes are discharged to the environment. Well-documented examples also exist where silver contamination in water and mud corresponds strongly with ecological damage to the environment (Hornberger et al., 2000; Brown et al., 2003).

SOURCE-PATHWAY-RECEPTOR-IMPACT

The complex behavior of silver contributes to the contradictory conclusions about its effects on human health and the environment:

- Different uses release silver in different forms and different quantities.

- Quantifying the mass of silver ultimately released to the environment (or to the body) from a given use is necessary to evaluate the risk associated with that use. Complex geochemical reactions determine how those releases translate to silver concentrations in food, water, sediments, soils or topical applications.

- Silver concentrations in the environment determine impacts. But concentrations in the environment are low compared with those of many other elements, adding to the challenge of obtaining reliable data on environmental trends. Similarly low concentrations of nanosilver might be expected where waste products from its uses are released, although nanoparticle-specific transport and accumulation mechanisms might also be expected.

- The environmental chemistry of silver metal influences bioavailability and toxicity in complex ways (where bioavailability is defined by the physical, geochemical and biological processes that determine metal uptake by living organisms). The influence of environmental chemistry on nanosilver bioavailability is a crucial question.

- Determining potential for toxicity is more complex than usually recognized. The type of test can have a strong influence on conclusions about silver's potential as an environmental hazard. Organisms are most sensitive when tested using long-term chronic toxicity tests and/or exposure via the diet (see later discussion). But such data are rare.

- Once inside an organism, silver may be highly toxic, but not necessarily so. The processes that influence internal toxicity (or biological detoxification) might be one of the most important considerations in determining risks from nanosilver.

- Ecological risk is ultimately influenced by toxicity at the cellular and whole-organism level, but that risk will differ from species to species.

In discussing how to evaluate risks from nanotechnologies in general, Owen and Handy (2007) referred to a "source-pathway-receptor-impact" as a unifying principle for risk assessment. Progressively evaluating each link in the source-pathway-receptor-impact chain is a systematic way to address potential risks from an activity. The questions to follow apply that approach to silver metal and nanosilver materials.

SOURCES: HOW MUCH SILVER IS RELEASED TO THE ENVIRONMENT BY HUMAN ACTIVITIES?

Silver is mined from the earth from deposits of the mineral argentite. Argentite occurs in lead-zinc and porphyry copper ores in the United States, and in platinum and gold deposits in South Africa (Eisler, 1996). Silver is also extracted during the smelting of nickel ores in Canada. Silver production from mining and smelting increased steadily through the last century. In 1979, silver was used mainly in photography (39%), electrical and electronic components (25%), sterling ware (12%), electroplated materials (15%) and brazing alloys and solders (8%). Recycling of the silver from such products is another major source of the metal. In 1990, the estimated world production of silver was 14.6 million kilograms (kg) (Eisler, 1996). In

2007, approximately 20.5 million kg of silver were mined worldwide (USGS, 2008).

Emissions to the environment of metals such as silver are influenced by commercial and industrial activities as well as by environmental regulations. Silver emissions peaked between the late 1970s and the early 1980s in the historically developed world (e.g., Europe, North America, Japan, Australia and New Zealand). After the 1980s, emissions began to decline in these jurisdictions with the passage and implementation of environmental legislation such as like the Clean Water Act in the USA in the 1970s. Industries and cities were forced to remove or capture contaminant materials, including silver, preventing their disposal to the atmosphere and especially to local water bodies. Many heavy industries, which release the largest masses of such contaminants, moved from the historically developed to the rapidly developing countries during the same period. More recently, use of silver in photography (one of the largest commercial uses) declined with the advent of digital photography (USGS, 2008).

In contrast to the historically developed world, developing countries whose economies are rapidly expanding (primarily in east and central Asia) have not kept pace with environmental regulations as their industries expand and demand for various products increases. Specific data on silver emissions to the environment in these jurisdictions are not available, but estimates for other contaminants are probably good indicators that silver emissions are increasing at a rapid rate (e.g., Jiang et al., 2006).

In 1978, most silver emissions came from smelting operations, photographic manufacturing and processing, the electronics industry, plating and coal combustion, along with a variety of smaller-scale domestic uses (Table 1; Eisler, 1996; Purcell and Peters, 1998). Because silver is so rare, the quantities produced and released to the environment seem small on a product-by-product basis, especially when compared with mass discharges of other metals. In 1978, the estimated loss of silver to the environment in the United States was 2.47 million kg, or 2,470 metric tons. Of that, about 500 metric tons were carried into waterways in runoff from soils, and 1,600–1,750 metric tons went to landfills (Purcell and Peters, 1998). While the silver in landfills is largely constrained and immobile and the silver in runoff is mostly part of the natural background, the most environmentally damaging silver was probably that going to the aquatic environment from human wastes, estimated to be about 250 tons per year (Eisler, 1996; Purcell and Peters, 1998). Table 1 accounts for the major sources of this silver release, including waste-

TABLE 1. MASSES OF SILVER DISCHARGED TO THE AQUATIC ENVIRONMENT FROM DIFFERENT SOURCES IN 1978.

Silver disposal to aquatic environments, 1978: USA	Kg silver per million people	Total discharges (metric tons)
Waste-treatment facilities	350	70
Photo developing	325	65
Photo manufacture	270	54
Metals production	20	1–10

1978 data from Purcell and Peters, 1998.

treatment facilities, photographic developing and photographic manufacturing and mining or manufacturing (Purcell and Peters, 1998). These loads were responsible for elevating concentrations of silver in the aquatic environment above the natural background level and for causing ecological effects from discharges that are discussed later.

There is substantial evidence that silver discharges declined considerably in the United States after the 1980s (e.g., Purcell and Peters, 1998; Sanudo-Wilhelmy and Gill, 1999; Hornberger et al., 2000). For example, the mass of silver discharged in 1989 and in 2007 from a well-studied publicly owned treatment works (POTW) at Palo Alto, California, in South San Francisco Bay has been compared (Hornberger et al., 2000) with the discharge from the entire urban area surrounding South Bay (Table 2). The silver-per-person discharged from both sites in the 1980s was similar to the estimated average discharges from waste-treatment facilities per person nationally (350 mg per person per year [Table 1]). Major improvements in waste treatment were implemented by all the local POTWs around the South Bay, as they were nationally, during the 1980s and 1990s. Probably more important, silver recycling was initiated for local industries, and the use of silver in photography declined considerably. The mass of silver released to South Bay in the wastes declined more than tenfold as a result of these changes.

In 2006, when silver releases were 6 kg per year, inputs to the Palo Alto POTW were 65 kg per year. This reflects the ability of sewage-treatment works to extract silver from effluents and retain it with an efficiency of about 90 percent (Lytle, 1984; Shafer et al., 1999). In studies of POTWs, 19–53 percent of the incoming silver associated with colloidal particles during treatment was removed by advanced filtration, indicating filtration is crucial to effectively removing silver. Despite the efficiency of silver removal, concentrations in the discharges to natural waters are correlated with silver in the incoming wastewater (Shafer et al., 1998). Discharges of silver both in the 1980s and 2007 (Table 2) were from POTWs that treated their effluents. The more silver that

TABLE 2†. DISCHARGES OF SILVER INTO SOUTH SAN FRANCISCO BAY FROM ONE WASTE TREATMENT FACILITY (POTW) AND FROM THE COMBINED POTW DISCHARGES FROM THE SURROUNDING URBAN AREA (SILICON VALLEY) IN THE 1980S AND IN 2007.

Facility	Kg silver released per year	mg silver released per person	Concentration in Bay (ng/L)
Palo Alto* 1989	92	415	
2007	6	27	
Silicon Valley** 1980	550	275	26-189 (mean = 113)
2007	40	20	6

† Silver released per person was determined by dividing the total discharge by the number of people served by the waste-treatment facilities.

* Data from Hornberger et al. (2000) and P. Bobel, Palo Alto Environmental Protection Agency (unpublished).

**Data from Smith and Flegal (1993).

entered these facilities, the more silver was lost to the environment. Sewage treatment helps, but it is not a cure for environmental risk if incoming loads are large enough.

PATHWAYS: WHAT ARE THE CONCENTRATIONS OF SILVER IN THE ENVIRONMENT?

Dispersal of silver into the environment is not necessarily an ecological risk. The concentration, environmental fate and ecological response are also important. The background concentration of every metal in soil and water is determined, in part, by erosion from Earth's crust. If the element is more abundant, its concentration is higher in undisturbed waters. Silver is an extremely rare element in the Earth's crust, which means that background concentrations are extremely low. Thus, the addition of only a small mass of silver to a water body from human activities will result in proportionally large deviations from the natural conditions.

Concentrations of most trace metals in waters are reported in parts per billion (ppb) or micrograms per liter (µg/L). Concentrations of silver are always in the pptr (parts per trillion) range, reported as ng/L. Table 3 and Figure 4 illustrate silver concentrations in different types of waters around the world at different times. The lowest concentrations of dissolved silver are found in the open oceans, where concentrations range from 0.03–0.1 ng/L (Ranville and Flegal, 2005). However, silver concentrations changed from 0.03 ng ng/L in 1983 to 1.3 ng/L in 2002 in surface waters from the open ocean off Asia (Ranville and Flegal, 2005). The distribution of the contamination followed a pattern that suggested wind-blown pollution aerosols were being carried to sea from the Asian mainland by the prevailing westerly winds. Ranville and Flegal (2005) concluded that the change reflected atmospheric inputs from the rapidly developing Asian continent, although the specific sources are not known. It was surprising that pollution inputs were sufficient to raise offshore silver concentrations by 50-fold. The change demonstrates the sensitivity of water bodies to changes in human inputs of silver, and suggests that local hot spots of substantial

TABLE 3. TYPICAL SILVER CONCENTRATIONS IN WATER BODIES OF THE WORLD.

Location	Silver concentration (ng/L)
Pristine Pacific Ocean	0.1 surface waters 2.2 deep waters
Oceans off Asia (2005)*	Changed from 0.03 to 1.3 in 20 years
South San Francisco Bay (2003)*	6
South San Francisco Bay 1980 1990**	 113 27–36
California Bight (nearest human inputs)***	4.5
Rivers in urbanized Colorado (2000)****	5–22
Effluents of Colorado POTWs (2000)****	64–327
"Protective" Ambient Water Quality Criteria	1,900–3,200

* Ranville and Flegal, 2005; **Smith and Flegal, 1993;
***Sanudo-Wilhelmy and Flegal, 1992; ****Wen et al., 2002.

TEXT BOX 2. How silver ions combine with other chemicals

The positively charged free silver ion (Ag^+) has a strong tendency to associate with negatively charged ions in natural waters in order to achieve a stable state. The negatively charged ions, or ligands, can occur in solution or on particle surfaces. In natural waters, five main inorganic, anionic ligands compete for association with the cationic metals: fluoride (F^-), chloride (Cl^-), sulphate (SO_4^{2-}), hydroxide (OH^-) and carbonate (CO_3^{2-}). Ligands also occur on dissolved organic matter. Equilibrium constants, also termed stability constants, define the strength of each metal-ligand complex. These constants can be used in models to predict silver speciation in solution or distribution among ligands. Speciation is driven by the combination of:

a) The strength of silver association with the ligand (if silver associates more strongly with one ligand than another, it is more likely to associate with the first); and

b) The abundance of the ligands. Ligands that are more abundant are more likely to associate with and bind the silver.

These properties work in combination. For example, at some point, an extremely abundant but weaker-binding ligand might outcompete a stronger binding but rare ligand. The specific complexes or precipitates of silver cannot be directly measured at the low concentrations in natural waters, but because their chemistry is quantitatively well-known, the distribution among inorganic ligands can be calculated from chemical principles with reasonable certainty. The outcome of the competition among ligands is more difficult to calculate from first principles if dissolved organic matter is present, because those ligands take many forms.

Speciation is typically more variable in freshwater than in seawater, because of greater variability in ligand concentrations. The composition of seawater is relatively constant; only concentrations of organic materials vary much. The silver chloro complex will always dominate in solution in seawater, although sulfide complexes may also occur (Cowan et al., 1985; Adams and Kramer, 1999).

silver contamination are likely to be developing on the Asian continent.

Dissolved silver concentrations have long been recognized as a characteristic marker of sewage inputs. In the late 1980s, there was a silver concentration gradient extending from the ocean off San Diego, California, into Mexican waters (Sanudo-Wilhelmy and Flegal, 1992). The source was the Point Loma waste discharge, which consolidates most waste from San Diego. Urbanized bays and estuaries showed similar levels of contamination. Concentrations up to 27–36 ng/L were determined to occur broadly in San Francisco Bay and San Diego Bay in the late 1980s (Flegal and Sanudo-Wilhelmy, 1993). In waters from the lower South San Francisco Bay, silver concentrations were as high as 189 ng/L in the late 1970s and early 1980s (e.g., Luoma and Phillips, 1988), when silver inputs from industry and waste-treatment facilities were elevated (Table 2). After upgrades of the treatment facilities, closure of a large photographic facility and instigation of silver recycling for the smaller photo processors (P. Bobel, personal communication), silver concentrations in the South Bay dropped to 2–8 ng/L (Squires et al., 2002). The important lesson from these studies is that when human activities mobilize sufficient silver, the contamination is readily detectable in large bodies of water. If inputs are controlled, the contamination may recede.

Fewer silver studies are reported for freshwaters than for marine or estuarine waters. Where data are available, concentrations are comparable to those found in urbanized estuaries, (Figure 4; Wen et al., 2002), but concentrations can vary widely. Concentrations

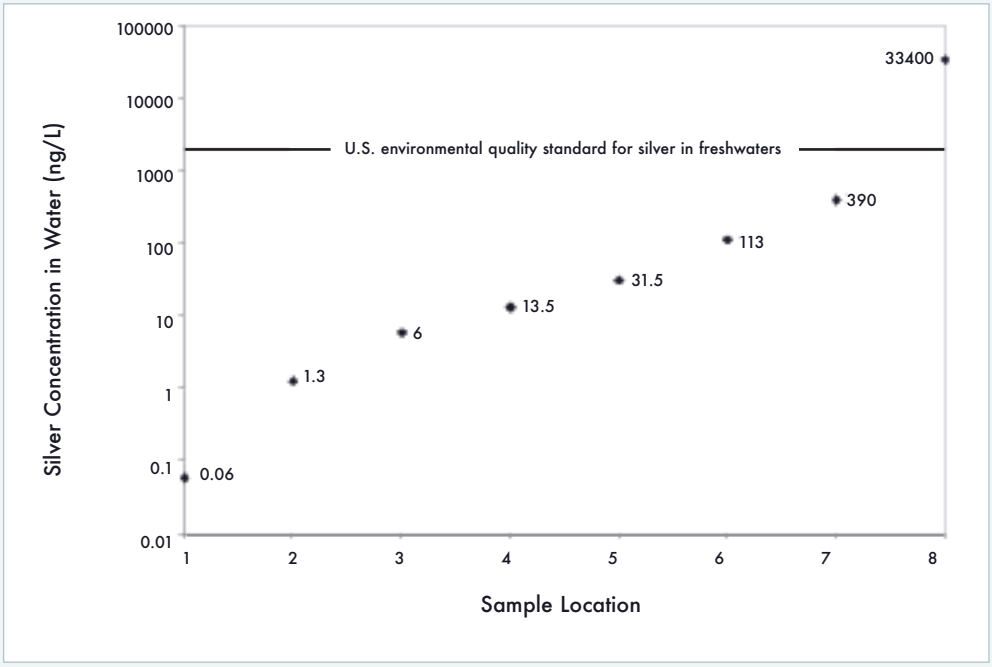
in effluents are much higher than are those in natural waters. Concentrations in urban effluents from three cities ranged from 64 to 327 ng/L; effluents from a photographic facility at the time (before 2000) contained 33,400 ng/L (Wen et al., 2002).

Environmental water quality standards provide guidelines for the upper limits for acceptable metal concentrations in water bodies. These regulatory limits are based on data from toxicity tests and on assumptions about dilution after discharge into the water body. They are enforced by comparing observations of environmental concentrations to the guideline. For example, North American ambient water quality criteria suggest that aquatic life will not be harmed if silver concentrations do not exceed 1,920–3,200 ng/L in streams and coastal waters (USEPA, 2002). The European Union does not list silver among its 33 designated "priority hazardous pollutants."[7] It is interesting that these regulatory guidelines, where they exist, are much higher than ever were found in even the most contaminated open waters during the period of greatest silver contamination (Figure 4), which is another contradiction in the silver story.

PATHWAYS: FORMS AND FATE

The form of silver in water is governed by the complex chemistry of the element and the nature of the water. Silver is among the met-

FIGURE 4. SILVER CONCENTRATIONS IN DIFFERENT WATERS GRAPHED ON A LOG SCALE



Sample Locations: Silver concentrations in different waters graphed on a log scale. 1. Surface waters of the Atlantic and Pacific Oceans in 1983 (median). 2. Surface ocean water off Asia in 2002 (one value). 3. Waters of San Francisco Bay in 2002 (median). 4. Streams in urban areas (median). 5. Waters of urbanized estuaries (San Francisco and San Diego Bays) in the early 1990s (median). 6. Average concentration near the southern terminus of San Francisco Bay in the early 1980s (median). 7. Effluents from cities in the 1990s (median). 8. Photographic effluents (one value). Data from Ranville and Flegal (2005), Smith and Flegal (1993), Squires et al., (2002), Flegal and Sanudo-Wilhelmy (1993), Wen et al., (2002) and USEPA (2002).

als that act as positively charged cations (Ag^+) in water. To achieve stability, the charged ion rapidly associates with negatively charged ions called ligands (Text box 2). A very small proportion of the total dissolved silver will also remain as the free ion (Ag^+), depending upon the concentrations of the different negatively charged ligands and the strength of the silver ion binding with each ligand. This distribution of silver between its ionic (Ag^+) and its ligand-bound forms is termed *speciation*. Silver forms especially strong complexes with free sulfide (-SH) ligands, and with the sulfide ligands that occur within organic materials dissolved in natural waters (Adams and Kramer, 1999). It is possible for dissolved sulfide and/or organic matter to complex essentially all the dissolved silver in freshwaters based on the relative abundance of (-SH) compared to silver concentrations (Adams and Kramer, 1999). Speciation has great influence on how much silver is available to affect living organisms. For example, silver complexed to a free sulfide is essentially unavailable for uptake by organisms.

Silver also interacts strongly with the chloride anion, but the interactions are complex. In freshwater, chlorides occur in low concentrations. But if there are more atoms of chloride present than atoms of silver, the silver quickly precipitates or falls out of solution as a solid compound, silver chloride. This compound is unavailable for uptake by organisms. The strong reactions of silver with free sulfides, dissolved organic materials and chloride can drive free silver ion concentrations to minuscule values in most freshwaters (Adams and Kramer, 1999).

Chloride occurs in very high concentrations in seawater (and thus in coastal waters and estuaries) because the salt in seawater is dominated by sodium chloride. Chemical principles predict that when chloride concentrations increase to about 10 percent of full-strength seawater, multiple chloride ions react with each silver ion to form complicated complexes that hold silver in solution (Cowan et al., 1985). The silver is more mobile and more reactive than it would be in fresh water because its most abundant form is an extremely strong silver-chloro complex (Cowan et al., 1985; Reinfelder and Chang, 1999).

Because silver accumulates in sediments, risk assessments must always consider the long-term implications of accumulation, storage, remobilization, form and bioavailability from sediments. The strongest reaction for silver, in both freshwater and saltwater, occurs with the negatively charged ligands in sediments (Luoma et al., 1995). Because ligands are so abundant in sediments and hold silver strongly, geochemical reactions tend to bind more silver ions to particulate matter compared to silver in solution. Between 10,000 and 100,000 ions of silver bind with particulate matter for every ion that remains in solution. Thus, concentrations on particulate matter containing organic material can be 10,000 times higher in sediments than in water (Luoma et al.,1995). Where silver concentrations in contaminated waters range from 25–100 ng/L (Table 2), silver concentrations in the sediments in the same locations range from 0.5–10 μg/g dry weight.

The availability of oxygen in sediments influences the form of silver bound to the particles (Text box 3). Strong complexes with organic material appear to predominate at the sediment surface, where oxygen is usually present and sulfides are rare (Luoma et al., 1995). Deeper within the sediments, where

oxygen is absent, silver associates with sulfide in an extremely stable form that is characterized by its lack of solubility in weak acids like hydrochloric acid (HCl) (Berry et al., 1999). Organically complexed silver is also present in many anoxic sediments, as evidenced by the presence of HCl-soluble silver (Luoma et al., 1995).

RECEPTOR: IN WHAT FORMS IS SILVER BIOAVAILABLE?

Toxicity is ultimately determined by the dose or exposure that a living organism receives. That is why environmental risk assessments and risk management formally consider both exposure and toxicity. Bioavailability is defined by the silver that is taken up by an organism from passing water over its gills or ingesting food, sediments or suspended material. Bioavailability is the sum of silver taken up from all these sources. Silver must penetrate the tissues of an organism before it can be toxic, so the bioaccumulated concentration is an indicator of the dose of silver to which an organism has been exposed.

The biological systems that transport materials across the boundary between an organism and its environment are complex.

TEXT BOX 3. Effect of sediment chemistry on bioavailability of silver from sediments

The presence or absence of oxygen has a strong influence on the form of silver in sediments. Oxygen is typically present in the water column of most natural waters. The contact of this oxygenated water with the sediment surface creates an oxygenated sedimentary surface layer. But deeper in the sediment, the oxygen is consumed by microorganisms faster than it diffuses into the sediments from the water column. All of the oxygen is used up, and the sediment becomes anoxic (without oxygen). The depth of the junction between the oxygenated zone and the anoxic zone can vary from mm to many cm depending upon the conditions in the sediment.

In the absence of oxygen, negatively charged free sulfide ions become abundant in most sediments. In the oxygenated zone of the sediments, silver is bound largely to organic materials. In the absence of oxygen, at least some of the silver becomes bound to sulfides (Berry et al., 1999). It is argued that if the number of available sulfide bonds (i.e., the molar concentration) exceeds the number of silver atoms, silver should not be bioavailable; it should be innocuous (Berry et al., 1999). Sulfides are orders of magnitude more abundant than silver in anoxic sediments—so low that bioavailability should usually be the case in much of the sediment column based upon this concept. And experiments convincingly show that bioavailability and toxicity of silver are greatly reduced in well-mixed, fully anoxic sediment. i.e., sediment with no oxidized surface layer (Berry et al., 1999).

The complexity is that almost all higher order animals require oxygen. Even animals that live within anoxic muds have mechanisms or behaviors that assure that they have contact with the oxygenated part of the sedimentary environment. If those organisms ingest particles and/or carry water across their gills from the oxygenated zone, silver will be bioavailable. If their contact is with particles from the anoxic zone, silver will be much less bioavailable. The exact outcome is thus highly dependent upon the nature of the sediment and how each organism experiences that sediment.

Field observations consistently show higher silver bioaccumulation in sediments contaminated with silver, whether or not the sediments are anoxic in the subsurface layers (e.g., Hornberger et al., 2000; Luoma et al., 1995). Laboratory experiments that allow animals to feed in sediments that contact oxygenated water at the surface also show that silver is bioavailable (e.g., Lee et al., 2000). There remains some controversy about how silver bioavailability is affected by the presence of anoxic sediments. Nevertheless, it is clear that silver bioavailability from sediments must be included in any assessment of risks.

The surfaces of cells and the surface lining of biological tissues are surrounded by a membrane system that must prevent unwanted substances from entering the cell and regulate entry of essential substances. Ion transporters are proteins that are selectively designed to take up essential ions based upon their metal charge and size, as well as their coordination and ligand preferences (Veltman et al., 2008). Nonessential metals such as silver are taken up by the transporters to the extent they mimic the characteristics of an essential ion. Silver ions are probably transported by a carrier system that controls the cell's concentration of sodium and/or copper (Bury and Wood, 1999). Silver uptake by the transporters (its bioavailability) is strongly influenced by the form of silver in the environment. One form favored for uptake by the transporters is ionic silver (Ag^{+}), because its properties are most similar to those of sodium (Na^{+}) and copper, which is transported as Cu^{+1}. Precipitated silver chloride, dissolved complexes between silver and sulphide or organic complexes are not recognized by these transporters (Bury et al, 1999; Hogstrand and Wood, 1998; Bianchini et al., 2002). Thus, precipitation or complexation in water almost completely inhibits silver bioavailability. Some authors have concluded that the bioavailability of dissolved silver in freshwaters, in general, will be low because reactions with sulfide and dissolved organic materials are so predominant (Ratte, 1999; Hogstrand and Wood, 1998).

Complexation with chloride in seawater does not eliminate bioavailability, however. Even though almost no free silver ion is present in seawater, rapid uptake of silver is observed (Engel et al., 1981; Luoma et al., 1995). Microscopic plants at the base of oceanic food webs (phytoplankton) bioaccumulate silver from marine waters to concentrations 10,000 to 70,000 times higher than those found in the water (Fisher et al., 1994). Uptake rates and the degree of silver bioconcentration by these organisms are exceeded only by mercury among the metals. This assures that high concentrations of silver will occur at the base of food webs wherever silver contamination occurs in estuaries, coastal waters or the ocean.

Bioaccumulation of silver from solution by marine invertebrates is also faster than for other trace metals, following the order:

silver>zinc>cadmium>copper>cobalt>
chromium> selenium (Wang, 2001).

When the soluble chloro complex is dominant, silver is taken up less readily than the free silver ion, for example, in fish or in invertebrates like mussels (Hogstrand and Wood, 1998). But uptake of the chloro complex is far greater than uptake when sulfide complexes are dominant or when silver is precipitated into its insoluble silver chloride form. In addition, at salinities greater than ~10 percent seawater, it is less likely that complexation with organic and soluble sulfides will reduce toxic effects; the extreme abundance of chloride ions makes the chloro complex a strong competitor for binding.

Luoma et al. (1995) concluded that, unlike in freshwater, the chemical reaction that dominates silver speciation in estuarine and marine environments also maintains substantial bioavailability. The result may be that the environmental "window of tolerance" for silver contamination in estuaries might be relatively narrow because of the strong responses of organisms to relatively small changes in exposure concentration.

TABLE 4. ASSIMILATION OF SILVER IN VARIOUS AQUATIC SPECIES.

Organism	Percent of ingested silver assimilated from food	Half-life of loss from body (days)	Transfer efficiency from food (percent)*
Invertebrate predators:			
Snail	69		
Snow crab	90	148	57
Marine oysters	44	70	11
Polychaete worms	24–34		
Marine clams	22–43	47–70	5–7
Marine mussels	14–23	13–35	1–3
Sea urchin	34	47	2
Zooplankton	17–43	1–3	0.2
Fish predators	9	30	0.04

*Uptake and loss of silver differ among species, the combination of which affects the likelihood that each species will accumulate a higher concentration of silver in its body than was in its prey (trophic transfer efficiency). Higher assimilation from food means more silver is taken up from food; and longer half-lives mean the silver is held longer by the organism before loss. High assimilation and longer half-lives result in a high potential to accumulate high concentrations of silver. Data from Wang et al., 1996; Wang, 2002; Reinfelder et al., 1997; Wang and Fisher, 1999; Reinfelder et al., 1998; Griscom et al., 2002; and Cheung and Wang 2005).

Silver associated with particulate organic matter can be taken up when those particles are eaten by animals. Digestion may generate free silver ions in digestive fluids of low pH. Silver may also combine with proteins and amino acids within the complex fluids of the digestive tract, or gut (Luoma, 1989). The gut membrane is capable of transporting amino acids and clusters of molecules of colloidal size, termed *micelles*. Silver will accompany these molecules as they are transported into the cells of the organism. Similarly, mechanisms exist to engulf particles and either digest them within the cell (intracellular digestion) or transport them through the membrane (endocytosis).

In the past decade researchers have quantified the importance of obtaining silver from contaminated food (Wang et al., 1996; Wang, 2002). In general, diet is a more important route of uptake than is uptake from solution. But the exact contribution of diet to bioaccumulation depends upon the efficiency with which silver is taken up by the gut (termed *assimilation efficiency*). An organism that takes silver up efficiently from food and retains it for long periods before excreting it is most likely to accumulate a higher concentration in its tissues than was present in its prey. *Biomagnification* is the term used when a predator accumulates a chemical to a higher concentration than occurs in its prey. Contrary to conventional expectations, many invertebrates have high assimilation efficiencies, slow loss rates and a high potential to biomagnify silver (Table 4; Reinfelder et al., 1997; Wang and Fisher, 1999). For example, when ingesting the microscopic aquatic plants (phytoplankton) that are their typical food, clams (*Macoma petalum)* from San Francisco Bay will take into their tissues 39–49 percent of the silver they ingest and accumulate concentrations five to seven times higher than in the phytoplankton (Reinfelder et al., 1998). As a result, exposure via diet explains 40–95 percent of the silver bioaccumulation by these animals (Griscom et al., 2002). Invertebrate predators seem to be especially efficient at assimilating sil-

ver from food and accumulate very high concentrations of the metal from their prey (Table 4). Cheung and Wang (2005) showed that a predatory snail, *Thais clavigera*, assimilated 60 percent of the silver in the prey it eats. Models predicted accumulation of silver to very high internal concentrations in the snail predator. An accompanying study in Clear Water Bay, Hong Kong, showed that, indeed, predator snails had five to ten times more silver in their tissues than did their prey (Blackmore and Wang, 2004).

In contrast, assimilation efficiencies are relatively low, or loss rates are high, for other organisms, including zooplankton and at least some fish. Predatory fish, for example, are inefficient at taking up silver from food and will not accumulate higher concentrations than are in their prey (Hogstrand and Wood, 1998; Wang, 2002).

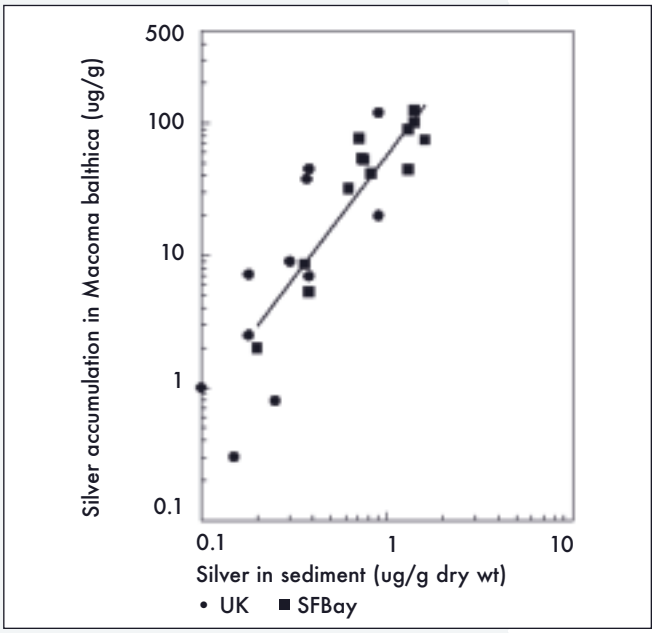
The tendency of silver to associate with sediments or to become associated with the complex particles in the water column does not necessarily eliminate bioavailability. Thus, sediments "store" a large reservoir of potentially bioavailable silver. Many animals also ingest sediments for food (Griscom et al., 2002), providing direct exposure to the bound silver. Invertebrates assimilate a lower percentage of silver from inorganic fractions of sediment than from the living or decaying material; nevertheless, the silver is bioavailable. Assimilation efficiencies of silver from bulk sediments, including inorganic materials, range from 11–21 percent in the clam *Macoma petalum.* Silver assimilation efficiencies from living plant materials by this clam are 38–49 percent. Ingestion of sediment was a more important source of silver than was uptake from water whether the source was sediments or plant materials (Griscom et al., 2002).

The form of the silver in sediments is important to bioavailability (Text box 3). Bioaccumulation of silver from sediments can be predicted from the concentration of silver extracted from the sediment with HCl, the weak acid that does not extract the silver from sulfides (Figure 5). This suggests organically bound silver is bioavailable, but sulfide-associated silver is not. A remarkably strong relationship occurs between weak acid extractable silver and silver concentrations in clams from mudflats (*Macoma spp.*), considering the diversity of conditions under which the data were collected. The figure also demonstrates that these clams bioaccumulate silver to higher concentrations than is found in the organic fraction of their sedimentary food.

IMPACT: TOXICITY OF SILVER

The inherent toxicity of silver determines its ranking as an environmental hazard, but the

FIGURE 5.



Silver concentrations in clams found in San Francisco Bay and the estuaries of southwest England correlated against silver concentrations in the sediments upon which the clams feed, measured in those sediments by extraction with a weak acid that eliminates silver sulfides (1N hydrochloric acid). Other extractions do not show a similar correlation.

definition of toxicity depends upon the organisms that are considered and the way toxicity is determined. It is well-known that silver is extremely toxic to bacteria. It is also among the most toxic of the metals to plants like phytoplankton, as well as to invertebrates and fish. Adverse ecological impacts have been observed in some well- studied instances of relatively moderate silver contamination in estuaries. However, silver is not especially toxic to humans or other mammals.

Factors such as the following influence the ability of silver to produce toxic effects:

- the ability be taken inside cells;
- the tendency to bind to biological sites that perform important functions;
- the degree to which the metal is excreted; and
- the degree to which the metal is sequestered in nontoxic forms inside cells.

Silver's history of use in medicine is tied to its antibacterial properties. A long history of study verifies that silver is a broad-spectrum and effective toxin to bacteria. The recent growth in uses of silver in the management of open wounds stems from the loss of effectiveness of many modern antibiotics because of the spread of antibiotic-resistant bacteria such as the staph bacterium (*Staphylococcus aureus)*.

Silver's antibacterial activity is strongly dependent upon the concentration of the silver ion. Silver nitrate dissociates readily, releasing free silver ions. Thus, silver nitrate has often been used in medical applications. Antimicrobial effects from other silver compounds are found only when the compounds are oxidized to release free silver ion. For example, bulk elemental silver, as in tableware or dishware, has antimicrobial activity only if oxidized silver species are present on the surfaces or within the silver metal. A higher surface area per unit mass will yield more oxidized silver.

Silver toxicity has been tested on many strains of bacteria. Silver inhibits the activity and growth of both gram-positive and gram-negative bacteria, as well as fungi (although fewer studies address the latter). There is less evidence that silver is toxic to viruses, despite some claims to the contrary. Silver is also toxic to strains of bacteria that can develop tolerance to other antibiotics (e.g., staph bacteria). For example, when bandages with and without "hydrocolloidal" silver were applied to human epithelium (isolated patches of reconstituted human skin), the silver-treated bandages killed gram-negative and gram-positive bacteria, including staph bacteria and antibiotic-resistant bacteria (Schaller et al., 2004). Atopic eczema (skin rash) can be related to or accompanied by colonization of the skin by staph bacteria. Gauger et al. (2003) compared the response of 15 patients to a silver-coated textile on one arm and cotton on the other for 7 days. They found a significantly lower number of the staph bacteria on the arm treated with the silver-coated textile during and at the end of the experiment.

Despite a large number of product studies like those above, the dose-response characteristics of silver toxicity to bacteria remains poorly understood. The concentration at which silver becomes toxic to the bacteria has not been studied carefully and is variable among experimental data that are available (Chopra, 2007). For example, two similar studies of the dose response of the pathogenic bacterium *Staphylococcus aureus* to silver showed thresholds of toxicity varying between 8 and 80 ppm (Chopra, 2007). Two other studies with another pathogenic bacterium, *Pseudomonas aeruginosa,* produced a similar range of toxicity of the

silver ion, from 8 to 70 ppm. The nature of the bacterial colony also influences the effectiveness of silver. Bjarnsholt et al. (2007) found that the bactericidal concentration of silver required to eradicate the bacterial biofilm was 10 to 100 times higher than that used to eradicate free-living bacteria. They concluded that the concentration of silver in many currently available wound dressings was much too low for treatment of chronic wounds infected by biofilms. Differences in silver delivery systems, different formulations of silver and different dressing materials also influence silver toxicity (Brett, 2006; Chopra, 2007).

Development of bacterial resistance to silver is less likely than the development of resistance to more selective antibiotics. The multiple mechanisms by which silver affects bacteria (Text box 4) make it more difficult for bacteria to manifest the multiple mutations necessary to produce resistant strains (Chopra, 2007). However there is no doubt that silver resistance can occur (Brett, 2006). Resistance to silver-based burn dressings has been reported, for example (Chopra, 2007). The genetic mechanisms of the resistance are not yet well-known. Dressings that release silver slowly are more likely to stimulate onset of resistance than are dressings that release high doses of bioavailable silver (Brett, 2006; Chopra, 2007).

Silver in any form is not thought to be toxic to the cardiovascular, nervous or reproductive systems of humans. Nor is silver considered to be a cancer-causing chemical (Drake and Hazelwood, 2005). Silver can be absorbed into the body through the lungs, gastrointestinal tract, mucous membranes of the urinogenital tract and the skin (Landown, 1996). If silver is ingested, the efficiency with which it absorbed is thought to be low (~10%; Drake and Hazelwood, 2005), although this may depend upon the form of silver ingested.

The limited occurrence of death from silver exposure or obvious signs of poisoning (systemic signs) in humans appears to reflect strong capabilities to sequester the metal in innocuous forms, often in tissues outside the functioning cells of organs (for more details see Text box 5). The most commonly reported response of humans to prolonged silver exposure is argyria or argyrosis. Both are characterized by pigmentation or discoloration of the skin, nails (argyria), eyes, mucous membranes or internal organs (argyrosis) by silver deposits (Text box 5). A skin color of gray, gray-blue or even black is symptomatic of these conditions.[8] Neither argyria nor argyrosis can be reversed, and both are incurable, although no obvious long-term health effects seem associated with either (Drake and Hazelwood, 2005).

Text box 4. Mechanisms of silver toxicity to bacteria

The mechanisms behind the biocidal action of silver are related to the interaction with thiol (sulfhydryl, –SH) groups in enzymes and proteins. Silver interferes with the functions that the protein normally performs when it attaches to such a ligand. Cellular respiration and transport of electrons across membranes are two examples of functions supported by enzymes with many sulfhydryl groups. Silver also inhibits DNA replication by interfering with DNA unwinding. In bacteria, silver induces oxidative stress at the cell wall, where many cellular functions are performed, affecting the bacteria's ability to respire and to maintain a balance of essential ions within the cell and thereby maintain an internal environment suitable for life. Thus, bacteria exposed to silver show inhibited growth, suppressed respiration and metabolism; they lose potassium and otherwise show suppressed transport of essential chemicals into and out of the cell membrane (Hwang et al., 2007).

Text box 5. Detoxification of silver

Detoxification of metals, including silver, is a normal process that has evolved in all organisms, presumably the result of evolving in the presence of metal ions naturally occurring in the Earth's crust. With silver, detoxification in humans appears to occur by precipitation of silver salts either as silver chloride, silver phosphate or silver sulfide within tissues. In people with argyria, the blue or gray skin discoloration is caused by the photoreduction to metallic silver during exposure to ultraviolet light (Eisler, 1996). Silver sulfide and silver chloride granules are deposited outside cells in the thin layer of connective tissue underlying the surface cells of many organs, termed the *basement membrane*. Macrophages, a type of white blood cell that takes up foreign material, also accumulate silver and prevent it from penetrating into cells (Baudin et al., 1994). Before storage as a stable mineral, silver first binds to proteins that contain a large proportion of sulfhydryl groups. The most common of these proteins are termed metal-specific binding proteins. These proteins then aggregate into the granular stored materials or are encased by lysosomes, the vesicles often used by the body to capture, hold or degrade foreign substances in an innocuous form. Silver deposits can be observed near peripheral nerves and the blood-brain barrier, but the deposits do not appear to have adverse effects on crucial membranes of the nervous tissue (Lansdown, 2007). If concentrations of a toxin get too high, lysosomes will break down and leak their toxins, however. The liver is an important organ for the synthesis of detoxifying proteins like metallothioneins, and that may be the reason silver tends to accumulate strongly in this organ. High concentrations of silver can also occur in the basement membrane of the digestive tract, which has a strong ability to accumulate, retain and eliminate the metal (Baudin et al., 1994).

In patients with argyria, deposits of silver are also found in the region of peripheral nervous tissues, small blood vessels (capillaries) or even near the blood-brain barrier (Lansdown, 2007). The silver in these regions is usually encased in a membranous vesicle (lysosomes) or as a nontoxic granule, preventing exposure to the more sensitive cellular machinery. Nevertheless, argyria appeared to be one reason for the curtailment of silver use once alternatives (antibiotics) were developed (Chen and Schleusner, 2007).

When toxicity does occur in humans, it is usually associated with exposure to a bioavailable form of silver and at very high doses. Exposure to metallic silverware poses no risk to human health because such products produce very little soluble silver or silver ions, for example. Acute symptoms of overexposure to silver nitrate are decreased blood pressure, diarrhea, stomach irritation and decreased respiration, but these require massive doses. Some chronic symptoms from prolonged intake of low doses of silver salts have been reported, including ulcers (Wadhera and Fung, 2005), fatty degeneration of the liver and kidneys and changes in blood cells (Drake et al., 2005).

Direct, systemic toxicity is not the only way that silver can affect human health. Hollinger (1996) predicted that subtle toxic effects would begin to appear as silver was increasingly employed in medical applications. He suggested that the implications of uptake of silver into the circulatory system (e.g., through ingestion or through wounds in the skin) should be further investigated. He also suggested that effects on delayed wound healing and possible local silver toxicity in specific organs be considered. A more recent study reported toxic effects of silver nitrate on the types of human cells involved in wound healing, i.e., fibroblasts and endothelial cells (Hidalgo and Dominguez, 1998). Prolonged exposure to silver nitrate produced dose-dependent cell loss at silver concentrations

of 0.4–8.2 ppm. The mechanisms of cell toxicity were similar to those of toxicity in bacteria, namely, depletion of energy reserves typical of effects on cell metabolism and effects on DNA synthesis. In a 2007 review of the literature on delayed healing, Atiyeh et al. (2007) concluded that "recent findings, however, indicate that the (silver) compound delays the wound-healing process and that silver may have serious cytotoxic activity on various host cells." However, they described the literature on silver as often contradictory with regard to both wound infection control and wound healing. Brett (2006) emphasized that such effects were not consistent with a long history of clinical successes in using silver bandages to treat burn victims. Atiyeh et al. (2007) suggested that the goal of a "practical therapeutic balance between antimicrobial activity and cellular toxicity" was elusive at the present state of knowledge. They concluded that "the ultimate goal remains the choice of a product with a superior profile of infection control over host cell cytotoxicity."

The ecological hazard of a chemical is determined by its persistence, its tendency to bioaccumulate and its toxicity. Silver is persistent in the environment because it is an element that can be neither created nor destroyed. Silver is one of the most toxic of the trace metals to many species, although the degree of toxicity is greatly influenced by how it is measured. It has a strong tendency to bioaccumulate to high concentrations in bacteria, humans and other organisms and to pass through food webs. It is biomagnified to higher concentrations in predators than in their prey.

The mechanisms of silver toxicity to higher organisms are much the same as those seen with bacteria. When a silver ion is taken up by fish from solution, for example, it perturbs the regulation of major ions in the gills by inhibiting sodium uptake (disruption of membrane transport processes). The inhibition of the animal's ability to regulate sodium and chloride at the gills perturbs the concentrations of major ions in the blood and affects internal fluid-volume regulation, among other fundamental life processes (Wood et al., 1999). Less is known about the mechanisms by which the silver ion is toxic to invertebrates, but disruption of metabolism through binding to sulfhydryl-rich enzymes and reduced growth would be expected.

The concentrations at which silver is toxic are determined by either short-term acute toxicity studies (mortality after 96 hours) or chronic toxicity studies (tests lasting many days or even months, and monitoring such signs as impairment of growth or reproduction). Chronic toxicity tests address responses that are symptomatic of stress, rather than immediate death. Chronic effects on an organism, like disruption of reproduction, slower growth or toxicity to early life forms, nearly always occur at lower concentrations than does acute toxicity to adults. But chronic stress is just as likely to eliminate a species as is mortality to adults. Thus, chronic tests reflect the most sensitive, but important, responses of organisms in nature.

Chronic toxicity tests, however, are much more difficult to conduct than are acute tests. They take more time, more maintenance and more complex logistics. Measuring sublethal endpoints is more difficult than counting dead organisms; thus, there are always less chronic data than acute data for a chemical contaminant. In many cases, the acute data alone are used to draw water quality regulations. Unfortunately, the studies of chronic silver toxicity are so few that USEPA has not defined a criterion for protecting species from chronic

TABLE 5*. RANGES OF TOXICITY TO SILVER IN VARIOUS TYPES OF TESTS WITH INVERTEBRATES AND FISH.

	Invertebrates Freshwater (ng/L)	Invertebrates Seawater (ng/L)	Fish Freshwater (ng/L)
Acute toxicity	850–3,000	13,300–27,000,000	5,000–7,000
Chronic toxicity	200–6,300		90–170 (trout) 370-650 (fathead minnow)
Invertebrates: eggs, embryo or larval	10	500–13,000	
Dietary exposure	50	100	

*Data from Luoma et al., 1995; Hogstrand and Wood, 1998; Wood et al., 1999, Bielmyer et al., 2006; and Hook and Fisher, 2001.

exposure. The lack of chronic toxicity data for silver is one explanation for the very high concentrations of silver defined by regulatory agencies as protective in natural waters (Table 3; Figure 4).

Whether designed to measure chronic or acute toxicity, traditional standardized tests have important limitations that greatly influence extrapolations to nature. Examples include:

- Short exposure durations. Acute tests are typically conducted for 96 hours, whereas organisms in nature are exposed for a lifetime (and presumably succumb at much lower concentrations).

- Only a few surrogate species are used for testing. The surrogates are not necessarily as sensitive as are many of the species in nature.

- Dietary exposure is not considered. For silver, this greatly affects determination of concentrations that are toxic (see later discussion).

Correction factors, or application factors, are incorporated into regulatory criteria to address tendencies to be overprotective (if geochemical conditions negate bioavailability) or underprotective (if diet is the crucial route of exposure) when applying toxicity testing results. The application factors are based upon professional judgment. The shortcomings of toxicity tests and the incorporation of professional judgment in the form of correction factors add uncertainties (and sometimes controversy) to water quality standards.

Among the standardized tests, a large body of evidence shows that the toxicity of the silver ion occurs at concentrations lower than those observed for every metal except mercury. The rank order of toxicity among metals for aquatic invertebrates, for example, typically shows greater hazards from mercury and silver ion than from copper, zinc, cadmium, nickel, lead or chromium. Species differ widely in their vulnerability to silver, but the rank order among metals is generally similar for most species.

The threshold of acute toxicity has been evaluated for more than 40 freshwater species and 25 marine species using the conventional standardized tests (Table 5; Wood et al., 1999). Toxicity thresholds are the concentrations at

which 50 percent of the organisms under investigation die. The most sensitive species include phytoplankton in freshwater and seawater, salmonids (e.g., trout) in freshwaters and early-life stages of a broad array of marine invertebrates, including oysters, clams, snails and sea urchins (e.g., summarized in Luoma et al., 1995).

Table 5 also shows how toxicity of dissolved silver as defined by chronic tests differs from toxicity determined in acute testing. Silver is most toxic when tested on developing life stages and especially toxic when delivered via food. When animals like microscopic zooplankton consume food contaminated by 50–100 ng/L silver, their ability to reproduce is inhibited (Hook and Fisher, 2001, 2002). In contrast, the toxic threshold observed in the traditional tests with dissolved silver is 10,000–40,000 ng/L (see Text box 6). Bielmyer et al. (2006) repeated these results with another species of common zooplankton, *Acartia tonsa,* and determined that 20 percent of animals had inhibited reproduction when fed diatoms (algae) exposed to 650 ng/L silver in seawater.

Silver toxicity to aquatic plants and animals is correlated with the concentration of "free" ionic silver. When sulfide and thiosulfate are present to complex the silver ion, toxicity declines remarkably. Embryos and larvae of fathead minnow, for example, are not affected until concentrations of silver reach 11,000,000 ng/L when sulfides are present in freshwater. In ligand free waters, silver is toxic at 370 ng/L. Hogstrand and Wood (1998) concluded that sulfide and thiosulfate offer greater than five orders of magnitude protection against chronic toxicity, reflecting the reduced bioaccumulation of the silver ion. Hogstrand and Wood (1998) concluded that "laboratory tests with silver nitrate almost invariably overestimate acute silver toxicity in the field because of the abundance of natural ligands which … markedly reduce its toxicity." They concluded that "it is doubtful if silver discharges in the freshwater environment would ever result in high enough silver ion levels to cause acute toxicity."

But not all complexes completely eliminate silver toxicity. Bielmyer et al. (2001) studied the effects of complexed silver on a sensitive freshwater zooplankton often used in toxicity testing, *Ceriodaphnia dubia.* They found inhibition of reproduction in 8-day tests at 10 ng/L when silver nitrate

Text box 6. Toxicity of silver in the diet

Hook and Fisher (2001, 2002) studied the effects of dietary exposure to silver on reproduction in zooplankton from both freshwater and marine environments. They exposed algal cells to a range of silver concentrations in water and then fed the algae to the zooplankton. When the zooplankton consumed algae exposed to 100 ng silver/L in marine waters and 50 ng silver/L in freshwaters, reproductive success was reduced by 50 percent in both cases. The acute toxicity of dissolved Ag in the marine species was 40,000 ng/L and in the freshwater zooplankton was 10,000 ng/L. One reason for the higher toxicity of silver in food was that the silver assimilated from food was accumulated internally in the zooplankton. Silver from food specifically accumulated in egg protein, depressing egg production in the zooplankton by reducing the ability of the organism to deposit protein in the yolk of the eggs and thereby inhibiting development of eggs and young (Hook and Fisher, 2001). This mechanism is consistent with silver's strong affinity for sulfhydryl complexation in essential proteins. In contrast, the silver from solution that was associated with the external surface of the animal had little adverse effect (Hook and Fisher, 2001).

(silver ion) was added to the medium. Silver in solution was just as toxic when bound to the sulfur-bearing amino acid cysteine as was the silver ion. Exposure to a more complex sulfur-bearing molecule reduced silver effects on reproduction to 600 ng/L, but did not "eliminate" toxicity. Similarly, toxicity is not eliminated by chloro-complexation in seawater, just as bioavailability is not eliminated (Table 5).

The concentrations of dissolved silver that are toxic in the most sensitive tests, including dietary exposures, are well within the range of silver concentrations observed in contaminated waters (Figure 4; Smith and Flegal, 1994). Bielmyer et al. (2006) noted that environmental standards designed to protect aquatic ecosystems (3,430 ng/L in freshwaters and 1,920 ng/L in seawater; USEPA, 2002) are well above the concentrations at which toxicity occurs in tests with feeding zooplankton, and would not protect such species. Data from sensitive testing protocols, like dietary exposures, were not considered when those standards were developed. The result seems to be a 10- to 100-fold underestimation of the toxic concentration of silver in many natural waters, especially the waters of estuaries, coastal zones and the oceans.

The ultimate test of whether a chemical is a threat to the environment lies in observations of toxicity in the waters where those chemicals are discharged. Evidence from nature can be controversial (Text box 7). On the other hand, historically, it was observations of nature, not toxicity testing, that originally detected the adverse effects of pesticides on birds; lead on children; mercury, DDT and PCBs on fish-eating birds; and the antifouling agent TBT on oysters and other invertebrates. All these chemicals are now recognized as powerful environmental toxins, and at least some uses of each of them have been banned.

Toxicity is usually manifested in complex ways in nature, and effects from one stress can be difficult to differentiate from another. One way to improve the likelihood of associating cause and effect in a field study is to use long-term data, in which a variable such as silver contamination changes slowly over time while unidirectional trajectories are absent in other aspects of the environment. As the Clean Water Act took force in the 1980s in the United States, it is likely that a number of such experiments in nature occurred as contamination receded (Sanudo-Wilhelmy and Gill, 1999). Only a few, however, were documented. Two such studies detected the disappearance of silver toxicity in San Francisco Bay as contaminated conditions

Text box 7. Challenges in separating cause and effect in a field study

A good example of the difficulty in distinguishing causes of an adverse effect from a pollutant comes from a 1984 study of mussels transplanted from a clean environment to a silver-contaminated environment in South San Francisco Bay (Martin et al., 1984). Where silver concentrations were highest in the bay, growth in mussels was adversely affected. However, other factors that could have affected feeding also changed as silver contamination increased. For example, silver-contaminated waters in the southernmost part of the bay also had higher suspended loads that could have reduced feeding by the mussels. Thus, it was not possible to conclusively tie the growth effects to silver alone, and this elaborate study had little impact on conclusions about silver toxicity in nature. Later studies showed that silver was indeed having adverse effects on organisms living in this region (Hornberger et al., 2000).

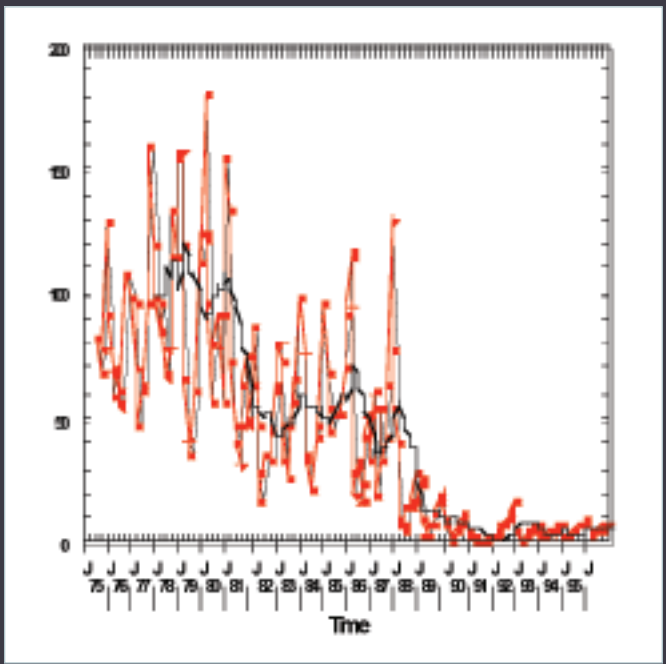
Text box 8. Effects of silver on invertebrates as observed in San Francisco Bay

Hornberger et al. (2000) reported on 32 years of monthly measurements on a mudflat 2 kilometers (km) from a domestic-sewage outfall beginning in 1975. Copper and silver were the major pollutants discharged from the treatment works in the 1970s. Improvements in treatment of the wastes from this facility were progressively implemented during the 1980s and 1990s, as mandated by the Clean Water Act. In response, Ag concentrations in clam tissues on a mudflat 1 km from the discharge declined from 106 µg/g to 4 µg/g over the 30-year period. Copper concentrations declined from 287 µg/g to 24 µg/g in clam tissues. Concentrations of both metals in sediments also declined. As noted earlier, concentrations of dissolved silver declined from an average of 113 ng/L in the late 1970s and 1980s (Smith and Flegal, 1993) to 6 ng/L in 2004.

Declining metals in the clams were strongly correlated to declines in waste discharges of the metals (Figure 6). Biochemical signs of stress were observed in surviving species during the period of contamination. But most important, reproduction persistently failed in the clams in the mid-1970s through much of the 1980s; the animals were not producing viable eggs and sperm. The adult clams apparently were present because successful reproduction occurred on other mudflats and a floating larval stage allowed individual clams to immigrate regularly to the contaminated mudflat. Tolerant individuals within the immigrants survived. The most convincing evidence that metals were the culprit in the stress response was that reproduction recovered and signs of stress disappeared as metal contamination declined into the 1990s. The community of animals living in the mud also changed with recovery from the metal contamination. Animals that fed on the mud directly (deposit feeders) and species that laid their eggs in the mud reappeared or increased in abundance once the contamination subsided. Other potential explanations of the biological and ecological changes were considered: food availability, sediment chemistry, salinity, temperature and organic chemical contamination. None of these potential confounding variables changed unidirectionally with the changes in clam reproduction and community characteristics over the 32 years (Shouse, 2002).

A later study with a similar long-term design showed effects of silver alone (Brown et al., 2003; Figure 7). This study covered the period 1990 to 1999, when a second species of clam was collected monthly from four locations in North San Francisco Bay. In this case, silver was apparently released from photographic processing at a nearby naval station. Silver concentrations were ele-

FIGURE 6.

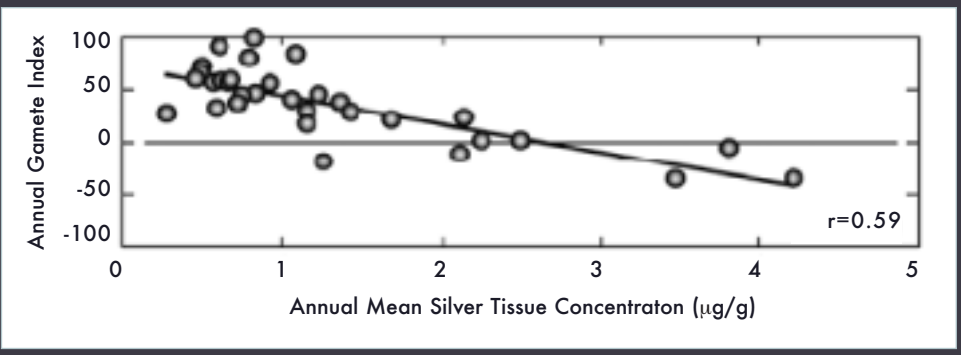


Silver concentrations in the soft parts of the clam *Macoma petalum* during the period 1975 to 1995 on a mudflat in South San Francisco Bay. Data points represent near monthly samples; bolded line is the 5-year moving average. Silver loadings to the environment, silver concentrations in water and silver in the biomonitor clam all declined as the Clean Water Act was implemented.

Text box 8. *(continued)*

vated in the bivalve *Corbula amurensis* for several years at two of the four stations in the study, and then the contamination receded. There was a higher frequency of reproductive failure in years when Ag contamination was greatest. An index of the annual average reproduction at all sites correlated strongly with the silver contamination in the tissues of the animals. Cross-cutting spatial and temporal sampling eliminated the likelihood that other sources of stress explained the change of reproductive maturity when Ag concentrations were high. This was the same effect observed in South San Francisco Bay and an effect similar to that observed when zooplankton were exposed to elevated silver in their diet (loss of reproductive capabilities).

FIGURE 7.



Correlation of the gamete index (reproductive maturity) with silver concentrations in the tissues of the clam, *Corbula amurensis*. Nine years of monthly samples at taken at four sites. Each point represents an annual average for each site. As silver contamination in tissues increased, the animals produced fewer mature eggs and sperm on an annual average basis.

improved (Text box 8). These studies showed that surviving invertebrates were unable to reproduce in the presence of silver contamination, but reproductive capabilities recovered when the contamination receded over a 30-year period. No other aspects of the environment showed a unidirectional trajectory of change like the metal contamination. Furthermore, the silver contamination that accumulated in sediments seemed to cause the reduced abundance or disappearance of species that either ingested sediments for food or laid their eggs in the mud. Those species also recovered when the contamination receded. The toxicity to the invertebrate animals occurred in the range predicted by the dietary exposure studies. The worst effects occurred at dissolved Ag concentrations estimated to be in the 100–200 ng/L range, which is 10-fold lower than the ambient water quality criteria (1,920 ng/L).

Field studies and dietary toxicity studies show toxicity and ecological effects are associated with silver concentrations in the environment in the ng/L range. Invertebrates are not as charismatic as some species, but they lie at the base of every aquatic food web. Changes in invertebrate communities result in changes in predator communities. Much of the silver released by a widespread consumer demand for photographic development and by industries and businesses was removed by waste-treatment facilities in the 1970s and 1980s. Much of the remaining dissolved silver discharged to the environment was undoubtedly complexed (not silver ion). Nevertheless, enough silver was discharged and bioavailable during that period, at the loadings shown in Table 1, to result in adverse ecological effects. There is no reason to think that a return to similar loadings to the environment would not result in similar effects.

III. EMERGING TECHNOLOGIES AND NANOSILVER

CONCEPTUAL FRAMEWORK

As noted earlier, rapid growth in capabilities for creating and manipulating materials in the nanosize range is leading to an explosion of ideas and nanosilver products. The source-pathway-receptor-impact concept (Owen and Handy, 2007) can be a useful framework for analyzing risks from these products (Text box 9), just as it is for silver itself. Environmental risks will depend upon the nature of the particle, the use of the product, the fate of the particle, the fate of the silver metal and the bioavailability and toxicity of both nanosilver and the newly added silver metal.

SOURCES OF NANOSILVER AND POTENTIAL DISPERSAL TO THE ENVIRONMENT

The environmental risks from emerging silver technologies will first depend upon the mass of nanosilver and silver (Text box 9) released to the environment. For most chemicals, governments have mandatory, uniform reporting requirements on mass releases of chemicals to

Text box 9. Factors that should be considered in evaluating environmental risks from nanosilver:

- *Sources* of nanosilver must be understood in order to manage risks. Regulatory programs ultimately will have to consider the mass of the nanoparticle emitted to the environment from specific sources in order to manage risks. Because silver itself is a toxin, the cumulative risks from the emerging technologies could also be influenced by the total mass of silver released.

- *Concentrations* in the environment determine risks. Environmental concentrations are therefore one basis for regulatory criteria. Concentrations are influenced by source inputs to the environment, fate in the environment and the characteristics of the water body. For example, when nanosilver is discharged to a water body via a sewage pipe, concentrations in the water are diluted to a degree determined by the characteristics of that water body but are also affected by interactions with particles and the fate of those particles. The concentrations that result determine any toxicity that will occur.

- The *pathways* of nanosilver in the environment also influence risk, as determined by chemical reactions with dissolved ligands, particles and sediments (mud).

- *Receptor: Bioavailability* of nanosilver is a crucial consideration in determining impacts. Bioavailability in this case is defined by the ability of organisms to accumulate into their tissues the form or forms of nanosilver delivered to it from the environment.

- *Impact: Toxicity* is determined by the internally accumulated, bioavailable nanosilver in each organism, not just the total concentration in the environment. However, biological factors also influence toxicity. If the organism can sequester the silver in forms that are not toxic (detoxification), then all the internal silver will not be biologically active and the contaminant will be less toxic. The forms of nanosilver that will be most toxic are those that are taken up readily from the environment, excreted slowly and/or are not sequestered internally in a nontoxic form.

- *Impact:* Effects on *ecological structure and function* are determined by how many and what kinds of organisms are most affected by nanosilver at the bioavailable concentrations that are present in the environment.

the environment. For example, the USEPA keeps a record of hazardous materials discharges through legislation such as the Toxics Release Inventory (TRI), wherein the industry that releases a chemical must report the mass that is released. Although such programs have a number of weaknesses (questionable data quality, infrequent syntheses), the loadings to the environment they define often provide a starting point for evaluation of environmental risks.

No mandatory reporting requirements exist for engineered nanoparticles. Voluntary programs were initiated in 2007 in the private sector (DuPont/Environmental Defense) and by some governments (USEPA and UK DEFRA nanoscale materials stewardship programs). However, some authors suggest that these do not include ingredients that typically lead to comprehensive reporting or high-quality data (Hansen and Tickner, 2007). Participation in these programs after their first year was very weak. A first-order need for conclusive risk analyses of nanosilver in the environment is consistent data from which to identify emissions from the new technologies or trends in those emissions.

The Project on Emerging Nanotechnologies database[3] provides anecdotal information about potential sources of nanosilver. The general information in the database and some of the information in the commercial websites themselves are instructive about some characteristics of the growing numbers of products containing nanosilver. The 240 products identified by Fauss (Fauss, 2008) in September 2007 were classified into types that are relevant for estimating environmental releases (for alternative classifications, see Blaser et al., 2008).

1. The most prevalent use was in products coated with a polymer containing nanosilver. Examples of these uses include nanosilver embedded onto handrails, medical devices, food storage containers, dressings for wounds and female-hygiene products. Polymeric silver is also called silver protein because the nanoparticle is complexed with a long chain molecule like gelatin, then added to the particular product.

2. The second most prevalent use was as "colloidal silver," which refers to nanosized silver particles (0.6–25 nm in this case) in a water suspension. These include solutions recommended for daily ingestion.

3. Spun silver is another prevalent application of nanosilver. In this case, the silver is integrated or spun into a fabric (e.g., cotton or synthetic fabrics), impregnating sheets, clothing, sportswear and other fabrics with the nanoparticle.

4. Nanosilver powder is used in a handful of products. For example, one manufacturer claims that its socks contain 100 times more silver than they actually need to work. Nanosilver powder is added, which comes out in shoes or in the first wash.

5. Ionic silver is intentionally generated by some products, including washing machines and dishwashers. These are not necessarily silver nanotechnologies, although some manufacturers suggest nanotechnologies are involved in effective generation of the silver ions. It is important the ionic silver technologies not be dismissed in terms of environmental risks, however, because they add to the total burden of silver discharged to the environment (one of the modes of risk).

Information on rates of increase in the number of nanosilver products is also anecdotal, but a number of sources are predicting rapid growth. Blaser et al. (2008) cited the Silver Institute as showing use of biocidal silver increased 500 times between 2000 and 2004.

Nanotechnology News reported the following in April 2006:[9]

> *Silver nanoparticles are emerging as one of the fastest growing product categories in the nanotechnology industry, according to Bourne Research, "silver nanoparticles may very well become the next 'it' product, much like antibacterial soaps took the consumer sector by storm a decade ago," said Marlene Bourne, principal analyst with Bourne Research. The medical sector was one of the first on board where end-uses have already migrated from burn dressings to surgical instruments and hand sanitizers. In addition, a recent study by a leading supplier of textiles to hospitals showed a dramatic reduction of infectious microbes in curtains embedded with silver nanoparticles. Sportswear manufacturers are also embracing its use to prevent odor in clothing. In the home, consumers can already find washing machines, refrigerators, HVAC filters, brooms and even food containers that employ silver nanoparticles to kill bacteria and limit mold growth—and this is just the beginning.*

Information from manufacturers' websites gives scattered, and often contradictory, information about the nature of the silver used. But some give at least anecdotal hints about concentrations of silver employed. Concentrations of 10 ppm silver are reported necessary for antibacterial effectiveness, so it is probably not unreasonable to assume this is the minimum concentration in products with silver sprays and mists, silver polymers and spun silver. One manufacturer cited the concentration of silver in its colloidal silver solution as 20 ppm silver,[10] although the range of concentrations in such products in other sources is reported as 3–1,000 ppm. Arizona State University researchers soaked six pairs of nanosilver socks in wash water and recovered from zero to 1.85 mg per sock. One pair of socks had no detectable silver. The lowest detectable mass of silver in a sock was 0.020 mg, and the highest was 31.2 mg (Benn and Westerhoff, 2008). The latter represent a maximum concentration in socks of about 1,358 ppm. This extreme variability is consistent with the variability in nanosilver dosing suggested for other products.

The silver polymers on products like handrails, for example, seem unlikely to release much silver to the environment because of their limited turnover. Similarly, products that end up in the terrestrial environment or as solid waste will be constrained in landfills (e.g., silver bandages) or in soils. It might be argued that medical applications like catheter linings, or even bandages, are unlikely to be a large source of environmental release because their use is limited compared to consumer products. Blaser et al. (2008) suggested that greater contact with water is most likely to result in the greatest release of silver to waste streams. The greatest risks would be expected from products that might be used by millions of people in ways that release silver directly to wastewaters. For example, coated products will present the greatest risk of dispersal if they are washed regularly and lose some of their silver (or

nanosilver) down the sewer. An example might be dishware or food storage containers. Spun silver products are likely to release some (nano) silver every time they are washed. In addition to the direct evidence of this (Benn and Westerhoff, 2008), one manufacturer advertises that the silver in its socks weathers their ability to fight microbes for 50 washings. This suggests that all the silver in the socks will eventually end up in the environment, most likely in domestic waste streams in the form of wash water. Silver ion generators release silver intentionally into wash waters, all of which will eventually end up in waste streams.

Is it possible that enough products, each releasing a small amount of silver, can eventually add up to an environmental hazard? An apt analogy is traditional photography. This was a technology used daily, most likely, by hundreds of millions of people. Each photograph that was processed released a tiny amount of silver as it was developed, and not all that silver could be recaptured or recycled, even in centralized facilities. The result was that almost every domestic waste stream contained high levels of silver contamination (Table 3; Figure 4). While no single nanosilver product is approaching the popularity of photography, the challenge lies in the diversity of products—ultimately, hundreds of dispersive products are conceivable. Many of these (e.g., antibacterial soaps) have potential interest to millions of people. Each would release (nano) silver into domestic wastewaters.

Another question is whether there is enough of this rare element available to support such a growth in technology. There was sufficient silver in circulation to support a release of 850 metric tons of silver per year to wastewaters and solid-waste facilities from the photographic industry in 1995 (Purcell and Peters, 1998). The market for antibacterial silver products is projected by some market analysts to grow to 110–230 tons of silver per year in the 25-member European Union by 2010 (Blaser et al., 2008); a similar-sized market might be expected in the USA. Sufficient silver was available in the past to support such uses.

Finally, it is argued that most nanosilver will be removed from wastewaters and deposited in sludges by waste treatment (Blaser et al., 2008). The silver in wastes from the processing of photographs, for example, was largely in the form of a strong thiosulfate complex. High concentrations of silver were typical of waste streams from photo processing facilities and were reflected in discharges from the waste-treatment plants that received such wastes (Table 1). The tendency of silver to associate with particulate material is exploited in sewage-treatment works to extract silver from effluents and retain it in sludges. Lytle (1984) compared six POTWs and found removal efficiencies of 75–95 percent. Silver concentrations in effluents ranged from <1 to 222,000 ng/L. More recently, Shafer et al. (1999) investigated removal of silver in five POTWs. More than 94 percent of the silver was removed in all five plants. Between 19 percent and 53 percent of the silver was associated with colloidal particles (50 nm–1,000 nm); however, that required filtration of the waste for removal, which is one of the most advanced forms of treatment. The rest was settled out as larger particles. In all cases, silver concentrations in discharges to natural waters correlate with silver in the incoming wastewater (Shafer et al., 1998). The more silver in incoming wastewater, the more silver that was lost to the environment. Sewage treatment helps, but it is not a cure

for environmental risk if incoming loads are large enough. In addition, the degree to which nanoparticles containing silver might be captured by waste treatment is unknown.

Proponents of the new technologies do not necessarily accept that there are environmental risks associated with their products (Text box 10). For example, one washing machine technology uses a silver anode assembly to release silver ions into a stream of tap water that is carried into the water supply of the machine. The goal is to achieve better bacterial control than conventional washing machines (although there is no evidence that bacteria are a problem in conventional machines). In March 2005, a press release from one of the machine's manufacturer claimed that "Samsung Silver Nano home electronic appliances including washing machine, refrigerator, air-conditioner, air-purifier and vacuum cleaner are all equipped with comprehensive features that kill bacteria, molds and even harmful influenza viruses during the filtering process." The Samsung website, downloaded in October 2007, had the following to say about the silver released: "Silver is present in nature and, as silver ions only attack bacteria (due to their cell structure) and not healthy organisms, it would be environmentally friendly" (http://ww2.samsung.co.za/silvernano/silvernano/washingmachine.html#). In late 2007, a new technology was introduced that can fit a silver ion generator to any automatic clothes washing machine.

MASS DISCHARGES TO THE ENVIRONMENT FROM NANOTECHNOLOGY

Very crude estimates of mass emissions of silver to the environment are, arguably, possible, if only to give a general sense of the nature of the potential environmental risks (see also Blaser et al., 2008). Factors to consider include:

- the nature of the potential sources
- the number of such sources and the potential for growth in that number
- the potential for dispersal to the environment
- the concentration of silver associated with each source

There is great uncertainty in such estimates, of course. First, as noted above, the quantities of nanosilver associated with individual

Text box 10. Controversies about the fate of silver nanoparticles

Quotes from NewsTarget.com, December 2006:

Silver is spread throughout the environment already. Taking silver from the environment, using it and having some of it return to the environment is no different than the use of any other metal from the environment, whether iron, copper, or whatever.

… free silver ions are needed to have an antimicrobial effect, but the ions will automatically bond with chlorine if they find their way into common drinking water, thereby rendering the ions inert. … Our patented Silver100 is a perfect case in point, where it took many years of development and achieved patent protection because it has a specific molecular structure to control the release of silver ions in microbial forms,

Once that occurs, the silver ions do not hang around. That's just the way the chemistry works.

products are not well-known. What is known suggests silver concentrations, even in similar products, are highly variable, as cited above for silver socks or colloidal silver solutions. Estimates should include scenarios that capture this variability. Maximum scenarios test the question of whether it is feasible for silver inputs from such products to be environmentally significant, for example. Second, it is not clear whether silver lost from any product will be in the form silver itself or of nanosilver. Estimates presented below consider only total silver releases, using the assumption that the baseline risk is from the loss of silver metal itself. Additional risks will occur if nanosilver is more toxic than the silver metal. Finally, it not possible even to estimate wastes from manufacturing plants in any reasonable way (Blaser et al., 2008). The only precedent is that silver manufacturing for photography released a quantity of silver approximately equivalent to photographic development (Table 1). If discharges from manufacturing plants add to the mass released from the products themselves, then an analysis of inputs from consumer products is a minimum estimate of discharges to the aquatic environment, perhaps by half.

Mathematically, the mass of silver discharged to the environment is estimated from the mass of silver in a product and assumptions about the rate at which that silver from that product enters the environment (Text box 11). Blaser et al. (2008) used a relatively complicated formula to determine releases to wastewaters, lumping types of materials and estimating water-contact times for each. In the approach taken here, we use data available on the silver content of known products that might discharge their silver to wastewaters. Their releases of silver are estimated from manufacturers' information. Releases from multiple products are calculated assuming similarity to products for which some data are available. Scenarios were devised for three types of new silver technologies:

1. Silver socks and similar consumer products that might be frequently washed to release nanosilver;

2. Silver Wash machines and similar silver-generating devices (dish washers, etc.); and

3. Swimming pools or other spa equipment that use silver as a bactericide.

The details of the calculations are described in Text box 11 and in Appendix A, Table A.1.

Table 6 shows the range of silver releases from the products and uses as described in Text box 11. Variability in the estimates from consumer products such as silver socks is extremely large, reflecting the great variability found in the quantity of silver in different socks. Table 6 shows that releases from socks (or products) containing the minimum silver that might kill bacteria are small compared to those from photography. Use of multiple products loaded to release as much as 31 mg silver (Benn and Westerhoff, 2008) could result in substantially more silver discharge to the environment than was the case for photographic development. Clearly, the quantity of silver used in each product will have an immense impact on the significance of silver releases to the environment, and could be a consideration in regulating these products.

One lesser-known but notable use of silver is for sterilizing swimming pools, spas, Jacuzzis and other containers holding 10,000 L or more

of water. Using the manufacturers' recommended concentration, pools, spas, and hot tubs could discharge 150 tons of silver per year alone for each million such containers, if each container was emptied only once per year. A silver load to the environment in the tons per year from pools is probably realistic (or an underestimate), in that 7 tons of pesticides were used in California swimming pools alone in 2003,[12]

Table 6 emphasizes that it is in the widespread use of products employing the new silver technologies, each of which itself seems intuitively innocuous, that the greatest environmental implications of the new silver technologies lie. No individual product releases silver at rates equal to those released by photographic development in the 1980s. But the sum of silver releases from a proliferation of

Text box 11. Estimating mass releases of silver from consumer products

There are several steps in calculating the mass of silver ($M_{Tsilver}$) released to wastewaters from nanosilver products. If the concentration in the product (C_{silver}) is known, then the mass of silver in the product, $M_{Psilver}$, in units of grams (g), kilograms (kg) or metric tons (T) of silver, is determined by:

$$M_{Psilver} = C_{silver} / M_{product} \quad (1)$$

where $C_{silver} / M_{product}$ is the concentration of silver in the product and $M_{product}$ is the weight of the product. For silver socks, we use two scenarios. The minimum scenario is that the concentration in the socks is that cited as the lowest bactericidal concentration: 10 µg/g. The mass of silver released in one year can be determined by assuming that some fraction (F_{year}) of the total silver in each product is released per year. The minimum sock scenario assumes that each pair of socks with 10 ppm silver will release one-fifth of its mass of silver per year if socks lose their silver in 50 washes, and each is washed 10 times per year. The maximum scenario uses the highest mass of silver lost from socks in a single wash, as observed experimentally: 0.031 g (Benn and Westerhoff, 2008). For Silver Wash machines, we assume a release of 0.05 g per year, as cited by one washing machine maker. For swimming pools, we assume the manufacturer's recommended concentration of 0.003 g/L and 10,000 liters per container, the volume usually cited for spas and similar uses (pools contain about 40,000 liters). We assume each container is emptied once a year.

A scenario for the number of products ($N_{product}$) in use also must be devised. One approach is to assume a given percentage (X) of the population of a jurisdiction uses the product where, for example:

$$N_{product} = X * N_{USA} \quad (2)$$

Scenarios are shown wherein 10 percent of the stated population and 30 percent of the stated population use silver socks; all U.S. households that are wealthy enough to hold equities buy Silver Wash machines , and 1 million swimming pools in the USA use silver as a biocide. Then, the total mass release from an individual product or use is:

$$M_{Tsilver} \text{ per year} = C_{silver} * F_{year} * N_{product} \quad (3)$$

To estimate what happens if silver technologies become the household standard, the total mass of silver discharged to the environment of a jurisdiction (MTsilver,) must be multiplied by an assumed number of products (P) where:

$$M_{TUSAsilver} = M_{Tsilver} * P \quad (4)$$

Reasonable scenarios are that 100 products will be used that resemble silver socks, 10 products exist that resemble Silver Wash machines, and 5 products could release silver like swimming pools (spas, hot tubs, Jacuzzis, silver filters for water purification, etc.). Finally, it is necessary to incorporate waste treatment. For this aspect of the scenario, the assumptions of Blaser et al. (2008) are used, in which 80 percent of waste is treated sufficiently to remove 90 percent of the silver.

TABLE 6.† COMPARISON OF DISCHARGES FROM SILVER NANOTECHNOLOGIES FOR SEVERAL DIFFERENT NEAR-TERM SCENARIOS FOR THE USA AND FOR SOUTH SAN FRANCISCO BAY.

Scenarios-USA	Silver discharged by this product (T)	X similar products (T)	After waste treatment (T)
Silver socks–10% of population; 10 pairs each	0.006-0.930	0.6-93*	0.17-26.0
Silver socks–30% of population; 10 pairs each	0.18-2.79	1.8-279*	0.50-78.1
Silver wash machines-Households holding equities (indicator of sufficient wealth)	2.85	28.5**	8.0
Swimming pools–1,000,000	30	150**	42
Maximum Scenario Totals		**457**	**128**
Photo Developing–1978	**65**		**20**
Scenarios: South San Francisco Bay	(kg)	(kg)	(kg)
Silver socks–10% of population; 10 pairs each	0.04-6.2	4-620*	1.1-174
Silver socks–30% of population; 10 pairs each	0.12-18.6	12-1,860*	3.4-521
Washing machines-10% of population	10	100**	28
Swimming pools	300	1,500**	420
Maximum Scenario Totals		**3,460**	**969**
Discharge to South Bay 1980			**550**
Discharge to South Bay 2007			**40**

† Historic silver discharges from a comparable consumer product, photo processing, are shown for comparison, as are total discharges into South Bay in 1980 and 2007.
* 100 similar products.
** 10 similar products.
** 10 similar products.
Key: T is metric tons; kg is kilograms.

different products could release much more silver than did photographic development. For example, the maximum scenario of releases to wastewaters for the three types of products we use as illustrations are projected to be as high as 457 metric tons per year for the USA, and 128 tons after waste treatment. Silver discharge from the metals and photographic industry in 1978 was 124 tons per year, for comparison. Blaser et al. (2008) estimated, in intermediate and maximum scenarios, that 9–20 tons of silver from biocidal uses would be discharged to

the European environment in wastewaters, with the remainder going to sewage sludge. Our scenarios for 100 consumer products like silver socks, used by 10 percent of the population, would yield similar releases. However, if products and technologies releasing higher concentrations of silver proliferate to a greater extent, much larger discharges may be possible. Blaser et al. (2008) also noted that increased silver concentrations in sewage sludges might increase environmental risks in the terrestrial ecosystems if and where sludges are used as biosolids to supplement fertilizers.

Blaser et al. (2008) concluded that emission reductions for silver in recent years in Europe could be reversed by 2010 by new releases from biocidal silver applications. It is possible to do a similar estimate using data from South San Francisco Bay, where the history of silver discharges to the environment after waste treatment is known. The maximum-discharge scenario in Table 6 shows that it is conceivable that bactericidal silver discharges, after waste treatment, could be twice those observed in 1980 (969 kg versus 550 kg per year), and could be 20-fold larger than discharge rates in 2007. More moderate scenarios (100 products like silver socks used by 10 percent of the population) could yield discharges ranging from insignificant to more than four times greater than 2007 discharges, depending on the silver content of the products. Silver technologies certainly also have the potential to reverse gains in removal of silver from human discharges to the aquatic environment in the USA. A return to conditions that are equal to or worse than those of the 1980s is conceivable if the present approach to managing this issue does not change.

Despite the very large uncertainties, the estimates point out that order-of-magnitude loading projections from emerging silver technologies are feasible at the present state of knowledge. Uncertainties lie as much in the variability of the products as in knowledge about them. It is also clear that potential cumulative release of silver from consumer products alone is likely to be within the order of historic releases of this persistent pollutant, especially if some products become as popular as some projections suggest. Waste-treatment facilities will capture some of the contamination with an efficiency that depends upon the form of the silver. But the possibility of a detectable impact on silver concentrations in the environment cannot be ignored. As larger numbers of products are used by larger numbers of households, the potential for environmental risks will increase. The concentration of silver in each product and the potential for dispersal (or release) of the generated silver ion or the embedded nanosilver seem a crucial requirement for reporting about a product or for its registration. The silver releases estimated here are probably the baseline levels of risk from the technology. Cumulative risks will depend upon releases from manufacturing and whether or not occurrence of silver as a nanoparticle adds to or is a multiple of the baseline. In a worst-case scenario, if the proliferation of silver in consumer products "takes off" and remains unregulated, every domestic waste-treatment plant could have a halo of silver contamination surrounding it. Some of these hot spots will be more intense than others. This was the characteristic of historic silver contamination (e.g., Hornberger et al., 2000).

The potential for significant mass releases of silver from the new technologies is not surprising news to the people who manage waste-treatment facilities. The Bay Area Clean Water Agencies (BACWA), representing 44 wastewater authorities throughout the San Francisco

Bay Area in California, requested in May 2007 that Samsung's Silver Wash machine be removed from the list of washers that qualify for a water-conservation rebate. Tri-TAC, a technical advisory group for POTWs in California, wrote a similar letter in January 2006 to the California Department of Pesticide Regulation. Tri-TAC is jointly sponsored by the California Association of Sanitation Agencies, the California Water Environment Association and the League of California Cities. The constituency base for Tri-TAC collects, treats and reclaims more than 2 billion gallons of wastewater each day and serves most of the sewered population of California. The dischargers were concerned about costs and contamination associated with increasing the loads of a persistent pollutant in their discharges to the local water body (P. Bobel, Tri-TAC member, personal communication). The same concerns were expressed in both letters (quote from the January 2006 Tri-TAC letter):

> Silver is highly toxic to aquatic life at low concentrations, and also bioaccumulates in some aquatic organisms, such as clams. Due to concerns about bioaccumulation and the placing of strict silver effluent limits in discharge permits, POTWs have implemented pollution prevention programs to identify and reduce silver discharges to sanitary sewer systems. These programs have been very successful in reducing POTW influent and effluent silver concentrations. However, widespread use of household products that release silver ions into sanitary sewer systems could greatly increase silver concentrations in POTW influents and effluents, leading to adverse effects on California waterways. POTWs are subject to Mandatory Minimum Penalties for the violations of their discharge permits that could result. … To allow the unrestricted usage of a product that intentionally releases silver into the environment would be an irresponsible neglect of the principles of environmental sustainability that should strongly influence such decisions.

In December 2007, several new products were released that include silver ion generators that can be fit to any standard washing machine. Tri-TAC wrote again to USEPA and state agencies, asking them to require registration of such products and requesting consideration of legislation to limit addition of toxics to consumer and commercial products. In November 2006, USEPA responded to these and other requests by regulating the Silver Wash machine as a pesticide under the U.S. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). Questions relating to what products such regulations apply and to how they will be applied to the rapidly growing plethora of new products remain to be resolved.

PATHWAYS OF NANOSILVER IN THE ENVIRONMENT

The nature or form of nanosilver could greatly influence its fate in the environment, and therefore its implications if released. Nevertheless, commercial institutions are often incomplete and inconsistent in the descriptions of their products.[13] For example, the term *silver colloid*, a common descriptor in silver health products, implies little specificity in the range of particle sizes. Some products explicitly define silver col-

loid systems as containing nanometer-sized particles (e.g., 1–25 nm) suspended in water. Other manufacturers claim that their products contain silver nanoparticles but list particle sizes beyond the nanoscale (e.g., 25–250 nm[14]). Still others state that their particles are ions or describe their product as containing "ionic particles." Few reference the scientific definitions of these terms (see Text box 1 for definitions). In fact, nanosilver is used in a wide array of forms. Silver coatings can be added to other nanoparticles like TiO_2 (Guin et al., 2007). Different-shaped particles may confer different reactivities (Pal et al., 2007). Charged functional groups or surface coatings can be added to silver nanoparticles to improve their dispersion in water. Nanosilver composites can also be synthesized by layering the particles onto organic carrier molecules that themselves are soluble (Balogh et al., 2001). These composites apparently can retain antibacterial activity and/or can also impart selectivity in physical or chemical interactions. The British Standards Institute (BSI) recently posted advice to manufacturers about what information to include on labels for nanomaterials: the size range of the materials; whether the nanoparticles are free or bound in a solid matrix; whether the product contains a mixture of various nanoparticles; the specific source of the nanoparticles; and a description of the specific function of the nanoparticles in the product, among other things. Complex interactions blur precise boundaries among macromolecules, nanoparticles, colloids and particles (Lead and Wilkinson, 2006), but differentiating nanoparticles using scientific definitions could provide at least general guidelines for the commercial sector (as in Text box 1).

Some of the above characteristics of a silver nanoparticle will determine which reaction pathway it will follow in the environment and thus its fate. For example, silver nanomaterials may

- stay in suspension as individual particles;
- aggregate;
- dissolve; or
- react with natural materials like dissolved organic matter or natural particulates.

Some nanosilver particles are engineered to remain in water as single particles (e.g., Lee et al., 2007). Charged functional groups or surface coatings can be added to improve water solubility and suspension characteristics. One company advertises 2- to 5-nm silver particles surrounded "by a polymer coating that makes them water dispersible."[15] If single nanoparticles in suspension prove to be a form of high toxicity, or if the silver nanoparticle proves to be of greater toxicity than silver itself, then persistence of the dispersed particle will affect its ranking as an environmental hazard. But the persistence of particles in dispersed form has not been studied on timescales relevant to the environment. Once a silver nanoparticle is delivered to an aquatic environment it will be subject to reactions in that environment indefinitely. The longer the particle or the traits that aid dispersal resist such reactions (over months, years or longer), the greater will be the buildup of such forms in natural waters.

The persistence of the particle itself is also likely to be an issue, but that, again, has not been studied. At least some formulations of silver nanoparticles dissolve or degrade in slightly acidic conditions and at temperatures not much above room temperature.[14] The presence of chloride or dissolved organic materials in the

water could accelerate the rate of nanoparticle dissolution if these ligands are abundant. Contact with strong dissolved ligands in wash water may also accelerate the rate at which silver is released from products like clothing.

Many nanoparticles have a tendency to cluster and attach to one another to form larger aggregates or agglomerates. BSI (2007) defines an *aggregate* as containing multiple strongly bonded particles and having a reduced surface area compared to the individual particles. An *agglomerate* is a collection of loosely bound particles or aggregates. Aggregation of nanosilver can be caused by a surface charge on the particle; typical of unmodified silver particles. Less concentrated suspensions of silver particles have fewer aggregated particles than more concentrated suspensions do. Some commercial entities suggest the threshold for the beginning of visible aggregation in silver "colloidal" systems is 12 ppm, or 12,000 ng/L.[16] The likelihood of aggregation in natural waters may be reduced by the dilute concentrations expected but may be increased in waste-treatment plants, where the materials could be concentrated by treatment processes. Water chemistry will also affect aggregation. Particles of all dimensions are more likely to aggregate as salinity increases, for example. Lee et al. (2007) found that one formulation of silver nanoparticles averaging 11.6 nm in size remained stable for 120 hours at the salinity found within the egg of a freshwater fish: 1.2 nanomolar (nM) sodium chloride (molar units reflect the number of atoms of sodium and chloride per liter of water). However, at 100 nM sodium chloride, the particles aggregated to an average size of 24.4 nm and lost some of their surface charge.

Aggregation reactions will have a strong effect on the fate of the silver nanoparticle. Aggregates and agglomerates are both more likely to ultimately settle to the sediments than are individual nanoparticles. Aggregation may reduce the effectiveness with which silver ions are released, if the surface area per unit mass declines. However the relationships between aggregation, surface area and ion release are complex. Despite the prevalence and importance of aggregation reactions, few commercial producers comment on this property.

Natural waters contain dissolved organic materials and natural particles of widely varying chemistries. Reactions of nanosilver with these natural materials also seem likely. Coating nanosilver particles with natural organic materials would be expected to reduce reactivity. Association with the particulates suspended in water could remove nanosilver from the water and either transport it with the suspended particulates or create concentrated deposits in sediments, analogous to the dominant reactions of the silver ion. Understanding such pathways will be crucial to connecting mass inputs of nanosilver to the environmental concentrations that are ultimately responsible for any potential toxicity.

The concentrations of silver or nanosilver in natural waters are likely to be within the same order of magnitude as were historic concentrations of silver metal during the times of elevated discharges, if mass discharges from new silver technologies rise to historic levels and reactions follow similar pathways. For example, concentrations of 26–189 ng/L in South San Francisco Bay, like those observed in the 1980s, might be expected to accompany a return to discharges of silver of the same order as occurred in the 1980s (550 kg/year; Table 2). If the new inputs are as reactive with particulate material as is silver metal, such discharges would increase sediment concentrations from the 2007 baseline of 0.2 ppm to the historic

highs of ~3 ppm in such a system. Concentrations of a similar magnitude were forecast by Blaser et al. (2008) in the Rhine River in response to their projected increases in use of bactericidal nanosilver. They forecast dissolved concentrations of silver in the Rhine of up to 40 ng/L (minimum-use scenario) and up to 340 ng/L (maximum-use scenario). They forecast maximum sediment concentrations of 2–14 ppm (μg/g) in the different scenarios, but suggested those estimates were high compared to data from contaminated natural waters. Thus, their conclusion that manufacturing, production and widespread use of silver wash machines, silver-impregnated clothing and dishware, silver-sanitized spas and swimming pools and a myriad of other products could reverse the present trend of declining silver concentrations in natural waters across the historically developed world. Even such coarse estimates provide a perspective from which to evaluate needs for environmental surveillance and toxicity testing for nanosilver.

Environmental surveillance is especially problematic for nanomaterials. At present, there are no fully developed monitoring dosimetry methodologies that can routinely detect nanoparticles or quantify their abundances in natural waters or sediments (Maynard et al., 2006; Owen and Handy, 2007), especially at concentrations in the ng/L range. Methods are, however, available for quantifying colloid abundance or characterizing metal speciation. Some of these might be adapted to quantifying nanoparticles in natural waters (Lead and Wilkinson, 2006), but most would be challenged to reliably and routinely detect ng/L changes in nanosilver concentrations.

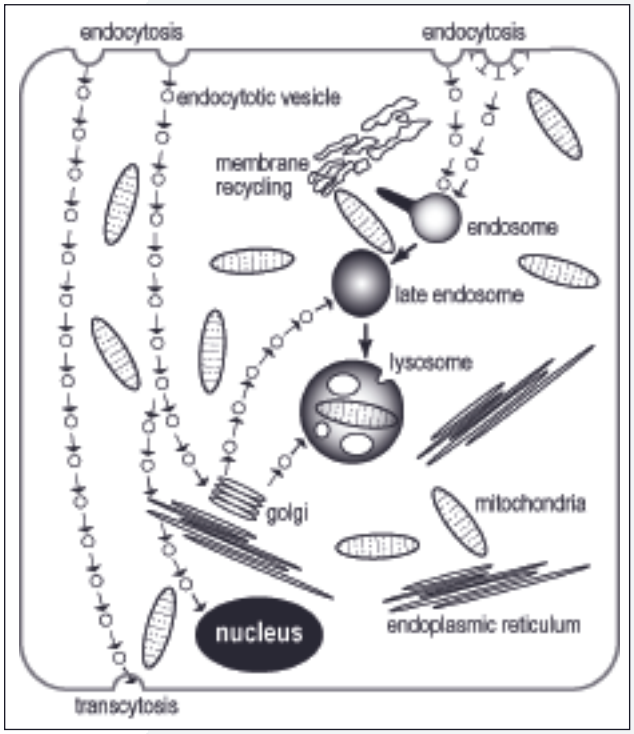
Monitoring silver itself might be an interim step in environmental surveillance for which very sensitive methodologies already exist. If inputs of silver nanotechnologies reach problematic levels, the baseline silver metal concentrations are likely to reflect that change. The environment is sensitive to new inputs because baseline concentrations of silver are naturally very low. Detecting a change in silver concentrations alone would not provide sufficient information about the nature of the silver contamination (e.g., is it nanosilver or silver); however, detection of changes in total silver concentrations might provide a first warning that discharges from new technologies are becoming environmentally influential.

IS NANOSILVER BIOAVAILABLE?

Bioavailability is a prerequisite to toxicity for nanosilver, just as it is for silver metal. Bioavailability is usually defined by the ability of the nanoparticle to penetrate into the organism; the bioavailable nanoparticle becomes toxic when its presence disrupts processes within cells. Toxicity can result from disruptions caused by the nanoparticle itself or it could occur because the nanoparticle delivers and releases silver, whose ions can disrupt processes. A nanosilver particle must also be considered bioavailable if it associates with and disrupts essential processes on the surface of the external membrane and/or delivers silver ions that do so. Although much remains to be learned, it is clear that mechanisms exist to allow nanosilver penetration into organisms and into their cells. These mechanisms, however, are probably different from those that control uptake of the silver ion.

It seems unlikely that nanoparticles of silver would mimic a major ion in a way similar to that in which the silver ion mimics sodium (although the nanoparticle could deliver silver itself to the transporter). However, a sec-

FIGURE 8.



Graphic representation of a cell showing critical cell machinery and the process of endocytosis (Moore, 2006).

ond transport system, the endocytotic system, is well suited as a portal for nanoparticle entry into cells (Text box 12). *Endocytosis* is the process whereby the membrane engulfs particles and transports them across the cell membrane and into the interior (Figure 8). It is the process that medical engineers exploit to get fluorescent nanoparticles into cells; thus it is known that nanoparticles can be taken up via such mechanisms. Endocytosis is common to the cells of all advanced organisms.

Exposures to nanosilver as free nanoparticles in water are the most common form used in most studies of aquatic organisms (Lee et al., 2007; Asharani et al., 2008). But silver nanoparticles are also likely to be incorporated into suspended particulate materials, taken up by plants and bacteria or bound onto sediments. Exposure could also occur when animals eat and digest such materials for food, or when predators eat prey that has taken up nanosilver. The membranes of the digestive tract have abundant carrier systems specifically utilized to transport nanosized materials, including amino acids, small proteins and

Text box 12. How nanosilver particles might enter cells

Endocytosis is the process by which materials ranging up to 100 nm in size enter cells. It is a likely mechanism by which nanoparticles are taken up. During endocytosis, cells absorb materials by engulfing them using the cell membrane. There are three kinds of endocytosis. In *phagocytosis,* the membrane folds around a larger object and seals it off. In *pinocytosis,* an infolding of the membrane engulfs solutes and single molecules such as proteins. *Receptor-mediated endocytosis* involves the formation of inward, coated pits with specific receptors. In all cases, the membrane forms a saclike vesicle, or endosome, into which the material is incorporated and then pulled into the cell. The endosomes can selectively concentrate some materials or exclude other materials during formation. Within the cell, the endosomes may merge with another saclike piece of cellular machinery, the lysosomes. These vesicles are specifically designed to break down the material or otherwise protect the other cell machinery from disruption by potentially toxic materials (Moore, 2006). If toxic materials become too concentrated within lysosomes, they can begin to leak toxins into the cell. Endosomal pathways also exist to deliver materials directly to the cell's organelles (the machinery within the cell that has specific functions). Examples of organelles include mitochondria, Golgi apparatus and the nucleus. Association of a nanoparticle with organelles is likely to disturb the functioning of those systems.[17]

micelles. Endocytosis is also common in the gut. Invertebrates like clams, mussels and oysters, for example, process much of their food in an organ system termed the *digestive gland*, or *hepatopancreas*. In some organisms, a high proportion of digestion occurs via engulfing of particles, termed *intracellular digestion*, where food is digested within the endocytotic vesicles within cells. Nutrients are then released into the cell.

Even though a mechanism for uptake of silver nanoparticles exists at the cellular level, it is important only if particles are retained sufficiently to accumulate within the cell. The physiological processes that govern how much nanosilver would be accumulated within an organism are the same as those that drive silver bioaccumulation: the sum of rates of uptake from food and water, balanced by the rate of loss. Mechanistically, the greatest risks will come from formulations or environmental conditions that induce high-uptake rates from food or water and/or for organisms unable to rapidly excrete the particles (with a low rate constant of loss). Thus, uptake is important, but nanoparticles that have a tendency to get trapped within cells can be expected to accumulate to high concentrations. Quantifying these basic physiological rates can provide a basis for comparing bioavailability and bioaccumulation of different types of nanosilver particles, different environmental conditions and different organisms. Comparisons with silver itself would also be informative. Protocols are well developed for comparing such rates (Luoma and Rainbow, 2005), but quantification of the rate will require methods that can trace nanoparticles as they are taken up. Nanoparticles synthesized with fluorescent markers, a radioactive label or an enriched stable isotope ratio, are potential methods of tracing the nanomaterials, although little experience exists with any of these in nanoparticle or nanosilver studies.

It remains uncertain whether uptake of nanosilver particles occurs into bacteria cells (e.g., Balogh et al., 2001). But bacteria and cyanobacteria (blue-green algae) are classified biologically in a unique kingdom, the prokaryotes. These organisms do not have capabilities for endocytotsis, so they may be less likely than are other higher-order organisms to pass nanosilver through their cell wall (Moore, 2006).

The organisms that are more highly evolved than bacteria (almost all other life forms, classified as eukaryotes) are capable of endocytosis. Thus, it is not surprising that nanosized particles can be taken up by these higher-order organisms. Most studies show transport into and retention by isolated cells in vitro. Fewer studies consider living organisms in vivo. Nanosized particles of sucrose polyester (Moore, 2006) or silicate fibrils (Koehler et al., 2008) were shown to be taken into the cells of the gills and the digestive gland of blue mussels (*Mytilus edulis*) after exposure of the whole organism. The smallest silicate fibrils appeared to pass across the gill cell membrane by diffusion, whereas larger particles were taken up by endocytosis (Koehler et al., 2008). The sucrose polyester was taken up by endocytosis only (Moore, 2006). Uptake of sucrose polyester into the cells occurred whether the nanoparticles were ingested or were suspended in water. The nanoparticles appeared to be taken into lysosomes within the cell after uptake in both studies.

Uptake of free, unaggregated nanosilver particles was recently demonstrated in the

embryos of zebrafish (Lee et al., 2007; Text box 13). Single silver nanoparticles in water were observed crossing the external tissue that protects the embryo via diffusion through unusually large pores. The particles then penetrated the embryo itself, although the mechanism was not clear. As the embryo matured to an adult, the nanoparticles were retained and spread through a number of major organs. Ultimately, a body of such work with a variety of organisms and conditions will be necessary for definitive conclusions about the processes involved. But the study with zebrafish (Lee et al., 2007) refuted the simplest null hypothesis: that risks from silver nanoparticles can be discounted because the particles are not available for uptake by organisms. Nanosilver is bioavailable, although details like rates of uptake and fate within the cell are less known.

The charge potential, surface area, surface structure, oxidation state and surface composition of nanoparticles that affect their chemical reactivity will also affect bioavailability, just as speciation and transformation affect bioavailability of silver metal. But beyond that general statement, little is known. USEPA's white papers on nanotechnology (USEPA, 2007) implied that metallo-nanoparticles were unlikely to be bioavailable, based on the assumption that only the free ion of metals such as silver are taken up. It seems unlikely that nanosilver would mimic a sodium ion, but the effects of the nature of the nanosilver particle on uptake by endocytosis are more complex and completely unknown. Changes in the form or stability of nanosilver during its residence in natural waters or within the complex gut environment also seem likely, but again, there are no studies and the implications for bioavailability are unknown. One of the challenges to delivering nanoparticle-based drugs, for example, is their tendency to aggregate in the gut, which increases their size and lowers their absorption (Florence, 2004; Ngo et al., in press).

An important property of silver nanomaterials appears to be an ability to increase accessibility of silver ions to organisms. Engineered silver nanoparticles can be thought of as a

Text box 13. Bioavailability of nanosilver to zebrafish *(Danio rerio)*

Lee et al. (2007) recently published one of the first authoritative works addressing bioavailability of a silver nanoparticle formulation using environmentally realistic concentrations of silver. The nanosilver was engineered so that it did not aggregate. Zebrafish embryos were bathed in water of the same salinity as occurs naturally in the egg (1.2 nM sodium chloride) with different concentrations of silver nanoparticles. The embryos were exposed over the entire period of development, beginning at a crucial life stage; the eight-cell cleavage stage. This cleavage stage is the most sensitive of several stages in the development of the embryo because its eight cells eventually proliferate into many cells that ultimately form the functioning organ systems in the adult organism. Disruption of one cell has great implications for normal development of the embryo into an adult. Lee et al. (2007) showed that single silver nanoparticles moved across the protective membranous tissue that protects the embryo from the external environment; termed the chorion. The nanoparticles were observed passing through large pores in the chorion, 1,500–2,500 nm in size. Pores also occur in the membranes that surround internal cells, but they are typically much smaller. The silver nanoparticles also made their way into the inner mass of the embryo, although the mechanisms by which they passed through that membrane were not visible. The single silver nanoparticles were retained by the embryo as it matured and were eventually found embedded in the retina, brain, heart, gill arches and tail of the mature fish.

large number of "potential" silver ions amassed in one "package" and modified by sophisticated nanometer-scale engineering in ways that might affect accessibility to cells. For example, Balogh et al. (2001) described "surface-modified poly(amidoamine) (PAMAM) dendrimers [that] were utilized as templates, nanoreactors or containers to pre-organize silver ions and subsequently contain them in the form of solubilized and stable, high surface area silver domains." Such a product delivers the package of silver ions to a target (e.g., bacteria). Efficient delivery of many silver ions to the exterior of bacterial cells appears to be one of the reasons nanosilver accentuates the effectiveness of silver as a bacterial biocide. The rate at which the silver ion is released from nanocomposites is about one order of magnitude higher than it is in microcomposites, because of the much larger specific surface area of the nanoparticles (Balogh et al., 2001). But effectiveness would also be accentuated if the nanoparticle protected the silver ions it carries from speciation reactions that would otherwise reduce bioavailability before the ions reach the organism. In that case, overall bioavailability from the nanoparticle could be much greater than for an equivalent number of free silver ions released to natural waters. Limbach et al. (2007) noted that nanoparticles, in general, could also be carriers for heavy metal uptake into human lung epithelial cells, accentuating the toxicity of the nanoparticle. They termed this a "Trojan horse-type mechanism." Moore (2006) also observed that the sucrose polyester nanoparticles carried with them another pollutant; the organic chemical poly-aromatic hydrocarbons (PAH). Uptake of the nanoparticles accelerated uptake of the PAH and increased its toxicity. It is conceivable that nanosilver particles could similarly act as a Trojan horse if they carried silver across the membrane via endocytotic processes and then released this toxin into the sensitive internal environment of the living cell.

It is also clear that nanosilver products can introduce silver to the human body. For example, silver colloidal solutions are promoted because they introduce silver to the bloodstream via intestinal uptake (Wadhera and Fung, 2005). Manufacturers claim that the silver circulates, eliminating germs in the blood, and is then excreted.[5] But deposition as silver chloride or silver sulfide in tissues certainly occurs to at least some degree, as evidenced by occurrence of argyria in people who take very high doses of the colloidal silver. It is unclear whether the form of silver in the blood in this circumstance is as a nanoparticle or a complexed silver ion. Innocuous deposition of silver metal in tissues is known, but circumstances that might result in carrying nanoparticles to organs where they might penetrate functional areas deserve greater investigation.

HOW DOES NANOSILVER MANIFEST ITS TOXICITY?

The environmental implications of the new nanosilver technologies will reflect the cumulative implications of exposure to the nanoparticles, and exposure to the toxic and persistent pollutant of which the nanomaterials are composed. As we have seen, the toxicity of silver metal has been studied, even though some aspects demand greater investigation. Whether silver occurring in the nanoparticle form poses additional risks remains poorly understood.

There is little question that many of the commercial products and medical devices containing nanosilver are toxic to bacteria, at least under ideal conditions. There is some controversy over the specifics, however. There is a

long history of positive experience with the effectiveness of silver in treatment of burns (Brett, 2006), with or without the addition of nanosilver. But questions arise about at least some claims about the effectiveness of using nanosilver to treat wounds. For example, Vermeulen et al. (2007) could find only three randomized controlled trials (in Cochrane's register of such trials) that rigorously tested efficacy of silver in treatment of wounds that were slow to heal (chronic wounds). The authors concluded that "there is insufficient evidence to recommend the use of silver-containing dressings or topical agents for treatment of infected or contaminated chronic wounds." At least some commercial products recognize the limitations of the new nanosilver treatments. For example, the literature accompanying a commercially available nano "silverhealing" bandage cites the broad-spectrum antibacterial effects of the silver ion that is released, but states the product "reduces the risk of infection from the very beginning, but cannot heal wounds that are already inflamed."

There is also little doubt that nanotechnologies can improve antibacterial capabilities compared to traditional uses of silver. One reason is that the new technologies allow manipulation of silver onto or into products where it could not be placed before (e.g., the lining of medical devices). The antibacterial function of the nanoparticle is to deliver silver ions to the bacteria in such locations. The characteristics of the nanosilver are also important. Correlations are found between the silver ion release rate of different formulations and toxicity to common bacteria such as *Escherichia coli* (Damm et al., 2008) or pathogens such as *Staphylococcus aureus* (Vallopil et al., 2007). Effectiveness is improved with reduced particle size. Smaller particles with larger surface areas deliver silver ions faster than larger particles with less surface area do (Lok et al., 2007). Aggregated particles with a reduced surface area also are not as effective as free particles. But formulations other than suspensions of free nanoparticles may also be effective antibactericides. For example, the silver composites studied by Balogh et al. (2001) were effective in maintaining silver in a form that can release the silver ion. The authors concluded that the nanosilver prevented complexation and/or precipitation of silver into unavailable forms before it contacted the bacteria.

The body of research on the antibacterial nature of various formulations of silver nanomaterials is growing rapidly as the quest for new applications continues. The research search engine Scopus found an average of 143 papers per year between 2002 and 2007 when queried with "silver" and "bacteria." The number of papers averaged half that between 1992 and 1997. A search of research specifically on nanotechnology related to silver and bacteria using the International Council on Nanotechnology (ICON) website returned 95 research papers published between 2003 and 2007. Further systematic study is needed, however, on dose response, how efficacy relates to particle properties, the influences of the exposure medium and the mechanisms by which nanosilver accentuates antibacterial capabilities (Brett, 2006).

The Royal Society (2004) identified free engineered or discrete nanoparticles as posing the greatest environmental risks. Most studies to date have focused on such free nanoparticles, and found some consistencies in the mechanisms by which they are toxic (see, for example, Text box 14). The data available suggest that the composition of such particles is also a consideration (Hussain et al., 2005; Lee et al., 2007), but that has received less emphasis. Even if the particle is not intrinsically toxic in the case of

nanosilver, the metal ion it releases is itself potentially a concern if the particle breaks down inside cells. For example, Hussain et al. (2005) found that ROS accumulation (Text box 14) in isolated liver cells from rats (in vitro) was accelerated more by nanosilver exposure than by exposure to nanoparticles of other compositions. They compared the toxicity of silver nanoparticles to that of nanoparticles of molybdenum, aluminum, iron oxide and titanium dioxide. The liver cells were exposed to the nanoparticles for 24 hours. Concentrations of 10–20 ppm nanosilver elicited increased ROS generation, but much higher doses were needed before other metals elicited similar effects. Among the other materials, molybdenum oxide was moderately toxic, but iron, aluminum, manganese and titanium oxides displayed less or no toxicity at the doses tested. The experiment could not resolve whether the presence of the nanoparticle or the release of silver was the ultimate cause of toxicity, but it was clear that particle composition (the presence of silver in the nanoparticle) affected toxicity.

As discussed earlier, most authors conclude that the toxicity of silver metal to humans is limited to local cell disruption, effects associated with extreme doses and/or the development of argyria. Whether these conclusions carry over to nanosilver is not known. If transport of nanosilver particles occurs in the bloodstream (Hollinger, 1996), then accentuation of silver ion release in damaging situations seems worth further investigation, based upon in vitro studies (e.g., Hussain et al., 2005). Similarly, if silver induces cell damage, thereby slowing the healing of wounds, it is important to determine to what extent nanosilver potentiates such effects. The decades of successful experience with silver in burn units with no evidence of cytotoxic effects (Brett, 2006) has not yet been achieved with nanosilver in the diverse uses for which it is being proposed.

Indirect effects on human health from improper use of nanosilver technologies might also deserve investigation. The effects of indiscriminately eliminating beneficial bacteria by long-term exposure to silver (whatever the form) may be important, but so far remain largely unaddressed. Sawosz et al. (2007) studied effects of ingestion of colloidal silver on the microflora and cell structure of the gut of quail. Exposures were for only 12 days at concentrations up to 25 ppm. At the highest doses, researchers saw increases in populations of lactic acid bacteria in the gut, but otherwise no major

Text box 14. Mechanisms of nanoparticle toxicity to cells

To evaluate toxicity to humans, cells cultured outside the body (in vitro tests) are often used. Such studies show evidence for altered behavior and toxicity in the nano range for many types of particulate material (Owen and Handy, 2007). One cause of nanomaterial-induced toxicity consistently found in such studies is generation of reactive oxygen species, or ROS (Oberdorster et al., 2005, 2007). Reactive oxygen species are generated as by-products of normal cellular function, but the cell's antioxidant defenses break them down. ROS accumulate when those antioxidant defenses are harmed or otherwise cannot keep up with ROS generation. ROS are harmful because they can damage cell membranes (membrane lipid peroxidation), leading to problems with transport systems. ROS can also affect the way proteins assemble and cause them to fragment, and they can cause damage to DNA (Ngo et al., in press).

disruption of gut microflora and no cell damage. Given the number of people chronically ingesting colloidal silver, and proposals to feed colloidal silver to animals commercially grown for human consumption, further systematic investigation of such effects seems warranted. Effects on human skin of chronic contact with silver-impregnated products may warrant consideration. Bacteria typically live on the skin in a harmless, mutually beneficial relationship, without causing any infection. Only when the skin is broken or damaged are bacteria associated with a risk of infection. If the normal bacterial flora are beneficial in preventing colonization by pathogens, or if they moderate the presence of other potentially dangerous organisms on the skin, then disruption of those benefits would deserve investigation.

In vivo studies of nanoparticle toxicity with living, higher-order organisms are just beginning. We know from experience with other contaminants that dose response in classical toxicity tests is difficult to extrapolate directly to toxicity in nature, yet this remains the dominant approach in the early experiments addressing ecological effects of nanosilver. Lee et al. (2007) cited some of the specific limitations of the approaches used to date, suggesting the science is unnecessarily revisiting approaches with serious constraints. For example:

1. Test species are typically dosed with much higher concentrations and for much shorter periods than would be expected in contaminated natural settings. Methodologies exist for tests that fully consider a stage in the life cycle or exposure from diet, but remain uncommon.

2. Studies with whole living organisms (in vivo) remain rare in the study of nanoparticles. An in vitro test with isolated cells is a powerful approach to address mechanisms and likelihood of toxicity, but it cannot address dose response. Realistic in vivo tests are necessary to determine what concentration in nature will be toxic.

3. Interdisciplinary study is essential. Nanoparticles can aggregate or change form during the experiment (Hussain et al., 2005), affecting exposure and effects. It is essential that nanoparticles be physically characterized and that any effects of residual chemicals added to promote stability be understood (Limbach et al., 2007; Asharani et al., 2008).

4. Nanomaterials are sometimes injected into organisms in vivo to avoid delivery issues like aggregation. This approach is highly invasive. Most important, it does not address bioavailability, distribution or transport within the organism (Lee et al., 2007).

Short exposures to high concentrations are an example of a pragmatic trade-off made to assure effects are observed, and that the experiment is completed on a timescale practical to the investigator. Such screening tests can show toxicity from nanoparticles is possible, but are ineffective in addressing the questions about effects in the ng/L range that will be most important for understanding implications of nanosilver. For example, Asharani et al. (2008) conducted one of the first studies of nanosilver effects on development of embryos of zebrafish. The experiment was informative in showing nanosilver accumulation into the embryo, occurrence of toxicity and how toxicity was expressed, but it was less useful in defining what concentrations might be of concern. The lowest exposure considered was 5,000 ng/L, at which some effects

were apparent. The exposure period was also not sufficient to include full development of all embryos.

In contrast, Lee et al. (2007) avoided the limitations associated with high concentrations, exposures of a limited duration and aggregation in tests of nanosilver toxicity to the embryos of zebrafish (see also Text box 13). They bathed a sensitive stage of the embryo in water chemically similar to the physiological environment of the embryo that was spiked with nanosilver. The particles were formulated to avoid aggregation and retained an 11 nm diameter during the experiment. The tests were conducted on a sensitive developmental stage of the organism and for the entire developmental period from the embryo to adult, which in zebrafish is only 120 hours. They used concentrations of nanosilver that were realistic in terms of expectation for contaminated waters. Their findings suggest nanosilver toxicity to zebrafish reproduction is feasible at environmentally realistic concentrations. For example, maturation of the zebrafish embryo was normal at 8 ng/L but was affected at the next highest dose levels that were used. Lee and colleagues (2007) conclude that "as nanoparticle concentration increases, the number of normally developed zebrafish decreases, while the number of dead zebrafish increases. As nanoparticle concentration increases beyond 19 ng/L, only dead and deformed zebrafish are observed, showing a critical concentration of Ag nanoparticles in the development of zebrafish embryo. … The number of deformed zebrafish increased to its maximum as nanoparticle concentration increased to 19 ng/L and then decreased as nanoparticle concentration increased from 19–71 ng/L because the number of dead zebrafish increased."

Different abnormalities also occurred as dose increased. Fin abnormalities and spinal cord deformities occurred at the lowest effective doses (beginning at 19 ng/L). Malformation of the heart and swelling (edema) of the yolk sac occurred at the next highest doses. At 44–66 ng/L, swelling of the head and eye abnormalities occurred. Both quickly resulted in death. Eye abnormalities included eyelessness and undeveloped optic cups with no retina or lens. Asharani et al. (2008) observed similar effects on zebrafish at the much higher doses.

Several aspects of the results of Lee et al. (2007) are especially important:

- Toxicity followed a dose response defined by the total concentration of silver. The number of particles or particle surface area may have co-varied with total silver, but total silver in the nanoparticles defined the effective dose adequately.

- Nanosilver toxicity occurred within a range of total silver concentrations that might be expected in contaminated natural waters (ng/L). Thus, the ng/L range expected in contaminated environments does not exclude the potential for adverse effects.

- Toxicity occurred at concentrations that are toxic to reproduction in other organisms when exposed to silver metal.

- The exposure to nanosilver affected the developmental process in the organism—a result similar to that observed for silver itself when accumulated from diet in other species (Hook and Fisher, 2001). Deformities of this nature are also typical of toxins that associate strongly with sulfhydryl groups and thereby influence the tertiary structure of proteins, an effect typical of silver metal.

- Toxicity testing across the range of concentrations and over time periods that are meaningful in natural waters was shown to be feasible.

A body of work will be necessary for definitive conclusions with regard to the concentrations at which nanosilver is toxic and the effects that might be expected. But it is clear that sophisticated alternatives to traditional tests of acute mortality from exposures in water at the ppm level are available. Such tests should be given the enhanced credibility they deserve as regulatory decisions are made.

Like Hussain et al. (2005), Lee et al. (2007) could not differentiate whether the adverse effects observed were caused by the nanoparticle or by the silver. Asharani et al. (2008) found nanosilver exposures were more toxic than equivalent silver ion exposures. But their seawater was made with natural sea salt, which would probably contain high concentrations of organic materials capable of complexing the silver ion and reducing its toxicity. Many of the signs of stress were consistent with silver toxicity in all these experiments, but that does not eliminate the possibility that nanosilver might potentiate the effects of the silver ion; as might occur if the nanoparticle acts as a vehicle to deliver the silver to the interior of cells (see earlier discussion). Moore (2006) also discussed delivery of contaminants into cells via the nanoparticle "container." These authors note that "exploitation of the … endocytotic routes of entry to the cell may allow pollutant nanoparticles to embed themselves within the functional machinery of the cell in ways that are toxicologically quite different from conventional toxic chemicals. Nanoparticles situated in the (organelles like) endoplasmic reticulum, Golgi, and endolysosomal system could conceivably act as foci for oxidative damage that could not be readily expelled from the cell and generation of radicals could lead to organelle dysfunction."

As noted earlier, complexation of the dissolved silver ion with sulfides or organic materials limits the silver available for transport across the membrane. But if organisms can mistakenly take up "a container of potential silver ions," then these natural protections are bypassed. Such a mechanism is supported by observations that both solubility and toxicity are retained in silver nanocomposites, even in the presence of sulfate or chloride ions (Balogh et al., 2001). Once trapped within a cell, nanosilver may deliver silver ions directly into the cell machinery. Much more study of this potential Trojan horse delivery system is necessary, but it is an interesting example of potential interactive implications of building nanomaterials with unique physical attributes from chemicals with known toxicity.

Finally, toxicity can be expected only if uptake occurs faster than the sum of excretion plus detoxification, whether it is silver, nanosilver or an interaction of the two that is toxic. Detoxification reactions in humans and invertebrates are known for silver. Silver can be taken up in high concentrations into the bodies of some bivalves, as it can in humans. But in some species, most of it is deposited in nontoxic, insoluble silver sulfide granules in basement membranes, away from crucial cell machinery (Berthet et al., 1992). In others, such mechanisms are less effective and animals are more vulnerable to toxic effects. For example, scallops are more vulnerable than oysters to silver toxicity because scallops detoxify a smaller proportion of the silver taken up (Berthet et al., 1992). Differences in detoxification capabilities among species remain a crucial uncertainty for most organisms, however.

IV. THE WAY FORWARD: CONCLUSIONS AND RECOMMENDATIONS

Silver is an effective antibacterial agent with a long history of use, but it also has a long history as an environmental pollutant. Nanotechnologies offer the potential for dramatic improvements in both traditional and new uses of silver. Great potential exists for invaluable uses in medical devices and water purification, to name two. But it is naïve to assume benefits will come from every imaginable nanosilver product without potential to cause harm. The sophistication of this new nanotechnology and its proliferation (largely uncontrolled) raise new questions of health and environmental impact.

The unique properties of nanomaterials present formidable challenges, both in terms of technical understanding (science) and of policy decisions (how to use the technology safely). Nanosilver illustrates the added challenge when nanoproducts are composed of materials that can be toxic themselves, at least in certain circumstances. Institutions need to rise to the challenges posed by these new combinations of physical and chemical traits, if safe, sustainable and beneficial nanotechnologies are to flourish.

Ultimately, policy decisions must be science based. As this report has shown, there is a wealth of knowledge on silver in the environment, and this knowledge provides a starting point for science-based decision making. We cannot afford to fall into the trap of assuming that because nano is new, we have no basis for managing its impacts. But nano does raise new questions, and a research strategy is necessary to address them. Some questions will need long-term exploratory research before answers are found. But opportunities also exist to address other questions in a timelier manner, if research is strategically targeted. Alone, neither bottom-up, principal investigator–led research nor a top-down wish list of research needs is likely to result in adequately targeted studies. A strategy is defined by mapping out what knowledge is needed and how we are to generate it, as well as identifying both basic research needs and immediate opportunities.

Addressing nanosilver specifically, the Owen-Handy (2007) framework is useful for characterizing the state of knowledge and thereby identifying where basic knowledge is sufficient to identify shorter-term opportunities for progress. The information generated by the source-pathway-receptor-impact framework also aligns well with the hazard and exposure data needed for risk assessment and management. Table 7 outlines some priority research goals that fall into that category of opportunities. An agenda that addressed these needs would quickly position better understanding and regulation of the impact of nanosilver. But that agenda is not short. Significant investment will be necessary to address just the immediate opportunities available to better manage this one set of nanoproducts.

History teaches us that a balance is needed between targeted, goal-driven research and research that is more exploratory. Understanding mechanisms, in the long term, will uncover the unasked questions and lead to

TABLE 7*. Critical research and (in some cases) policy goals for ensuring rapid improvements in the safe use of nanosilver.

	Goal
Source	Develop terminologies that will allow nanosilver physicochemical properties to be related to behavior, and potential impact
	Classify products by their potential to lead to human exposure and dispersal of silver in the environment
	Establish consistent registration and reporting requirements for nanoproducts, and thereby begin generation of data from different sources of nanosilver and silver.
	Quantify silver loads to the environment (and the form of the loading) from individual nanosilver products as well as cumulative loads as the number of products grows.
Pathways	Develop methods to detect and programs to monitor nanosilver, or silver as its surrogate, in natural waters, sediments and soils.
	Mobility, persistence and transformation: Investigate the physical/chemical interactions of different nanosilver formulations in natural waters, sediments and soils. Important data gaps include knowledge of stability of different types of nanosilver on long timescales, effects of water chemistry on reactivity and bioavailability, as well as the likelihood and nature of associations with natural particulates.
Receptor	Understand if and how nanosilver particles penetrate the membranes of higher-order organisms. Understand how particle characteristics affect transport.
	Adapt existing methodologies and compare uptake rates from diet and water, as well as rate constants defining excretion for critical species and cell lines. How efficiently is nanosilver transferred from prey to predator via diet? Compare rates between nanosilver and silver or among formulations of nanosilver (bioaccumulation).
	Investigate whether different physicochemical reactions in the environmental influence the bioavailability of nanosilver. In particular, do nanosilver particles deliver silver ions directly to nontarget organisms and thereby increase bioavailability by protecting the silver ions from speciation reactions that reduce bioavailability?
	Determine if biological traits influence bioaccumulation of nanosilver and thereby make some species more likely than others to accumulate high levels of the nanomaterials.
Impact	Understand the implications or impacts of nanoparticles once they are inside cells (in vitro). Questions include: • How stable are particles in the intracellular environment? • Do mechanisms for detoxification exist and what controls the rates? • What is the nature of the disturbance (ROS generation, DNA instability, disruption of reproduction or successful development) • Is the disturbance from the particle itself or the release of silver? • Can particles be excreted once they penetrate into a cell?
	In humans, are there direct effects from uptake of nanosilver into the bloodstream? Does nanosilver slow wound healing, and how is that balanced by improved antibacterial activity? Are there indirect effects on human health from exposure to silver products, such as skin disturbances from disruption of bacterial populations? Are there intestinal problems from ingestion of nanosilver or from disruption of bacteria in the gut or collection of nanodebris? What are the implications of transporting nanosilver particles in the bloodstream? Is deposition of nanosilver similar to deposition of silver itself?

*While some goals will be achievable sooner and some later, these are all opportunities for relatively rapid advancement. Research should begin as soon as possible, within the framework of a nanotechnology risk research strategy, if science-based decisions are to be made on the safe use of nanosilver.

unforeseen solutions. But where our basic knowledge points to clear questions, we must take advantage of immediate opportunities to obtain information critical to decision making in the short and medium terms.

Implementing long-term basic research and exploiting short- and medium-term opportunities is not enough, however. Adequately addressing the challenges presented by a rapidly growing silver nanotechnology will require a broader plan, the ingredients of which are so far in short supply. That plan must be characterized by interdisciplinary collaboration at an unprecedented level; an investment of resources comparable to the potential for economic benefits from the new opportunities; collaboration among agencies, stakeholders and universities; and international collaboration that involves sharing of talent and resources. Linkage between research and decision making is also fundamental to moving policies for managing environmental risks forward as fast as the growth of the commercial uses. Translating research into decisions in government, industry and among consumers remains a challenge in all of environmental science. But progress in this regard is essential if we are to learn and to teach others how to use nanosilver wisely. Nanosilver is only one of a plethora of nanotechnologies rapidly advancing into the commercial market.

In moving forward, there are a number of obvious needs where research and policy connect:

- Integrate nanosilver risk research needs into a unified, multi-agency, stakeholder-vetted nanotech dialogue. Participation of all interested parties in defining the questions and timetables is important. Generating the interest of talented scientists from the private sector, the agencies and the universities in working across historically sacred boundaries is essential.

- Assign responsibilities, resources and timelines for implementing the research strategy, and clearly identify mechanisms that will lead to better and more effective translation of the new knowledge into decision making.

- Integrate research among international research programs to leverage resources and ensure timely and relevant progress. This must include finding ways to cross what often seem to be impenetrable impediments to resource sharing among international institutions.

- Develop and share appropriate terminologies to underpin research and oversight.

- Define clear rules for defining a product's ingredients that take into account its unique physical and chemical attributes. Use that information to track production, use and environmental release/dispersal data.

- Assess what information is needed to oversee safe use of nanosilver, over and above that for managing the impact of ionic silver (or non-nanosilver).

- Assess the relevance and shortcomings of current silver-relevant regulations.

The unique properties of nanomaterials present some formidable technical obstacles to better understanding environmental and health risks (Maynard et al., 2006; Oberdorster et al., 2005). The knowledge base is limited, the technical challenges are great and the growth of commercial applications is rapid. But as we

have shown in the case of nanosilver, existing knowledge provides a powerful baseline from which to identify research priorities and begin making scientifically defensible policy decisions. Systematic evaluation of that baseline for a number of specific nanomaterials might be a first step. The sophisticated advances in engineering nanosilver products have created new challenges to accompany the new opportunities. All institutions need to rise to these challenges if we are to see the benefits the new technologies promise.

APPENDIX A

DETAILS OF CALCULATIONS FOR DIFFERENT SCENARIOS OF SILVER RELEASE INTO THE ENVIRONMENT.

(SEE TEXT BOX 11 FOR AN EXPLANATION OF THE CALCULATIONS.)

	C	$M_{product}$	C_{silver}	N	X	$N_{product}$	$F_{product}$	Per product $M_{Psilver}$	P	$M_{10(0)}$ products
USA	(µg/g)	(g)	(g)	(# people)		(units of product)	per yr.	(T/yr)	(#)	(T/yr)
Socks - 10 pair/person	10	100	0.001	300,000,000	0.1	30,000,000	0.2	0.01	100	0.6
10% of population			0.031	300,000,000	0.1	30,000,000	0.2	0.19	100	18.6
Socks - 10 pair per person	10	100	0.001	300,000,000	0.5	150,000,000	0.2	0.03	100	3
50% per year			0.031	300,000,000	0.5	150,000,000	0.2	0.93	100	93
Socks - 10 pair per person	10	100	0.001			1,000,000	0.2	0.00	100	0.02
1 million people			0.031			1,000,000	0.2	0.01	100	0.62
Washing machines			0.05			1,000,000	1	0.05	10	0.5
Washing machines			0.05			57,000,000	1	2.85	10	28.5
Swimming pools	10	10	10			1,000,000	1	0.03	10	0.03
Palo Alto										
Socks	10	100	0.001	250,000	0.1	25,000	0.2	0.00	100	0.0005
				250,000	0.1	25,000	0.2	0.00	100	0.0155
Washing machines			0.05	250,000	0.5	125,000	0.2	0.001	10	0.0125
			0.05	250,000	0.2	50,000	0.2	0.001	10	0.005
South Bay										
Socks - 10 pair/person	10	100	0.001	200,000	0.1	2,000,000	0.2	0.00	100	0.004
10% of population			0.031	200,000	0.1	2,000,000	0.2	0.001	100	0.124
Socks - 10 pair per person	10	100	0.001	200,000	0.5	1,000,000	0.5	0.001	100	0.05
50% per year			0.031	200,000	0.5	1,000,000	0.5	0.016	100	1.55
Socks - 10 pair per person	10	100	0.001	200,000	0.5	1,000,000	0.2	0.000	100	0.02
1 million people			0.031	200,000	0.5	1,000,000	0.2	0.006	100	0.62
Washing machines			0.05	200,000	0.2	400,000	1	0.020	10	0.2
Washing machines			0.05	200,000	0.1	200,000	1	0.010	10	0.1
Swimming pools	3	1000	0.03			1,000,000	1	0.030	10	0.3
	C	**$M_{product}$**	**C_{silver}**	**N**	**X**	**$N_{product}$**	**$F_{product}$**	**Per product $M_{Psilver}$**	**P**	**10 Products $M_{TUSAsilver}$**

$M_{product}$ is mass of product. $M_{Psilver}$ is mass of silver in product. N is population of the area of interest (in this case, 300 million is used for simplicity), N_{PA} is 250,000 people for the city of Palo Alto; N_{SV} is 2 million people for Silicon Valley. X is fraction of the population using the product. $N_{product}$ is the number of products. $F_{product}$ is the fraction of silver in the product released per year. P is the number of products. $M_{Tsilver}$ is the total silver released to the environment.

ENDNOTES

1. Scorecard: The pollution information site. Available at http://www.scorecard.org/chemical-groups/one-list.tcl?short_list_name=pp USEPA, Office of Water. Water Quality Standards Database. Available at http://www.epa.gov/wqsdatabase/
2. In 2007, the market for nanotechnology-based products totaled $147 billion. Lux Research projects that figure will grow to $3.1 trillion by 2015 (Lux, 2008;).
3. Available online at www.nanotechproject.org/inventories/consumer/
4. Available online at http://www.nanotechproject.org/inventories/consumer/
5. Available online at http://www.newstarget.com/020851.html
6. http://www.silver-colloids.com/Tables/Experiment.PDF
7. Environmental Quality Standards for the European Union Available at http://www.wecf.de/cms/articles/2006/07/eu_statements.php
8. Pictures are available online at http://images.google.co.uk/images?hl=en&q=argyria&um=1&ie=UTF-8
9. Available at http://www.azonano.com/news.asp?newsID=2172
10. Available at http://www.purestcolloids.com/bioavailability.htm
11. Available at http://www.ici.org/shareholders/dec/05_news_equity_rpt.html
12. Available at http://www.pesticideinfo.org/DS.jsp?sk=65011#working
13. For example http://bio-alternatives.net/buysilver.htm?gclid=CJC02Jn 8u48CFQVrgwod2AxteA
14. Available at http://www.jrnanotech.com/index.html.
15. Available at www.nntech.com.
16. Available at http://bio-alternatives.net/buysilver.htm?gclid=CJC02Jn8u 48CFQVrgwod2AxteA .
17. The simplest explanations of these processes may be found online at http://en.wikipedia.org/wiki/Endocytosis and http://en.wikipedia.org/wiki/Endosome

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Size Dependent and Reactive Oxygen Species Related Nanosilver Toxicity to Nitrifying Bacteria

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The intrinsic slow growth of nitrifying bacteria and their high sensitivity to environmental perturbations often result in cell growth inhibition by toxicants. Nanoparticles are of great concern to the environment because of their small size and high catalytic properties. This work sought to determine size-dependent inhibition by Ag nanoparticles and evaluate the relationship between the inhibition and reactive oxygen species (ROS). Nanoparticles with an average size range of 9−21 nm were synthesized by varying the molar ratios of BH_4^-/Ag^+ in the solution. The resulting ROS generation was quantified in the presence and absence of the bacteria while the degree of inhibition was inferred from specific oxygen uptake rate measurements, determined by extant respirometry. By examining the correlation between nanoparticle size distribution, photocatalytic ROS generation, intracellular ROS accumulation, and nitrification inhibition, we observed that inhibition to nitrifying organisms correlated with the fraction of Ag nanoparticles less than 5 nm in the suspension. It appeared that these size nanoparticles could be more toxic to bacteria than any other fractions of nanoparticles or their counterpart bulk species. Furthermore, inhibition by Ag nanoparticles as well as other forms of silver (AgCl colloid and Ag^+ ion) correlated well with the intracellular ROS concentrations, but not with the photocatalytic ROS fractions. The ROS correlations were different for the different forms of silver, indicating that factors other than ROS are also important in determining nanosilver toxicity.

Introduction

While nanotechnology has great potential for beneficial environmental uses (*1–5*), the explosion of nanotechnology-enhanced products raises concerns regarding the adverse effects of nanoparticles on human health and the environment (*6–8*). Nanoparticles with their high surface/volume ratio are more reactive with the increased catalytic properties (*9*) and therefore could become more toxic than the bulk counterpart (*10*).

Nanosilver (Ag nanoparticle) is one of the most commonly used nanomaterials because of its strong antimicrobial activity (*10–12*). The mechanisms by which Ag nanoparticles kill microorganisms are, however, largely unknown and the mode of antimicrobial action by nanosilver is not clear. Possible mechanisms by which Ag nanoparticles inhibit microbial growth include particle attachment to cell membranes, causing the changes of membrane permeability and redox cycle in the cytosol, intracellular radical accumulation, and dissipation of the proton motive force for ATP synthesis (*10, 12–14*). Evidence from scanning transmission electron microscopy also shows that smaller particles (<10 nm) may enter the cell directly to inhibit microbial growth (*12*). For comparison, the inhibitory effect of Ag^+ is believed to be due to its sorption to the negatively charged bacterial cell wall, generating reactive oxygen species and deactivating cellular enzymes, disrupting membrane permeability, and ultimately leading to cell lysis and death (*15–17*).

The reactive oxygen species (ROS) including singlet oxygen ($^1O_2$), superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH•) are generated constantly through *exogenous* (extracellular) and *endogenous* (intracellular) processes as part of aerobic life on the earth (*18*). While singlet oxygen is often generated following absorption of energy (light), other ROS are formed at the one-electron steps in oxygen reduction (*19*):

$$O_2 \xrightarrow{e^-} O_2^{-\cdot} \xrightarrow{e^- + 2H^+} H_2O_2 \xrightarrow{e^-} OH^- + OH^{\cdot} \xrightarrow{e^- + 2H^+} 2H_2O \quad (1)$$

Exogenously, nanoparticles of TiO_2 and ZnO with large surface areas and highly reactive catalytic sites can produce photocatalytic ROS in the presence of near-UV light (*20, 21*). Even without UV light irradiation, nanoparticles of transition metal oxides were capable of generating ROS monitored through fluorescence measurements (*9*), and Ag nanoparticles were able to produce ROS detected by electron spin resonance spectroscopy (*22*).

Endogenous ROS are produced inside the cells. Normally cells are able to reduce oxygen to water through their eletron transport chains and protect themselves from ROS damage through the use of enzymes such as superoxide dismutases (SOD, to convert superoxide to hydrogen peroxide) and catalases (to convert hydrogen peroxide to water and oxygen) (*23, 24*). Under unfavorable environments such as hypoxia or in the presence of toxins, oxidation stress occurs and endogenous ROS accumulation can damage cellular constituents and disrupt cell functions. Recent studies suggested that ROS generation by Ag nanoparticles or Ag^+ ions is responsible for the strong bactericidal activity (*22, 25*), although a quantitative estimation was not carried out.

The objectives of this study were to quantitatively determine the size dependent nanosilver toxicity to nitrifying organisms and evaluate the relationship between the cell growth inhibition and reactive oxygen species (ROS). Ag nanoparticles of different sizes were synthesized to evaluate the size effect on microbial growth. The photocatalytic and intracellular ROS concentrations due to silver exposure were determined and correlated with nanosilver toxicity. Nitrifying bacteria were chosen as model microbes because they are sensitive to a number of environmental conditions such as pH, dissolved oxygen concentration, and temperature, and are therefore susceptible to inhibition (*26*). A quantitative description of the nanoparticle sizes and ROS production that may affect nanosilver toxicity will ultimately contribute to a mechanistic understanding of nanosilver toxicity for appropriate use and disposal of the nanotechnology-enhanced products in the environment.

Materials and Methods

General Experimental Design. Aliquots of nanosilver suspensions of different sizes and concentrations were spiked in enriched nitrifying cultures for microbial growth inhibition tests. Aliquots of the same suspensions were used for UV/vis

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characterization and size distribution analysis by transmission electron microscopy. ROS formation following exposure to Ag nanoparticles was determined in the presence and absence of nitrifying cultures. For comparison purposes, Ag^+ ions and AgCl colloids (average size = 0.25 μm) were used as reference materials. The results of nanoparticle sizes and ROS measurements were correlated with the degrees of inhibition by Ag nanoparticles, AgCl colloids, and Ag^+ ions.

Nitrifying Culture. Nitrification involving the oxidation of ammonium ions to nitrites and nitrates by nitrifying bacteria is commonly observed in municipal wastewater treatment plants and the natural realm. Enriched nitrifying bacteria were cultivated for more than 180 days in a continuously stirred tank reactor (14 L) operated at solids retention time (SRT) of 20 days and hydraulic retention time (HRT) of 1 day as described earlier (*27*). The reactor was fed with an inorganic medium containing ammonium (8.3 mM, NH_4NO_3) as the sole energy source and requisite macro- and micronutrients. Low concentrations of anions such as chloride and sulfate were present in the reactor to minimize their complexation potential with Ag^+ ions. Sodium carbonate (0.5 M) was intermittently added to maintain the reactor pH at 7.5 ± 0.1 and fulfilled both carbon and alkalinity requirements. The effluent concentrations of NH_4^+-N and NO_2^--N (<1 mg/L) and NO_3^--N (~ 380 mg/L) indicated complete nitrification. The nitrifying cultures were periodically withdrawn from the reactor for ROS measurements and inhibition tests.

Silver Nanoparticles and Silver Bulk Species. Silver nanoparticles were made from 0.25 mM $AgNO_3$(EM Science) by adding different concentrations (0.025, 0.05, 0.09, 0.15, 0.3 mM) of sodium borohydride ($NaBH_4$, Sigma) with polyvinyl alcohol (PVA) as a capping agent. Sodium borohydride was added into a 0.06% (wt) PVA solution, and silver nitrate was then rapidly injected at room temperature. The freshly prepared nanoparticle suspensions were referred to as parent suspensions and used for size characterization and cell growth inhibition studies.

Silver nitrate (Fisher Scientific) was used to provide free Ag^+ ions in the solution. Silver chloride colloids were prepared by vigorous mixing (700 rpm) 1 mL of a 14 mM silver nitrate standard solution and 1 mL of a 28 mM sodium chloride solution with 18 mL of distilled water.

Nanoparticle Characterization. Aliquots of the prepared nanosilver suspensions were periodically scanned from 250 to 700 nm to check the characteristic surface plasmon absorption band of Ag nanoparticles at approximately 400 nm using a UV−vis spectrophotometer (Cary 50, Varian, CA). The concentrations of Ag nanoparticles in the suspension were inferred from the difference between the measured concentrations of Ag^+ ions using a silver ion/sulfide selective electrode (Denver Instrument, CO) and the total Ag^+ ions added initially. Transmission electron microscopy (TEM, JEOL 1400) was utilized to identify the nanoparticles and determine their size distribution. The nanosilver suspension was added to a standard carbon-coated TEM grid and images of the samples were taken at an accelerating voltage of 100 keV. The histograms of nanosilver size distribution were generated from TEM images using ImageJ, a free, Java-based image processing package available at http://rsb.info.nih.gov/ij/.

Cell Growth Inhibition Measurements. Aliquots of nitrifying cultures (60 mL) were collected from the nitrifying bioreactor and the microbial growth rates were inferred from specific oxygen uptake rate measurements in triplicate using a batch extant respirometric assay (*26*). MOPS [3-(*N*-morpholino) propanesulfonic acid] was added to maintain the solution pH at approximately 7.5 during ammonium oxidation. The biomass suspensions were amended with Ag nanoparticles, AgCl colloids, or Ag^+ ions at final total concentrations of 0.05−1 mg/L Ag. Every batch respirometric test was accompanied by a positive control (e.g., untreated nitrifying biomass only). The biomass suspensions were aerated with pure oxygen gas before NH_4^+-N (10 mg N/L as NH_4NO_3) was added. A decrease in the dissolved oxygen (DO) level in the respirometric vessel due to nitrification was measured by a DO probe (YSI model 5300A, Yellow Springs, OH) and continuously monitored at 4 Hz by an interfaced personal computer. The inhibition of nitrifying bacterial growth, or nitrification inhibition, was inferred from the difference between the measured specific oxygen uptake rate in the absence ($SOUR_{control}$) and presence ($SOUR_{sample}$) of the Ag species (eq 2), as reported earlier (*26*).

$$\%\text{inhibition} = \frac{(SOUR_{control} - SOUR_{sample})}{SOUR_{control}} \times 100\% \quad (2)$$

Intracellular ROS Determination. Intracellular ROS concentrations were determined using an established fluorescence assay with modification (*9*). Aliquots of nitrifying cultures were removed from the bioreactor and centrifuged at × 10 000 rpm for 15 min. The pellet was then resuspended in a loading buffer solution containing 10 μM H_2DCFDA (dichlorodihydrofluorescein diacetate, Invitrogen, OR), 20 mM MOPS, 1 mM NH_4NO_3, trace metals, and 4 mg/L K_2HPO_4 to mimic normal growth and incubated at room temperature for 30 min. After the loading buffer solution containing H_2DCFDA was removed via centrifugation, the pellet cells were inoculated with prewarmed growth medium, amended with nanosilver (average size: 15 nm) or silver bulk species at predetermined concentrations, and plated into 96-well plates. The fluorescence of the cells from each well was measured using a microreader (VICTOR^3, PerkinElmer, CT) with 485 nm excitation and 535 nm emission filter. Fluorescence data were taken automatically after 30 min incubation. Hydrogen peroxide (30%, Fisher Scientific) was used as a standard for ROS measurements (see Supporting Information Figure S1), and intracellular ROS concentrations due to silver exposure were determined in H_2O_2 unit.

Photocatalytic ROS Determination. Photocatalytic ROS was measured using APF [3′-(p-aminophenyl) fluorescein], a new ROS indicator with greater specificity (mainly sensitive to $OCl^-$and $OH\cdot$) and higher resistance to light-induced oxidation than H_2DCFDA. According to the vender's protocol (Invitrogen, OR), superoxide and hydrogen peroxide fluoresce at 3 orders of magnitude lower than hydroxyl radical using APF. Therefore, photocatalytic ROS was mainly related to $OH\cdot$ production, which was determined as molar unit of OCl^- in the solution. The APF was added at a final concentration of 5 μM to a series of Ag nanoparticle (average size: 15 nm) or Ag bulk solutions of various concentrations (C = 0.05 to 1 mg/L Ag) and plated into a 96-well plate. Initial fluorescence of the solution in each well was measured using the microreader with 485 nm excitation and 535 nm emission filter described above. The 96-well microplate was placed on the laboratory bench under the room light for 30 min and the fluorescence was measured again. The percentage of fluorescence increase was calculated by comparing the fluorescence before and after light illumination as described previously (*28*). Hypochlorite (6% NaOCl, Fisher Scientific) was used as a ROS standard for photocatalytic ROS measurements (see Supporting Information Figure S2).

Results and Discussion

Size-Dependent Nanosilver Toxicity. Silver nanoparticles with an average size range of 9−21 nm were synthesized by varying the molar ratios (R) of BH_4^-/Ag^+ due to the changes of $NaBH_4$concentrations (Table 1). The synthesized Ag nanoparticles had different average sizes (Figure 1A), UV−visible spectra (Figure 1B), and size distributions (Figure

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Nanoparticle Silver Released into Water from Commercially Available Sock Fabrics

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Manufacturers of clothing articles employ nanosilver (n-Ag) as an antimicrobial agent, but the environmental impacts of n-Ag release from commercial products are unknown. The quantity and form of the nanomaterials released from consumer products should be determined to assess the environmental risks of nanotechnology. This paper investigates silver released from commercial clothing (socks) into water, and its fate in wastewater treatment plants (WWTPs). Six types of socks contained up to a maximum of 1360 μg-Ag/g-sock and leached as much as 650 μg of silver in 500 mL of distilled water. Microscopy conducted on sock material and wash water revealed the presence of silver particles from 10 to 500 nm in diameter. Physical separation and ion selective electrode (ISE) analyses suggest that both colloidal and ionic silver leach from the socks. Variable leaching rates among sock types suggests that the sock manufacturing process may control the release of silver. The adsorption of the leached silver to WWTP biomass was used to develop a model which predicts that a typical wastewater treatment facility could treat a high concentration of influent silver. However, the high silver concentration may limit the disposal of the biosolids as agricultural fertilizer.

Introduction

The burgeoning nanotechnology industry is quickly producing nanomaterials that are being incorporated into consumer products. As of 2007, the Project on Emerging Nanotechnologies at the Woodrow Wilson International Center for Scholars had compiled a list of more than 500 consumer products that claim to include some form of engineered nanoparticle (*1*). Of these products, about 20% contain silver nanoparticles. Socks, paints, bandages, and food containers incorporate nanosilver (n-Ag) to exploit its antimicrobial properties. In clothing such as socks, n-Ag may restrict the growth of odor-causing bacteria (*2*−*10*).

Despite the growing commercialization of n-Ag, little is known about the environmental effects of widespread use of products containing silver nanoparticles (*11*). *Ionic* silver is highly toxic to aquatic organisms (*12*−*14*), and the United States Environmental Protection Agency (USEPA) has set water quality criteria values for silver in salt and fresh water at 1.9 and 3.4 ppb, respectively. The USEPA has also instituted a secondary drinking water standard for silver of 100 ppb. Toxicity and exposure data for *nanoparticle* silver, however, is currently lacking (*15*−*17*). Studies have demonstrated the toxicity of nanoparticle silver to bacteria (*3, 5, 6, 8, 10*), suggesting that the antimicrobial effects of silver may be detrimental to aquatic ecosystems. Therefore, it is important to characterize (as colloidal or ionic) and quantify the silver released from commercial products.

The ubiquitous use of commercial products containing n-Ag could potentially compromise the health of many ecosystems. For example, household washing of clothing containing n-Ag may release silver into sewer systems. Since more than 70% of the U.S. population is served by public sewers (*18*), most of the n-Ag from consumer products would enter a municipal wastewater treatment plant (WWTP). The n-Ag present in sewage may partition onto wastewater biomass and be removed at a WWTP, only to re-enter the environment via agricultural land application of wastewater treatment biosolids. If n-Ag proves to be difficult to remove in a wastewater treatment system, n-Ag remaining in the treated effluent stream may enter surface water environments, potentially disrupting numerous biological ecosystems.

This paper investigates n-Ag release from commercial clothing (specifically, socks) into water, as well as the form of this silver and the adsorption characteristics that determine its fate in WWTPs. The amount of n-Ag in the sock fabric was quantified before determining the concentration and form (nanoparticle or ionic) of the silver released during repeated washings of the socks with distilled water. Batch adsorption isotherm studies were conducted with wastewater biomass and two sources of silver: (1) silver released from the socks into the wash water (nanoparticle or ionic), and (2) reagent ionic silver. These isotherms were then used with a partition model for wastewater treatment to evaluate the amount of silver that could be present in the treated effluent or WWTP biosolids.

Materials and Methods

Acid Digestions of Sock Fabric. Six brands of commercially available socks were purchased (Table 1) based on the manufacturers' claims that the socks contained nanoparticles of silver. A modified digestion method was used to quantify the amount of silver in the socks (EPA SW 846 Method 3050B). An air-dry mass (100− 500 mg-dry) of each sock was submerged in a solution of 5 mL of ultrapure reagent grade nitric acid (6901-05, JT Baker, Phillipsburg, NJ) and 5 mL of deionized water. After a watch glass was placed over the digestion beaker, the solution was heated to approximately 100 °C and allowed to react. Nitric acid was added in 2 mL aliquots until the bulk of the sock material was digested. The digestion solution was allowed to cool, and then 3 mL of 30% hydrogen peroxide (HX0635-2, EMD Chemicals Inc., Gibbstown, NJ) was added to complete the digestion process. Again the digestion beaker was heated to 100 °C, and hydrogen peroxide was added in 1 mL aliquots until effervescence was minimal, indicating completion of the digestion. The digestion solution was cooled, filtered through a glass fiber filter (Qualitative #2, Whatman) and diluted to 100 mL. Silver was quantified by inductively coupled plasma optical emission spectroscopy (ICP-OES iCAP 6000, Thermo Scientific).

Washing of Socks in Water. Socks were placed in 1 L amber glass bottles with 500 mL of ultrapure water (Millipore Inc.). The bottles were agitated for either 24- or 1-h contact times on an orbital shaker table at approximately 50 rpm. The 24-h contact time was chosen to allow sufficient opportunity for the socks to leach silver. The 1-h contact time is more representative of a "real world" washing machine cycle, though the quantities of leached silver from both

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TABLE 1. Sock ID/Characterization and Silver Content

				acid digestion analysis			wash analysis
sample ID	sock company	description (color)	price per pair	mass of silver per mass of sock (μg Ag/g sock)	average sock mass (g)	total silver in sock (μg)	cumulative silver released after 4 24-h washings (μg)
1a	Sharper Image	lounge sock (green)	$2.47	25.8	29.3	756	836
1b	Sharper Image	lounge sock (blue)	$2.47	57.8	27.3	1578	1845
2	Sharper Image	athletic (white)	$1.65	2.1	28.6	60	bdl
3	Fox River (Xstatic)	casual (black)	$13	1358.3	23.0	31,241	165
4	Arctic Shield (E47)	over-the-calf boot sock (green)	$14	35.9	58.6	2104	bdl
5	Zensah	basketball (black)	$13	bdl	24.2	bdl	bdl
6	AgActive London	casual (black)	£6.99	0.9	21.9	20	19

contact time experiments are comparable. After the specified contact time, the socks were removed, excess water was wrung out into the glass bottle, and the socks were placed in new bottles for the next washing. Each brand of sock was washed at least 3 consecutive times for either 1 or 24 hours. All 1 L glass bottles were acid washed with 10% HCl (HX0603-3, EMD, Gibbstown, NJ) or 10% HNO_3, triple rinsed with distilled water, and air-dried prior to use.

One brand of sock (1b) was washed with City of Tempe tap water (conductance ~1000 μmhos/cm) using the above procedure and a single 24-h contact time. This was done for comparison with socks washed in ultrapure water.

The effect of detergents on silver released from socks into domestic wastewaters was not addressed in this research. Although it is acknowledged that most people use soaps when washing their clothes, the goal of this study was to obtain data on the interaction of n-Ag from socks with distilled and tap water. Additional research is required to identify the effect of detergents on the quantity and form of silver released from socks into domestic wastewater streams.

Separation of Nanoparticle and Ionic Silver Species. Three approaches were employed to separate the nanoparticle form of silver from ionic silver and to quantify both forms. First, washwater samples were centrifuged at 15 000 rpm ($F = 24\,900g$) for 20 min, but this procedure did not remove all colloidal silver from suspension. Second, the silver in the washwater samples was size-separated using membrane filters (Pall) of 0.4, 0.1, and 0.02 μm pore diameter in either a 25 mm syringe filter or a 45 mm vacuum pump apparatus. The 0.4 μm filter cleared extraneous material from clogging the smaller-pored filters. The 0.02 μm filter is the smallest pore size commercially available for a syringe filter apparatus and can be used as a rough estimate for a threshold between ionic and nanoparticle silver. Third, a silver ion specific electrode (ISE) (Accumet Silver/Sulfide, Fisher) was used in combination with a pH/mV meter (Φ 250 series, Beckman) to measure free Ag^+ ions of the unfiltered 1-h washes of socks 1b and 3.

Scanning and Transmission Electron Microscopy Analyses. Scanning electron microscopy (FEI XL30 EFSEM with EDX capabilities) and transmission electron microscopy (JEOL JEM-2010F TEM/STEM with EDX capabilities) were used to confirm the presence of silver nanoparticles in the sock material and in the washwater samples, respectively. The sock material was ashed at 550 °C in a programmable muffle furnace (Fisher Scientific), then prepared on an SEM stub. Two methods of stub preparation were used: (1) the ashed sock material was lightly dusted onto the carbon tape of the SEM stub surface, and (2) the ashed sock material was suspended in distilled water and subsequently evaporated in droplets onto the carbon tape of the SEM stub. Energy-dispersive X-ray analysis (EDX) was used to confirm the elemental presence of silver in the electron micrographs.

For TEM imaging, the sock washwater was first evaporated to concentrate the nanoparticles, thus increasing the probability of identifying them on the TEM stub. Drops of the concentrated sock washwater were then evaporated on the TEM stub for analysis. EDX was used to confirm the presence of silver in the micrographs.

Adsorption Experiments with Wastewater Biomass. Batch adsorption isotherms quantified the potential removal of silver (nanoparticle and ionic, combined) from the washwater by wastewater treatment system biomass (activated sludge). Isotherm studies were conducted on two types of silver-containing water solutions: the sock washwaters and a reagent ionic silver solution. The latter was prepared using a plasma standard solution of Ag^+ (1000 ppm Ag^+, 5% HNO_3, Cat. no. PAGN-100, Manchester, NH) and distilled water.

Wastewater biomass for the isotherm studies was collected and prepared in two ways. First, biomass was sampled from a bench-scale reactor. This reactor was conditioned with return activated sludge (RAS) from a local WWTP and operated without wasting of biosolids (i.e., long sludge age). Second, a fresh sample of biomass was collected from the RAS line of a local wastewater treatment facility. A 5 mM $NaHCO_3$ solution was used to rinse the biomass before spiking it into the batch isotherm experiments.

For each batch sample in the isotherm test, 40 mL of the silver solution of interest was spiked with a dose of the biomass stock solution, and the sample was diluted up to 50 mL with ultrapure water. The initial silver concentrations in the batch experiments ranged from 60 to 500 ppb. Doses between 0.5 and 6 mL (0.17−2.23 mg of dry biomass) of the biomass stock solution were used in the samples.

All experiments were conducted within a pH range of 5.8−7.4. The pH values of the experiments using sock washwater were not adjusted. However, the ionic silver stock solution experiment was adjusted from pH 3.5 to pH 6.1 using 1 N NaOH (7708, Mallinckrodt, Paris, KY). The samples were allowed to mix on a shaker table at 45 rpm for 1 h of contact time, making these quasi-equilibrium experiments, allowing ample time for adsorption to take place while limiting the time for the biomass to produce additional surface area for adsorption. Then they were filtered through a 0.4 or 0.45 μm membrane filter (Pall) to remove any suspended biosolids. The filtrate was then analyzed for total silver by ICP-OES.

Results

Quantification of Silver in Sock Material and SEM Confirmation of Nanoparticle Size. Acid digestion of the sock material indicated that 5 of the 6 types of socks contained detectable levels of silver ranging from 2 to 1360 μg-Ag/g-sock (Table 1). SEM confirmed the presence of silver nanoparticles in socks 1a, 3, and 4 (not shown). Figures 1 and 2 show SEM/EDX images of representative samples from

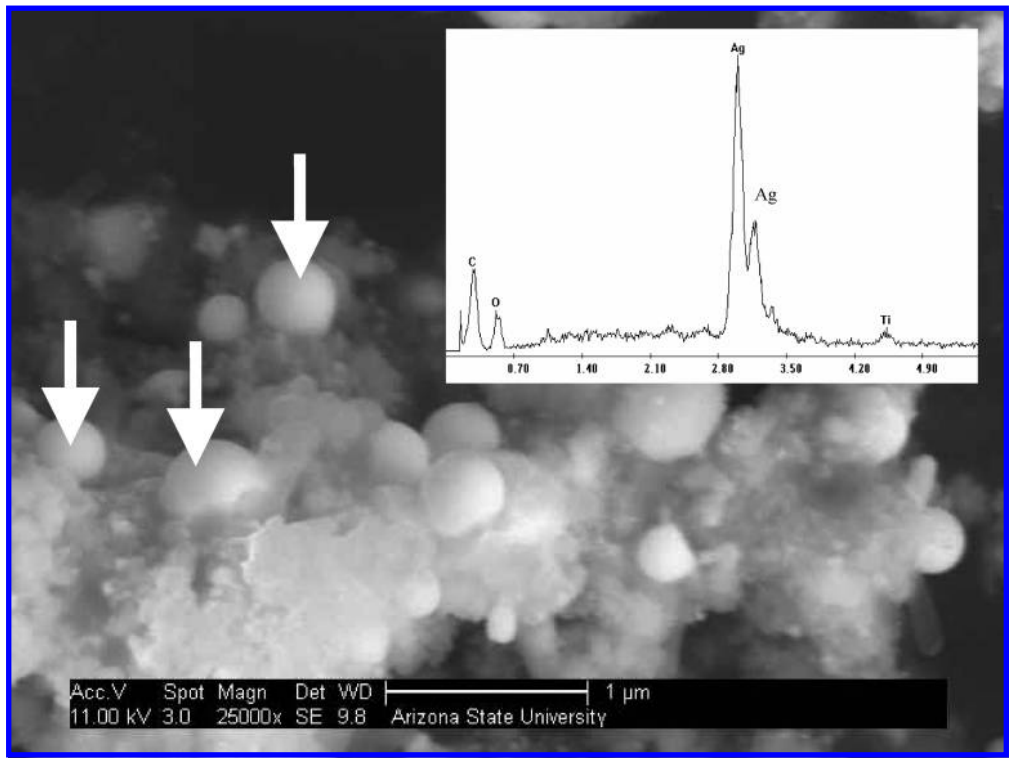

FIGURE 1. SEM image of ashed sock 3 material showing spherical silver particles on the order of 100 nm in diameter. Inset: Representative EDX analysis of the spherical silver particles marked with white arrows.

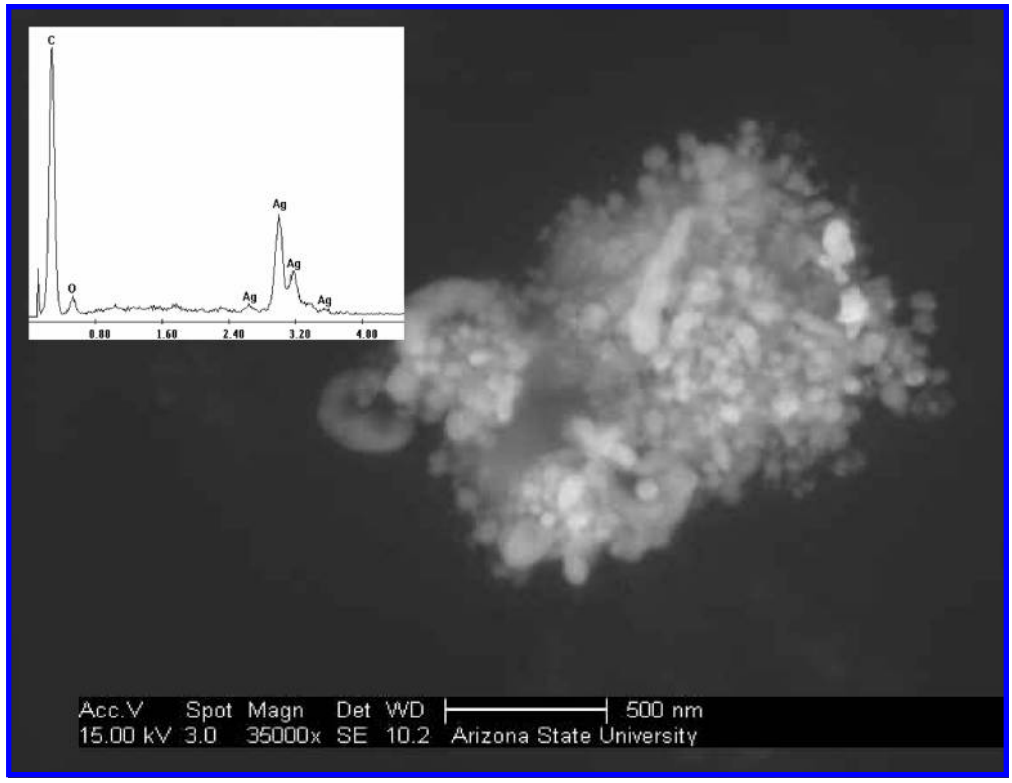

FIGURE 2. SEM image of ashed sock 1a material showing agglomerated silver nanoparticles. Inset: Representative EDX analysis of points within the mass confirms a majority of silver particles. The carbon peak is attributed to residual sock fabric and/or the carbon tape used to mount the sample.

socks 3 and 1a, respectively, after ashing. The carbon and oxygen peaks of the EDX analyses can be attributed to the surrounding residual sock material and/or the carbon tape used for SEM stub preparation. Particles of elemental silver with diameters of 100−500 nm exist within the three types of socks. The silver particles in sock 1a do not appear nearly spherical like those of sock 3, but are irregularly shaped and much smaller (<100 nm). Although the melting point of pure

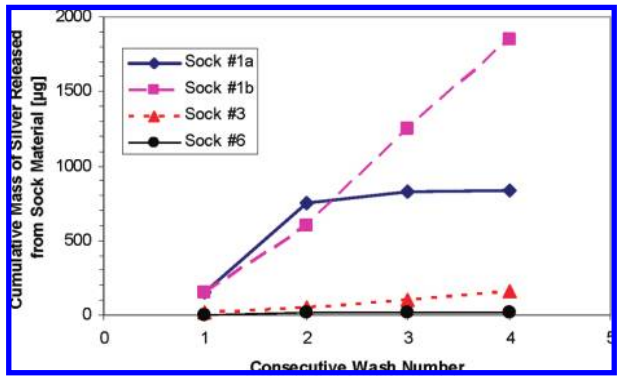

FIGURE 3. Cumulative mass of silver released from three sock types (four socks total) into four consecutive 24-h washings in distilled water.

silver is 962 °C, the ashing of the sock material at 550 °C may have sintered smaller n-Ag particles into larger diameter spheres. However, this preparatory step was necessary to remove the bulk of the sock fabric to obtain a clear image of the n-Ag.

Release of Silver into Washwater. Three of the six sock sample types (1a and 1b, 3, 6) detectably leached silver into the ultrapure wash water (1- or 24-h contact time). Figure 3 presents the cumulative mass of silver released during four sequential 24-h washes of the four sock samples. Silver was steadily released throughout the washes. Socks 1b and 3 were still releasing significant amounts of silver after four washes, whereas socks 1a and 6 had leached almost all of their silver by the fourth wash. The silver content in the 500 mL washwaters ranged from 3 to 1300 ppb (1.5−650 μg of silver). The 24-h wash simulations leached comparable (i.e., same order of magnitude) silver amounts as 1-h wash simulations. After three washes, two socks of 1b leached a total of 1245 and 1020 μg during the 24-h (Figure 3) and 1-h washes (Table 2), respectively. Similarly, two socks of 3 leached 100 and 390 μg in the 24-h (Figure 3) and 1-h washes (Table 2), respectively.

A comparison of socks based on the amount of silver leached relative to the silver content of the sock (Table 1) suggests that fundamental differences in the manufacturing processes of the socks control the amount of silver that is released into the washwater. For example, socks 3 and 4 contained relatively large amounts of silver (31 242 and 2105 μg, respectively), yet released only small percentages (<1%) of their total silver into the wash water, while socks 1a, 1b, and 6 released nearly 100% of their silver content in four consecutive washes. Additionally, the socks release their silver into the washwater at different rates. Socks 1 and 6 released the most silver during the second wash, while sock 3 steadily increased its release of silver throughout subsequent washes. These data would suggest that the various sock types have different longevities in which the silver continues to function as an antimicrobial agent. For example, because most of the silver, if not all, contained in socks 1a and 1b is leached in the first four washes, one might assume that these socks would not perform as well as socks 3 and 4 at preventing odor-causing bacteria growth.

A fresh sock 1b sample was washed once with City of Tempe tap water (*19*) (conductance ~1000 μmhos/cm) for 24 h to investigate the effect of water quality on the release of silver from the sock. The potential of water to corrode metals is related to many water quality parameters, but in general, as buffering capacity and alkalinity increase, water corrosivity decreases (*20*). After one wash, sock 1b had released 15 μg of silver into the tap water as compared to 155 μg of silver released into the ultrapure water (Figure 3). This result may indicate that tap water is less aggressive than ultrapure water at stripping silver from the sock fabric. Therefore, these experiments in ultrapure water may be an overestimate of the amount of silver that could be leached into domestic wastewater streams. The silver in the tap water solution could not be characterized as nanoparticle or ionic because of interferences with the ISE and the probable formation of silver salts during SEM/TEM sample preparation.

Silver Characterization of the Sock Wash Solutions. TEM and EDX analysis of the colloids in the wash water of sock 1a (Figure 4) indicated the presence of silver material with diameters from one to a few hundred nm. These particles are in the same size range and are irregularly shaped like the particles in the SEM image of the sock 1a material. Thus, at least some of the n-Ag is released into the washwater as nanoparticles; not just as dissolved ionic silver.

Table 2 summarizes the colloidal and ionic characterization of the silver leached into the washwaters. Very little silver was separated by filtration into the size range of 20−100 nm. For sock 1b, about 70−90% of the silver was characterized as ionic by the ISE depending on the number of washings, which suggests that 75−100 μg/L of n-Ag may be present in the washwaters. Therefore, it should not be assumed that all of the silver initially released from socks is in the dissolved ionic form.

The first wash of sock 3 released nearly all silver as colloidal, as confirmed by the agreement of the filtration and ISE data. Subsequent washes of sock 3 contained increasing amounts of ionic silver. Thus, the ultrapure water in which these experiments were conducted may be corrosive. In experiments using commercial n-Ag (Aldrich) in ultrapure water, the ionic silver in solution increased over time as measured by ISE. This suggests that n-Ag is oxidized into a dissolved ionic form when subjected to prolonged exposure in water.

Partitioning of Ionic and Nanoparticle Silver to Wastewater Biomass. Quasi-equilibrium batch adsorption isotherm experiments were conducted using various sock wash solutions or reagent ionic Ag^+ solution. Silver partitioned onto the biomass and was fit with the Freundlich isotherm equation ($q = KC^{1/n}$), where C is the equilibrium silver concentration after exposure to wastewater biomass (Figure 5). The values for the Freundlich adsorption capacity parameter, K, ranged from 3.4 to 17 (μg-Ag/g-biomass)(L/

TABLE 2. Nanoparticle and Ion Separation for Silver in 1-hr Washes via Filter Analysis and Ion Selective Electrode

		percent of total silver			
sock ID	total silver in wash water (μg)	passing 0.4 μm filter	passing 0.1 μm filter	passing 0.02 μm filter	[Ag^+] from ISE
1b, first 1-hr wash	145	93	93	86	72
1b, second 1-hr wash	275	98	91	85	76
1b, third 1-hr wash	600	83	83	81	86
3, first 1-hr wash	80	7	3	2	5
3, second 1-hr wash	160	53	53	53	25
3, third 1-hr wash	150	97	90	87	69

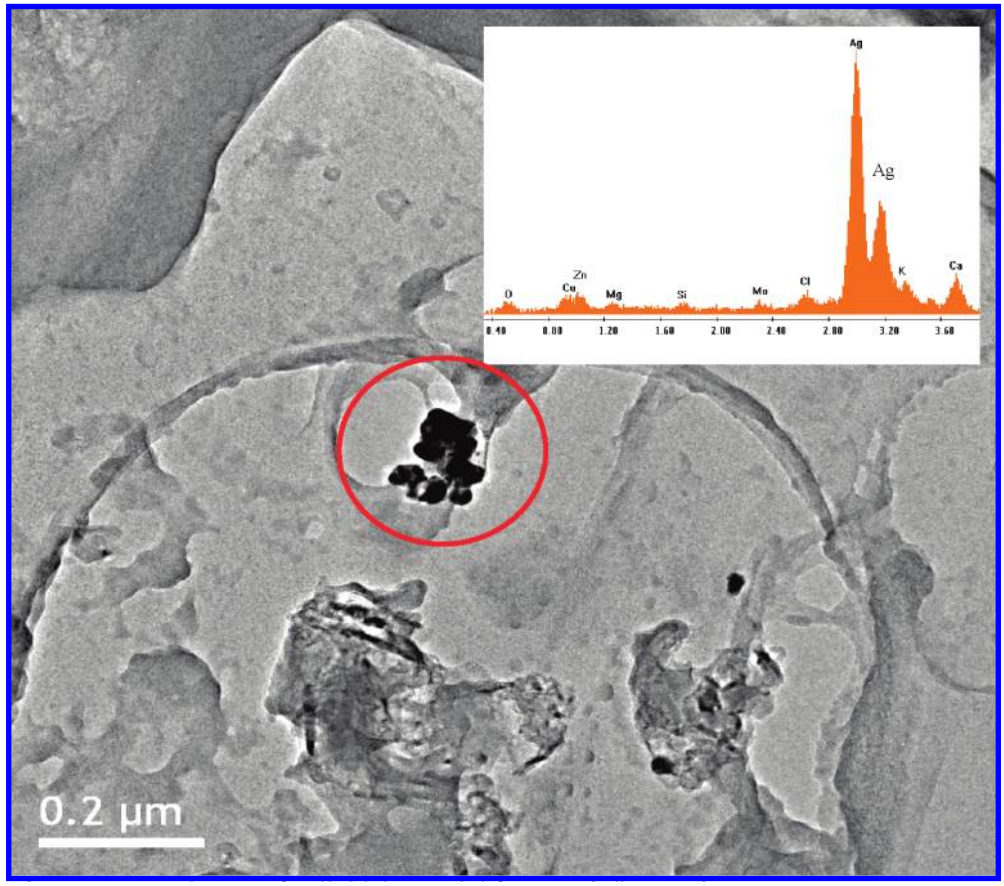

FIGURE 4. TEM image of colloidal material from sock 1a washwater. Inset: EDX confirmation that the dark particles within the circle are predominantly silver.

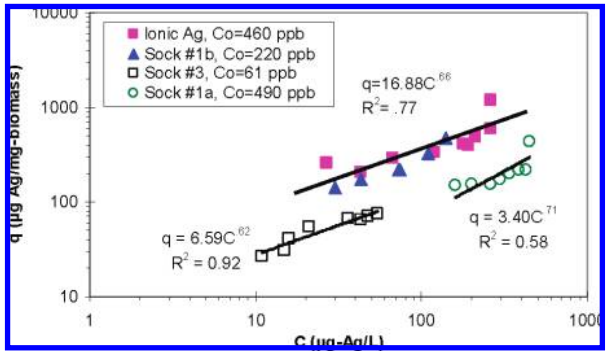

FIGURE 5. Batch adsorption isotherms for the wash solutions of three socks (1b, 3, 1a) and an ionic silver solution (Ionic Ag). Initial silver concentrations varied from 61 to 490 ppb, and pH values ranged from 5.8 to 7.4.

μg-Ag)$^{1/n}$. The slopes of all of the isotherms are very similar, yielding an average Freundlich adsorption intensity parameter, $1/n$, of 0.66 (unitless).

Although the presence of colloidal silver was confirmed in washwater samples, the isotherm experimental data suggest that the silver leached from the socks adsorbs to biomass in a manner similar to that of ionic silver. The isotherm of reagent ionic silver solution overlaid that of sock 1b wash water, which contained mostly Ag^+ based on the ISE measurement (Table 2). Sock 3 leached low percentages of Ag^+ in washes 1 and 2, but the isotherm experiment for this sock was conducted with washwater that had been stored for 4 weeks, possibly giving the colloidal silver time to solubilize into ionic silver. Removal of Ag^+ from wastewater can be attributed mainly to precipitation with chloride and adsorption to biomass, which can be hindered by complexation with dissolved organic matter (*21*).

Two wastewater biomass preparations were used for the isotherm experiments. Figure 5 presents data for isotherms conducted with biomass from a full-scale WWTP and a laboratory-scale bioreactor, which are represented by closed and open data points, respectively. Since the isotherm studies conducted on wash water from socks 1b and 1a used full-scale WWTP and laboratory-scale biomass, respectively, the difference between the closed and open data points is most likely related to the different biomass samples. However, the difference in isotherm results could also be attributed to interactions between dye from the socks and the leached silver because socks 1b and 1a differ in color.

Although adsorption characteristics may change in real environmental matrices (i.e., WWTP mixed liquor, municipal sewage, etc.), this adsorption data can be used to estimate the performance of a WWTP. The Freundlich isotherm nonlinear relationship was applied to the general fate model for sorption (*22*) to determine the amounts of silver contained in wastewater biomass or in treated effluent. A steady-state mass balance of a WWTP with nonlinear sorption of an adsorbate can be expressed as

$$C = C_0 - \left(\frac{X\tau KC^{1/n}}{\theta}\right) \quad (1)$$

where C is the effluent concentration of silver, C_0 is the influent silver concentration, K and $1/n$ are the Freundlich adsorption parameters, and X, τ, and θ are operational parameters of a WWTP (mixed liquor suspended solids, hydraulic and solids retention time, respectively). The common values used for these model parameters are provided as Supporting Information. Figure 6 presents

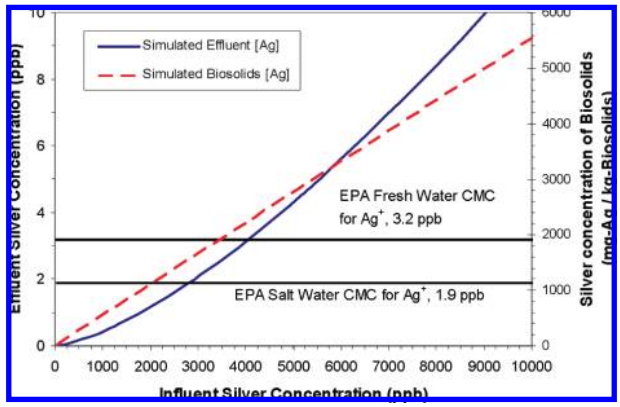

FIGURE 6. Model results illustrating the removal of influent silver for a typical WWTP (model parameters: Freundlich *K* and $1/n = 9.0, 0.7$; $\tau = 0.5$ d; $\theta = 5$ d; $X = 2000$ mg/L). The silver concentration in the treated effluent would exceed the USEPA salt and fresh water Criteria Maximum Concentrations (CMCs) at influent concentrations of 2900 and 4250 ppb, respectively. The concentration of silver in the waste activated sludge flow is represented by the dashed line and the secondary *y*-axis.

simulations of the silver concentrations exiting an activated sludge reactor using common WWTP design conditions and average values for K and $1/n$ (9.0 and 0.7, respectively). Using a common municipal WWTP influent silver concentration of 5 μg/L, the model results in an effluent silver concentration of 0.01 μg/L, and the wasted biosolids silver concentration is 2.8 mg-Ag/kg-biosolids. The effluent from the wastewater treatment facility would exceed the USEPA water quality criteria for salt water and fresh water at influent silver concentrations of about 2900 and 4250 ppb, respectively. These influent concentrations are 3 orders of magnitude higher than those commonly observed for municipal WWTPs (*21*). The treated effluent would not exceed the 100 ppb secondary drinking water standard for silver until the influent concentration reached approximately 45 400 ppb (not shown graphically).

Based upon the existing water quality criteria for silver, the model suggests that wastewater treatment plants are capable of removing a much higher silver load from a wastewater stream than should be reasonably expected from an increased number of consumer products containing silver nanoparticles. Model simulations were conducted by varying two WWTP operation parameters: the MLSS concentration was varied from 2000 to 4000 mg/L and the ratio of θ:τ was varied from 5 to 20. Using the 1.9 ppb salt water CMC as the maximum allowable effluent concentration, a MLSS concentration of 2000 mg/L with an θ:τ of 20 would treat an influent silver concentration of 1460 μg/L. Similarly, a MLSS concentration of 4000 mg/L with an θ:τ of 5 would treat an influent silver concentration of 11 600 μg/L (a table of the model results can be found in the Supporting Information). However, the concentration of silver in the biosolids is worthy of some consideration. The model suggests at an influent silver concentration of 180 μg/L, the silver concentration in the biosolids would exceed the 5 mg/L Toxicity Characteristic Leaching Procedure (TCLP) by the USEPA. An increase in consumer use of n-Ag could therefore restrict municipal wastewater treatment facilities from exporting their biosolids as fertilizer for agricultural lands.

Additional Implications

New analytical techniques that distinguish between nanomaterials (metal, metal-oxide, quantum dots, etc.) and dissolved ionic species at relevant concentrations in environmental matrices are important for the advancement of nanotechnology. Methods such as capillary electrophoresis (*23*) and size-exclusion chromatography (*24*) have the potential to separate nanomaterials from ionic species, but detection methods suited for environmental matrices and relatively low concentrations are limited. With the capability to separate and quantify nanomaterials, exposure data can be obtained during ecotoxicity studies to facilitate environmental risk assessment studies. The unknown environmental risks are currently preventing scientists and the public from fully supporting the advancement of nanotechnology (*25*), but new analytical techniques can answer these questions, thus allowing nanotechnology to develop at a pace acceptable to all stakeholders.

Acknowledgments

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Supporting Information Available

WWTP model parameters and a table summarizing the multiple simulations of the model. This material is available free of charge via the Internet at http://pubs.acs.org.

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Troy M. Benn* and Paul Westerhoff: Nanoparticle Silver Released into Water from Commercially Available Sock Fabrics

1. Page 4137, Figure 5. The Freundlich isotherm data describing the adsorption of silver to wastewater treatment biomass was

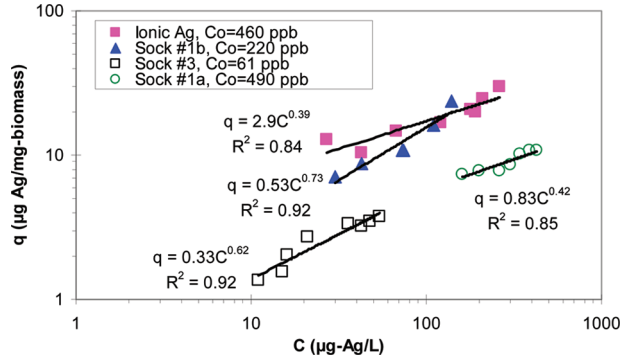

FIGURE 5. Batch adsorption isotherms for the wash solutions of three socks (#1b, #3, #1a) and an ionic silver solution (Ionic Ag). Initial silver concentrations varied from 61 to 490 ppb, and pH values ranged from 5.8 to 7.4.

erroneously calculated. The correct data are presented here in Figure 5.

2. Pages 4136−4137. The values for the Freundlich adsorption capacity and intensity parameters, K and $1/n$, were mistakenly reported. The correct values of K ranged from 0.33 and 2.9 (μg-Ag/g-biomass)(L/μg-Ag)$^{1/n}$. The correct average value for $1/n$ is 0.54 (unitless).

3. Page 4138, Figure 6. The silver concentration in the effluent of a WWTP would exceed the EPA salt and fresh water criteria maximum concentrations (CMCs) at lower influent concentrations than initially reported.

4. Page 4138. The revised WWTP model for silver removal leads to the following corrections:

- Using a common municipal WWTP influent silver concentration of 5 μg/L, the model results in an effluent silver

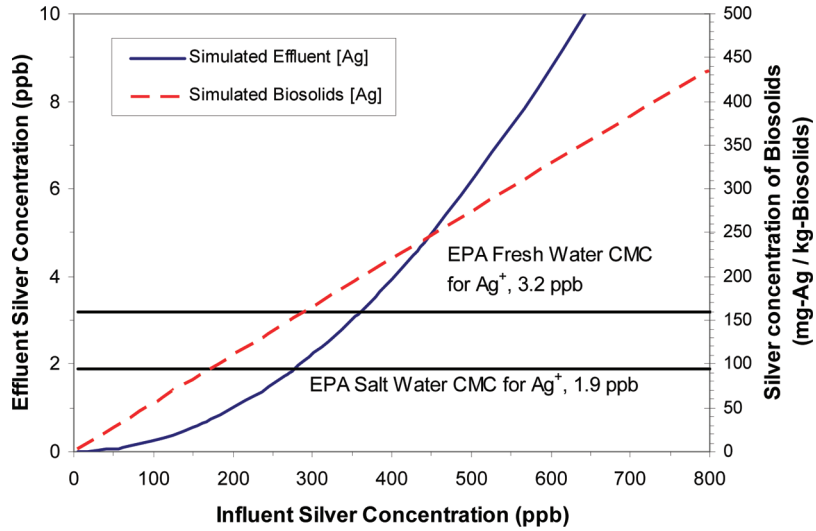

FIGURE 6. Model results illustrating the removal of influent silver for a typical WWTP (model parameters Freundlich K and $1/n$ = 1.0, 0.5; τ = 0.5 d; θ = 5 d; X = 2000 mg/L). The silver concentration in the treated effluent would exceed the EPA salt and fresh water criteria maximum concentrations (CMCs) at influent concentrations of 280 and 360 ppb, respectively. The concentration of silver in the waste activated sludge flow is represented by the dashed line and the secondary y-axis.

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concentration of 0.001 μg/L, and the wasted biosolids silver concentration would be 2.8 mg-Ag/kg-biosolids.

- WWTP effluent would exceed the EPA CMC for salt and fresh water at influent silver concentrations of about 280 and 360 ppb, respectively. These influent concentrations are 2 orders of magnitude higher than those commonly observed for municipal WWTPs.
- WWTP effluent would not exceed the 100 ppb secondary drinking water standard for silver until the influent concentration reached approximately 2100 ppb (not shown graphically).
- By controlling mixed liquor suspended solids (MLSS) from 2000 to 4000 mg/L and the θ:τ ratio from 5 to 20, a WWTP could treat a range of influent silver concentrations between 140 and 1110 μg/L to less than 1.9 μg/L.

Supporting Information Available

Table S1 showing WWTP maximum allowable influent silver concentrations, C_0 (ppb), that would result in an effluent silver concentration lower than 1.9 ppb as a function of operational parameters MLSS and θ:τ. This material is available free of charge via the Internet at http://pubs.acs.org.

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Estimation of cumulative aquatic exposure and risk due to silver: Contribution of nano-functionalized plastics and textiles

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ABSTRACT

Products with antimicrobial effect based on silver nanoparticles are increasingly used in Asia, North America and Europe. This study presents an analysis of risk to freshwater ecosystems from silver released from these nanoparticles incorporated into textiles and plastics. The analysis is presented in four stages; (i) silver mass flow analysis and estimation of emissions, (ii) assessment of the fate of silver in a river system and estimation of predicted environmental concentrations (PECs), (iii) critical evaluation of available toxicity data for environmentally relevant forms of silver and estimation of predicted no-effect concentrations (PNECs), and (iv) risk characterization. Our assessment is based on estimated silver use in the year 2010, focusing on the Rhine river as a case study. In 2010, biocidal plastics and textiles are predicted to account for up to 15% of the total silver released into water in the European Union. The majority of silver released into wastewater is incorporated into sewage sludge and may be spread on agricultural fields. The amount of silver reaching natural waters depends on the fraction of wastewater that is effectively treated. Modeled PECs in the Rhine river are in satisfactory agreement with monitoring data from other river systems. Because a complete characterization of the toxicity of environmentally relevant silver species is lacking, only a limited risk assessment is possible at this time. However, our study indicates that PEC/PNEC ratios greater than 1 cannot be ruled out for freshwater ecosystems, in particular sediments. No risk is predicted for microbial communities in sewage treatment plants.

1. Introduction

The use of silver nanoparticles incorporated in consumer products has become common in the last years because of the biocidal effect of the silver ion. Industry makes use of this new technology in food contact applications, in the interior of automobiles such as steering wheels and in building materials such as sanitary tubing and coverings. Another field of application for products with antimicrobial effect based on silver ions is medical equipment such as catheters, infusion systems and medical textiles (The Silver Institute, 2001; Markarian, 2002; Simpson, 2003; Markarian, 2006.). Worldwide, markets for silver-containing nano-functionalized products have started to grow significantly (The Silver Institute, 2001; Markarian, 2006; Rundle, 2006).

The increasing use of antimicrobial silver nanoparticles requires an environmental risk assessment for such products. Here we evaluate the impacts of silver released from silver-containing plastics and textiles on freshwater ecosystems. The relevance of the topic is given by the EU directive concerning the

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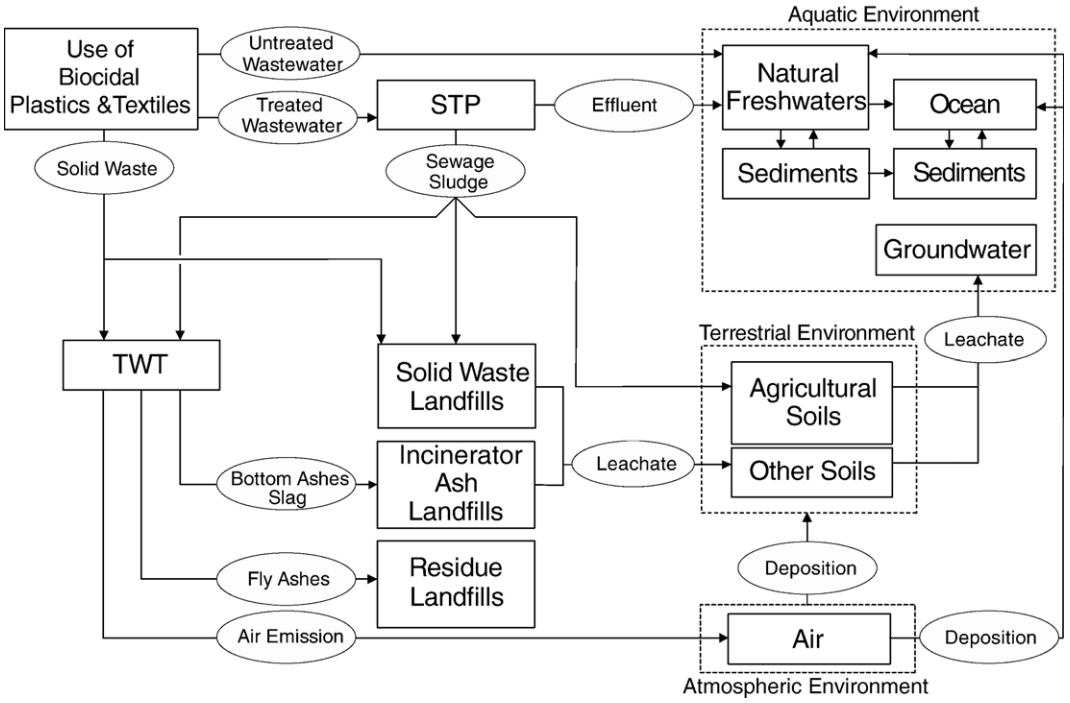

Fig. 1 – Overview of silver flows triggered by biocidal plastics and textiles. Arrows represent silver flows; dashed lines indicate different environmental spheres. TWT = thermal waste treatment; STP = sewage treatment plant.

placing of biocidal products on the market (European Parliament and Council, 1998) and the recent announcement of regulation of silver nanoparticle technology by the US Environmental Protection Agency (Weiss, 2006). There are projections of strong growth in the silver nanoparticle market for the coming years (HeiQ, 2006).

The biocidal mechanism of silver-containing products results from a long term release of silver ions (Ag^+) by oxidation of metallic silver (Ag^0) in contact with water (Kumar et al., 2005). In plastics and textiles silver nanoparticles are embedded in the polymer matrix; another possible approach is to apply a silver-containing surface coating to the polymer (Marini et al., 2007). Here we focus on silver released from nanoparticles embedded in the polymer and assume that only silver ions and not entire nanoparticles are released. The Ag^+ ion inhibits the enzymes for the P, S, and N cycles of nitrifying bacteria (Ratte, 1999). In addition, ionic silver can block DNA transcription and interrupt bacterial respiration and adenosine triphosphate (ATP) synthesis (Kumar et al., 2005).

The antimicrobial spectrum of silver is extensive, and silver has also been reported to be effective against a variety of virus types (Han et al., 2005). Silver ions also have fungicidal and algicidal effects (Ratte, 1999).

Silver is classified in the "soft" metal group and binds very strongly with reduced sulfur groups such as thiolates and sulfides (Stumm and Morgan, 1996). Because reduced sulfur is found in most natural waters (Kramer et al., 2007), free silver ions exist only at very low concentrations in the aquatic environment.

The objectives of this study are (i) to estimate, in comparison to current silver releases from various other applications, the incremental amount of silver that will be released to the environment by biocidal plastics and textiles in a near-future scenario, and (ii) to evaluate for the total of silver releases from biocidal and other applications whether predicted silver concentrations in freshwater and sediments could have adverse effects. Releases from other biocidal applications of silver such as cosmetics or exterior paints are not considered because they are very difficult to quantify.

Our assessment is presented in four stages. First, the system boundaries are defined, mass flows of silver are quantified, and three emission scenarios are defined. Second, the behavior of silver in natural freshwater is reviewed, and a mass balance model is applied to calculate predicted environmental concentrations (PECs) for freshwater and freshwater sediments. PECs are also estimated for sewage treatment plants (STPs) and sewage sludge. The uncertainty of the results is assessed and predicted concentrations are compared to empirical data. Third, toxicity data for environmentally relevant silver compounds are compiled and predicted no-effect concentrations (PNECs) are determined where possible. Finally, the potential for risk caused by the release of silver into freshwater is evaluated using all available data.

2. Emission scenarios

2.1. *Temporal scale and spatial boundary*

The market for silver-containing biocidal products is undergoing rapid expansion. The Silver Institute (2001) estimated that the use of silver as a biocide would increase by about 500 times between 2000 and 2004 in Europe (with an estimate of 30 t/yr for 2004). We selected the year 2010 in order to take into

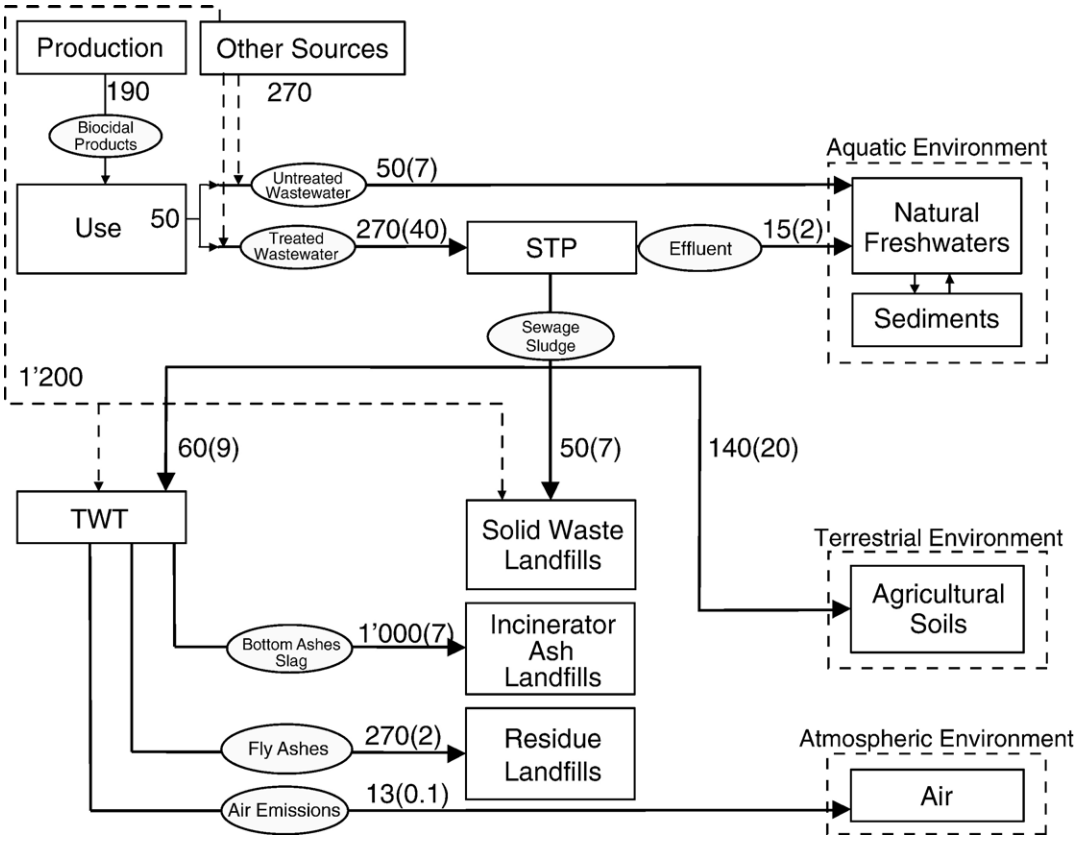

Fig. 2 – Quantified mass flows of silver triggered by the use of biocidal products and by other silver uses (intermediate scenario, values in tonnes per year; values in brackets are amounts originating from the use of biocidal products). Thin arrows represent flows caused by biocidal plastics and textiles. Dashed arrows are silver flows from other sources. Thick arrows are used where mass flows of biocidal products and other silver sources come together. Production of silver-containing plastics and textiles is not considered. Mass flows into the marine ecosystem and silver in leachates and aerial deposition are not quantified.

account this anticipated growth but to still have a reliable economic data base. The European market for silver-containing products is projected to reach 110–230 t of silver by 2010 and stabilization is expected for approximately 2015 (HeiQ, 2006). Prospective estimates of the amount of a chemical used in new applications are not given in the scientific literature but have to be derived from projections of companies active in such a new market. Accordingly, the estimated amounts of silver used in biocidal applications are relatively uncertain. However, it is possible to deal with these uncertainties within the framework of our assessment. First, we cover the range of 110–230 t/yr by three different emission scenarios, see below. Second, when there are more reliable data available, this information can be incorporated into the silver mass flux diagram presented in Fig. 2. On this basis, also the results from the river model used in Section 3 can be updated because the model output is a linear function of the releases used as model input.

The spatial domain of our study is the 25 member countries of the European Union (EU25).

2.2. *Mass flows of silver into the environment*

Fig. 1 shows the routes for silver entering the environment from silver-containing plastics and textiles. The use of biocidal products releases silver ions into wastewater, which is either treated in a sewage treatment plant (STP) or directly discharged into natural waters. The small fraction of silver that is not removed with sewage sludge reaches natural waters via STP effluents. Sewage sludge is either applied to agricultural soils, disposed of in solid waste landfills or incinerated in thermal waste treatment (TWT) plants. If applied on fields, silver mainly stays in the top layer of soils (Hou et al., 2005). Landfilling of sewage sludge may allow silver to leach into subsoil and groundwater. In the incineration process, silver ends up in slag and bottom and fly ashes; emissions to air are minor (1% of amount leaving TWT plants, see Fig. 2). Disposed biocidal plastics and textiles are either incinerated or deposited in solid waste landfills.

Immediate release to the environment arises from STP effluents, from untreated wastewater, and from silver contained in sewage sludge that is spread out on agricultural fields. Pollution of soil and groundwater via leachate might pose a delayed risk that is not quantified here.

Emissions arising from the production of biocidal products are ignored in this assessment. Solid waste from silver-containing biocidal products is not included, either, because the stock in use in 2010 is disregarded here. Deposition from the atmosphere to water surfaces is neglected (it amounts to

Table 1 – Parameter values in the three emission scenarios (EU25; 2010)

Parameter	Explanation	Unit	Minimum scenario	Intermediate scenario	Maximum scenario	Reference
POP	Population	millions	469	464	460	Lanzieri (2006)
$k_{release}$	Ag^+ release rate	g Ag^+ g Ag^{-1} d^{-1}	$3 \cdot 10^{-5}$	$1 \cdot 10^{-3}$	$1 \cdot 10^{-3}$	Kumar et al. (2005)
$Ag_{products}$	Silver in biocidal products	t a^{-1}	110	190	230	HeiQ Materials Ltd. (2006)
$Ag_{textiles}$	Silver in textiles	t a^{-1}	12	18	30	
$Ag_{plastics}$	Silver in plastics (total)	t a^{-1}	100	170	200	
$Ag_{plasticsH2O}$	Silver in $plastics_{H_2O}$ [a]	t a^{-1}	50	120	150	
$Ag_{plastics_{dry}}$	Silver in $plastics_{dry}$ [b]	t a^{-1}	50	53	50	
$t_{water\ contact}$	Period of:	d				
	– Washing textiles		1	2	4	
	– Wearing textiles		4	17	87	
	– Water contact $plastics_{H_2O}$		365	365	365	
	– Water contact $plastics_{dry}$		0	15	91	
f_{STP}	Fraction of wastewater treated in STP	–	0.90	0.85	0.80	Eurostat (2006); ECB (2003, p. 58)
$f_{removal}$	Fraction of silver removed from water in STP	–	0.99	0.94	0.85	Shafer et al. (1998); Daxbeck et al. (2002)
$f_{agriculture}$	Fraction of SS [c] used in agriculture	–	0.46	0.56	0.66	EEA (2001); Maurer (2006)
f_{TWT}	Fraction of SS directed into TWT plants	–	0.30	0.25	0.20	
f_{SWL}	Fraction of SS deposited in solid waste landfills	–	0.24	0.19	0.14	
$WW_{percapita}$	Wastewater produced annually per capita	m^3 a^{-1}	180	70	50	Maurer (2006); ECB (2003, p. 62)

[a] $Plastics_{H_2O}$ is the category of plastics with substantial water contact e.g. sanitation tubes or medical catheters.
[b] $Plastics_{dry}$ is the category of plastics without substantial water contact e.g. computer keyboards, door handles or car steering wheels.
[c] SS = Sewage sludge.

approximately 5% of 13 t/yr released to air, compared to 65 t/yr directly released to water systems). Marine ecosystems are not considered.

2.3. *Assumptions for emission scenarios*

Three silver emission scenarios were created for EU25 in the year 2010, see Table 1. The uncertainty contained in the underlying assumptions is represented by building a Minimum, an Intermediate and a Maximum emission scenario. The Intermediate scenario considers the most probable assumptions and the Minimum and Maximum scenarios represent the assumptions that result in lower and elevated PECs, respectively.

2.3.1. *Silver emission into natural waters*

The amount of silver released annually into wastewater by the use of biocidal plastics and textiles ($Ag_{WW,biocidal}$) is estimated from the silver in biocidal plastics and textiles ($Ag_{products}$) multiplied by the release rate of silver ions from these products ($k_{release}$) and the period the products are in contact with water ($t_{water\ contact}$):

$$Ag_{WW,\,biocidal} = Ag_{products} \cdot k_{release} \cdot t_{water\ contact} \quad (1)$$

For units of all parameters, see Table 1. Due to different periods of water contact, plastics and textiles are treated separately. Plastics are further divided into products designed to be used in contact with water ($plastics_{H_2O}$) and products without pronounced water contact ($plastics_{dry}$). $Plastics_{H_2O}$ are assumed to release silver ions via water contact during 365 days per year, d a^{-1}, under all emission scenarios; examples are medical catheters and sanitation tubes. $Plastics_{dry}$ are, for example, computer keyboards, door handles, car steering wheels and mobile phones, which are expected to have water contact for less than 25% of the year. For textiles, the ion release in the washing process (1–4 d a^{-1}) and via human sweat during dermal contact (4–87 d a^{-1}) is considered for the annual period of water contact.

Kumar et al. (2005) examined the silver ion release rates ($k_{release}$) of rectangular polyamide samples doped with additives containing nano-sized silver and fully submerged in an aqueous medium. They report varying release rates depending on the type of additive that has been filled into the polymer. The lowest release rate was found for elemental silver and is applied in the Minimum scenario ($3 \cdot 10^{-5}$ g Ag^+ g Ag^{-1} d^{-1}). The highest release rate – used for the Intermediate and Maximum emission scenarios – was observed when zeolite acts as the carrier of silver ($1 \cdot 10^{-3}$ g Ag^+ g Ag^{-1} d^{-1}). Most polymers such as polyester, polypropylene, polycarbonate or polyvinyl chloride are more crystalline than polyamide, which results in decreased water permeability and hence a lower silver ion release rate (Radheshkumar and Münstedt, 2006). Therefore the rates used here represent the upper range of expected values for plastics. In contrast, silver release from textiles may be underestimated because a

Table 2 – Silver (I) mass flows in tonnes per year for the three emission scenarios

Parameter	Explanation	Minimum	Intermediate	Maximum
$Ag_{WW,\ biocidal}$	Silver released into wastewater from biocidal products	0.5	50	60
$Ag_{WW,\ other}$	Silver in wastewater originating from sources other than biocidal products	190	270	350
Ag_{WW}	Silver in wastewater	190	320	410
$Ag_{WW,\ untreated}$	Silver in untreated wastewater	20	50	80
$Ag_{WW,\ STP}$	Silver entering STPs	170	270	330
$Ag_{STP\ effluent}$	Silver in STP effluents	1	15	50
$Ag_{water,\ input}$	Silver reaching natural waters	20	65	130
Ag_{SS}	Silver in sewage sludge	170	255	280
$Ag_{SS,\ agriculture}$	Silver in sewage sludge used in agriculture	80	140	190
$Ag_{SS,\ TWT}$	Silver in sewage sludge undergoing TWT	50	60	60
$Ag_{SS,\ SWL}$	Silver in sewage sludge deposited on solid waste landfills	40	50	40
$Ag_{waste,\ other}$	Silver in solid waste originating from sources other than biocidal products	700	1200	1700
Ag_{SWL} [a]	Silver deposited in solid waste landfills	800	1250	1800
Ag_{TWT} [b]	Silver directed into TWT plants	800	1260	1700
Ag_{slag} [b]	Silver ending up in slag	600	1000	1300
$Ag_{fly\ ashes}$ [b]	Silver directed into fly ashes	160	270	370
Ag_{air} [b]	Silver emitted to atmosphere	8	13	17

[a] Amount based on the assumption that 100% of solid waste is deposited in solid waste landfills.
[b] Amount based on the assumption that 100% of solid waste is incinerated.

rectangular polymer specimen has a smaller surface area than a textile fiber.

For silver released from coatings, higher release rates (per unit amount of silver) have been reported (Marini et al., 2007). However, for coatings there is enormous diversity in properties and for some coatings the release rate could even be lower than for silver in bulk polymers. Therefore, coatings in themselves need more release rate data for a reliable assessment of silver releases.

The final silver input into receiving waters ($Ag_{water,input}$) is determined by the amount of silver predicted in wastewater (Ag_{WW}), the fraction of wastewater treated in STPs (f_{STP}) and the fraction of silver that is removed in the treatment plant ($f_{removal}$)

$$Ag_{water,\ input} = Ag_{WW} \cdot (1 - f_{STP}) + Ag_{WW} \cdot f_{STP} \cdot f_{removal} \quad (2)$$

The European Chemicals Bureau (ECB, 2003, p. 58) estimates that 80% of the inhabitants in EU25 are connected to a sewage treatment plant. Eurostat (2006) reports that in 2002 86% of wastewater was treated. Thus a range of 80–90% is taken for f_{STP}. Silver is efficiently eliminated from wastewater in STPs. The following fractions of removal are used: 99% in the Minimum, 94% in the Intermediate (both from Shafer et al., 1998) and 85% in the Maximum scenario (Daxbeck et al., 2002).

2.3.2. *Silver emission into terrestrial environment*

Silver from biocidal plastics and textiles produced in 2010 enters the terrestrial ecosystem via sewage sludge. According to the European Environment Agency (EEA, 2001) 55% of sewage sludge was expected to be used for agriculture and soil conditioning in 2005, 25% undergoes a thermal waste treatment and 20% is disposed in solid waste landfills. We assume that the relative shares of the different treatments estimated for the EU15 in 2005 will be similar for EU25 in 2010 (M. Maurer, 2006). To cover possible differences in the amounts of sewage sludge applied to fields, we use shares of 46% and 66% of sludge applied to fields in the Minimum and Maximum scenario, respectively. The only available measurement of silver in a thermal waste treatment plant (Schneider, 1987) indicates that 77% of silver ends up in slag and bottom ashes, 21% in fly ashes and 1% is emitted to the atmosphere.

2.4. *Silver emissions from other applications*

It is not only biocidal plastics and textiles that cause silver emissions. Our estimate of the contribution of biocidal products to total silver releases is based on a comparison of estimated releases from biocidal applications to estimates of total silver release reported by Lanzano et al. (2006) for silver in European sewage and solid waste.

Data for sewage sludge (Lanzano et al., 2006) include silver from photo laboratories, film and print production, dentists and hospitals, galvanic and electroplating work, the production of circuit boards and catalyst production. These estimates have a low to medium reliability. Lanzano et al. (2006) did not include silver from water disinfection estimated by The Silver Institute (2001). We estimated amounts of silver in wastewater from sources other than biocidal products (see Table 2) for EU25 in 2010 by extrapolation based on population size.

The silver amount contained in solid waste (700–1700 t a^{-1}; Table 2) is based on Lanzano et al. (2006). Sources are municipal solid waste (circuit boards, old black-and-white films, photographic prints, dental fillings, non-recycled coins and old silverware and silver-oxide batteries), waste from electrical and electronic equipment, industrial waste (photo laboratories, film and print production, dentists and hospitals), hazardous waste (silver-oxide batteries and waste from dentists) and imported silver waste. Again values are extrapolated via demographic data to EU25 in 2010. Two extreme management practices are analyzed for the solid waste treatment: 100% incineration or 100% landfilling.

2.5. Results

Table 2 summarizes the estimated silver mass flows in all three scenarios. The situation for the Intermediate scenario is visualized in Fig. 2. The total amounts of silver finally reaching natural waters are 20 t a^{-1} (Minimum), 65 t a^{-1} (Intermediate), and 130 t a^{-1} (Maximum scenario). In the Minimum scenario biocidal products only constitute 0.3% of the total mass flow, whereas in both the Intermediate and the Maximum scenarios this share rises to 15%. This difference is caused by lower silver release rates and lower amounts of plastics in contact with water that were assumed in the Minimum scenario, see Table 1. The percentages do not include silver released from the stock of biocidal products in use in 2010 (see Section 6 for a discussion of this assumption). In all three scenarios the product category causing the highest silver release is $plastics_{H_2O}$ with a share of 88–97% in $Ag_{WW,biocidal}$.

Immediate silver emissions to the terrestrial ecosystem are estimated to be 80–190 t Ag that are contained in the sludge used as soil conditioner (0.3–15% of this silver is released from the use of biocidal products). 8 t a^{-1} (Minimum), 13 t a^{-1} (Intermediate) and 17 t a^{-1} (Maximum scenario) are emitted to the atmosphere if 100% of solid waste is incinerated.

3. Exposure assessment

3.1. Environmentally relevant silver species in freshwater

It is the high toxicity of the silver ion to aquatic organisms that causes concern about environmental effects of the metal. However, in the past few years it has been observed that forms other than Ag^+ are predominant in the aquatic environment and that the free ion occurs only in extremely low concentrations (Kramer et al., 2002, p. 6).

Silver binds extremely strongly to reduced sulfur (Bell and Kramer, 1999). Measurements in various aquatic environments show that sulfide concentrations typically exceed silver concentrations by three orders of magnitude (Kramer et al., 2002, p. 6). Rozan et al. (2000) found micromolar concentrations of sulfides in oxic river waters. They concluded that iron, zinc, and copper stabilize sulfide in natural waters by the formation of metal sulfide clusters. Sulfide may also be stabilized by metals associated with natural organic matter (Manolopoulos (2001) cited in Bowles et al., 2002). Thiols might be another source of reduced sulfur in freshwater, but Adams and Kramer (1999a) could detect inorganic sulfides in nanomolar concentrations only in sediment pore waters and not in the water column. Adams and Kramer (1999a) concluded that due to the formation of extraordinarily strong sulfide complexes, silver should outcompete most other metals for the available sulfide ($Hg^{2+} > Ag^+ > Cu^+ > Pb^{2+} > Zn^{2+}$). Therefore, in freshwater systems, silver is expected to be bound to sulfide either in the form of colloidal silver sulfide clusters or as a silver sulfide surface complex on organic matter. In interstitial waters of sediments it might also be prevalent as organic silver thiolate complexes. In STPs silver thiosulfate complexes – originating from the photoprocessing industry – are mostly converted into insoluble silver sulfide (Ag_2S) (Lytle, 1984). Ag_2S is seen as the ultimate form of silver in the natural environment due to its high stability (Bell and Kramer, 1999).

Silver sorbs strongly to suspended particles. Therefore the main part of silver is found in the particulate phase (>0.45 μm) and reported solid–water partition coefficients for silver are on average $10^{5.3}$ L kg^{-1}. Accordingly, silver is easily removed in STPs by sludge, and in aquatic systems it is rapidly incorporated into sediments, from which a long-lasting resuspension into the water column is possible (Kramer et al., 2002; Wang et al., 2003). Much of the silver present in the dissolved phase (<0.45 μm) is associated with colloids (Shafer et al., 1998).

Hereafter, we use the term “silver sulfide” to refer to all different types of silver compounds containing reduced sulfur.

3.2. Methods for estimating PECs in freshwater

To assess whether the expected silver load may inhibit the microorganisms in sewage sludge, we assumed that the concentration in STPs is equal to the concentration in wastewater (C_{WW}, μg L^{-1}). C_{WW} results from dividing the amount of silver in inflows of European STPs ($Ag_{WW,STP}$) by the product of wastewater generated per person and year ($WW_{percapita}$), the fraction of wastewater treated in an STP (f_{STP}), and population (POP):

$$C_{WW} = \frac{Ag_{WW,STP} \cdot 10^9}{WW_{percapita} \cdot POP \cdot f_{STP}} \quad (3)$$

Values of 50 and 180 m^3 a^{-1} are used for $WW_{percapita}$ in the Maximum and in the Minimum scenario, respectively, see Table 1 (M. Maurer, 2006). For the Intermediate scenario, a value of 70 m^3 a^{-1} is taken (ECB, 2003, p. 62).

In order to validate the mass flow analysis from the previous section, we calculated the concentration of silver in dry sewage sludge according to ECB (2003):

$$C_{ss} = \frac{f_{removal} \cdot Ag_{WW,STP}}{2/3 \cdot SM_{infl} \cdot WW_{percapita} \cdot POP \cdot f_{STP} + SS_{surplus} \cdot POP \cdot f_{STP}} \quad (4)$$

$Ag_{WW,STP}$ is the amount of silver found in inflows of STPs (see Table 2), SM_{infl} the concentration of suspended matter in inflows of STPs (0.45 kg m^{-3}) and $SS_{surplus}$ (0.011 kg d^{-1}) the surplus sludge that is produced per inhabitant and day. Values for the emission scenario-dependent parameters $f_{removal}$, $WW_{percapita}$ (m^3 d^{-1}), POP and f_{STP} are given in Table 1.

We selected the Rhine, a large European river receiving a high amount of treated wastewater, as a representative case study to estimate PECs in the aquatic environment resulting under the three silver emission scenarios. Steady state water and sediment concentrations are estimated with a river model (Scheringer et al., 1999). The model parameters chosen represent the characteristics of the Rhine from Basel (Switzerland) to Lobith (Netherlands), 700 km downstream where the river splits up in three parts. In the model, water is transported through 70 boxes of constant length (10 km) and of increasing volume (up to a factor of 4). Each box consists of a compartment of moving water (W1), a compartment of stagnant water (W2) and a compartment representing the top layer of the sediment (Sed). The volume of the sediment is 5% of the stagnant water, which is set to 12.5% of the moving water body. The compartments are

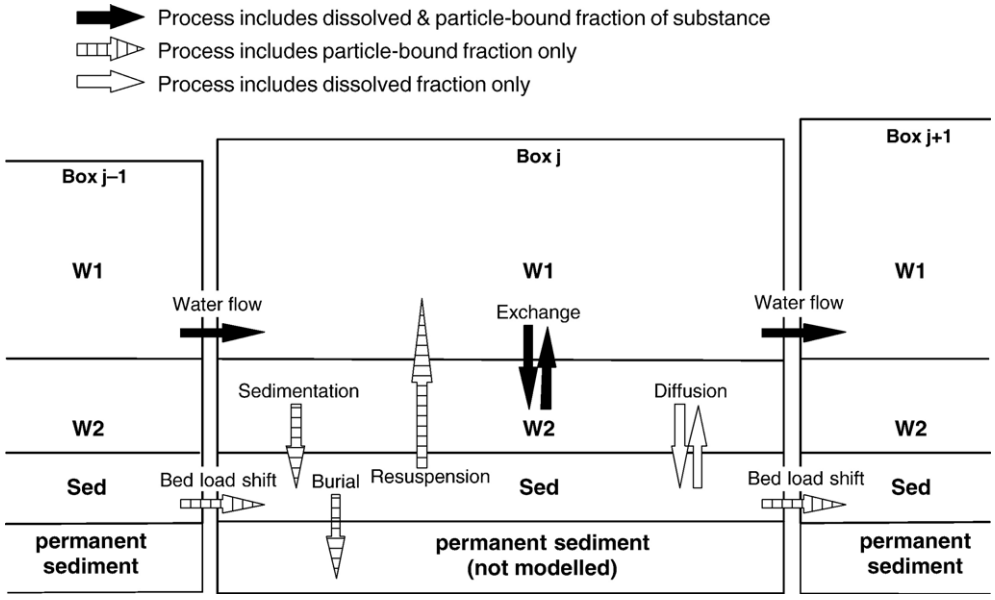

Fig. 3 – Processes simulated in the Rhine river model. W1 is the moving water, W2 the stagnant water and Sed represents the top layer of the sediment.

assumed to be completely mixed. The model includes the following processes (see Fig. 3): downstream transport of dissolved and particle-bound silver with moving water; bulk exchange between moving and stagnant water; sedimentation of particles from stagnant water into the top sediment layer; resuspension of particles into moving water; burial of the mineral fraction of particles into the permanent sediment; and diffusive exchange of the dissolved fraction of silver sulfide between stagnant water and interstitial water in the sediment. The model version used here also includes a bed load shift downstream that is described by splitting the original sediment burial process into a burial and a horizontal sediment transfer process at a ratio of 1:9. This represents the average effect of high water flow during spring and subsequent downstream transfer of sediment. The sedimentation mass flux is equal to the sum of resuspension, burial, and sediment transfer to the box further downstream.

The behavior of silver sulfide in water is characterized by the partition coefficient K_p which is defined by the ratio:

$$K_p = \text{metal in particulate matter}\left(\mu g\,kg^{-1}\right)/\text{metal in filtrate}\left(\mu g\,L^{-1}\right)$$

Empirical partition coefficients determined for total silver in river waters range from $10^{4.0}$ to $10^{6.6}$ L kg^{-1} based mostly on a size cutoff of 0.4 μm, i.e. silver sulfide particles below 0.4 μm are included in the filtrate (Kramer et al., 2002). In the river model an average value of $10^{5.3}$ L kg^{-1} is used. Silver sulfide levels expected in the interstitial water ($C_{Sed,interstitial}$) result from dividing the silver concentration in sediment (C_{Sed}) by the partition coefficient (K_p).

$$C_{Sed,\,interstitial} = \frac{C_{Sed}}{K_p} \quad (5)$$

Different amounts of silver were released to the moving water compartment of the model at representative sites along the river. ICPR (2005) estimates 50 million inhabitants living in the Rhine watershed. If silver emissions are assumed to be proportional to population, 11% of total silver reaching natural waters in EU25 is released to the Rhine: 2 t in the Minimum, 7 in the Intermediate and 15 t in the Maximum scenario. Based on Spiess (2005), 45 cities with more than 10,000 inhabitants were selected along the Rhine between Basel and Lobith. The silver input quantity is adjusted for every city relative to population and silver enters the model at the box representing the city's position.

The uncertainty in the model outputs is assessed by running the model for the three emission scenarios and by performing first-order error propagation for the model itself (MacLeod et al., 2002). First-order error propagation is especially appropriate for preliminary simulations that are done for large geographic areas when input parameters might vary within the system. We use confidence factors (Cf) to express the uncertainty in input parameters and model results, where 95% of possible values in a distribution lie between the median multiplied by Cf and the median divided by Cf.

3.3. *Predicted environmental concentrations*

To avoid conversion of the amounts of silver released to the model into amounts of various silver sulfide species, we express all model results in terms of silver concentrations. Expected silver concentrations in STPs are 2 μg L^{-1} in the Minimum scenario, 9 μg L^{-1} in the Intermediate and 18 μg L^{-1} in the Maximum scenario (see Table 3). For STP inflows in southern Wisconsin, USA, Shafer et al. (1998) report a range of 1.78 to 4.27 μg Ag L^{-1} in three STPs treating common wastewater and 24.0 to 105 μg L^{-1} in two plants receiving high silver loadings from industrial discharges. Because for the derivation of C_{STP} silver releases from industrial and common household sewage in combination were used, it is

Table 3 – Predicted environmental concentrations in the aquatic environment

Compartment	Unit	Minimum scenario	Intermediate scenario	Maximum scenario
Sewage treatment plants	$\mu g\ L^{-1}$	2	9	18
River water	$ng\ L^{-1}$	40	140	320
River sediment	$mg\ kg^{-1}$	2	6	14
Interstitial water of the sediment	$ng\ L^{-1}$	9	30	70

reasonable that our estimated concentrations lie between the concentrations measured for common wastewater and for industrial discharges, see also Fig. 5.

The predicted silver sulfide concentrations in anaerobically digested, dry sewage sludge (7, 24 and 39 mg Ag kg^{-1} DW) are similar to measured data (see Fig. 5), indicating that the aquatic silver emissions are sufficiently well characterized.

River model results for the moving water and the top layer of the sediment are displayed in Fig. 4. The silver concentrations in water along the course of the Rhine are estimated to range between 4 and 40 ng L^{-1} (Minimum), 10–140 ng L^{-1} (Intermediate) and 30–320 ng L^{-1} (Maximum scenario). Corresponding values for the sediment are 0.04–2 mg kg^{-1} in the Minimum, 0.1–6 mg kg^{-1} in the Intermediate and 0.3–14 mg kg^{-1} in the Maximum scenario. In both water and sediment, a downstream accumulation of silver sulfide is observed; in the water maxima occur 600 km downstream from Basel, and in the sediment at 660 km. For a conservative risk characterization maximum silver levels are used as PECs, see Table 3. In the interstitial water of the sediment 9 ng L^{-1} (Minimum), 30 ng L^{-1} (Intermediate) and 70 ng L^{-1} (Maximum scenario) are calculated.

Ranges of parameter values and corresponding confidence factors used in the uncertainty analysis are given in Table 4, which also shows the sensitivity of silver concentrations in water and sediment to changes in input parameters. The confidence factors obtained for the silver concentrations in water and sediment are 4.2 and 18, respectively. With this confidence factor, the 95th percentile of the predicted silver concentration in water is 1350 ng L^{-1}. This value corresponds to data measured about 1 km downstream from a treated photoindustry effluent (1107 ng L^{-1}; Wen et al., 2002). Generally, the model results are in agreement with the wide range of empirical data for silver in river waters (<0.01–148 ng L^{-1}; Fig. 5).

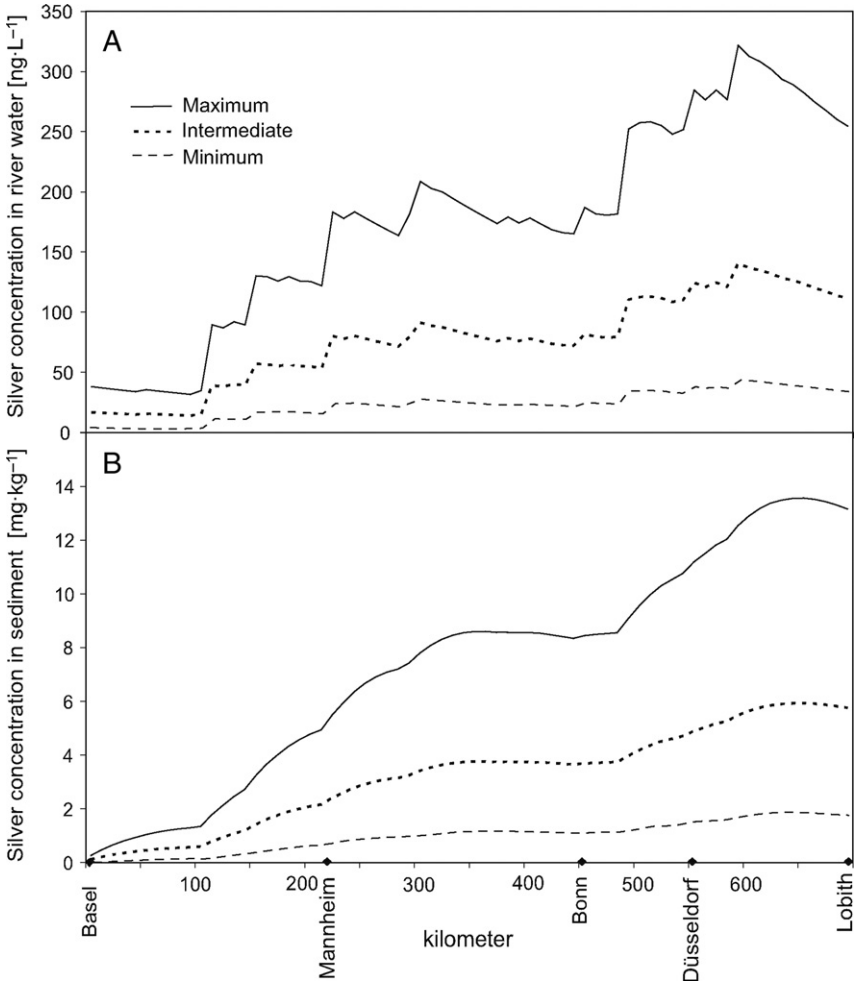

Fig. 4 – Predicted silver concentrations in the Rhine river between Basel and Lobith for three emission scenarios. (A) Concentrations of silver in moving water and (B) in top layer of the sediment.

Table 4 – Confidence factors and sensitivities of selected parameters for the Rhine model

Parameter	Explanation	Unit	Range	Cf	S_{W1} [a]	S_{Sed} [b]
U	Water flow velocity	m s^{-1}	$5.70 \cdot 10^{-1}$–$1.64 \cdot 10^{0}$ [c]	1.7	$4.66 \cdot 10^{-1}$	$6.79 \cdot 10^{-1}$
[SPM]	Concentration of suspended particulate matter	kg m^{-3}	$1.10 \cdot 10^{-3}$–$1.48 \cdot 10^{-2}$ [d]	12	$1.61 \cdot 10^{-2}$	$9.82 \cdot 10^{-1}$
ρ_{Sed}	Sediment density	kg m^{-3}	$1.00 \cdot 10^{3}$–$2.50 \cdot 10^{3}$ [e]	1.8	$2.55 \cdot 10^{-2}$	$9.96 \cdot 10^{-1}$
Φ	Porosity of sediment	–	$7.50 \cdot 10^{-1}$–$9.50 \cdot 10^{-1}$ [f]	1.1	$2.30 \cdot 10^{-2}$	$8.96 \cdot 10^{0}$
u_{sed}	settling velocity of SPM	m s^{-1}	$1.16 \cdot 10^{-6}$–$3.47 \cdot 10^{-5}$ [g]	10.8	$5.50 \cdot 10^{-1}$	$2.92 \cdot 10^{-1}$
μ_{resusp}	Resuspension rate	kg m^{-2} d^{-1}	$2.20 \cdot 10^{-5}$–$4.60 \cdot 10^{-4}$ [h]	4.6	$1.35 \cdot 10^{-2}$	$1.58 \cdot 10^{-2}$
K_p	SPM–water partition coefficient	L kg^{-1}	$10^{4.0}$–$10^{6.6}$ [i]	20	$1.76 \cdot 10^{-1}$	$2.24 \cdot 10^{-1}$

SPM: suspended particulate matter.
[a] $S_{w1} = \frac{\Delta Output/Output}{\Delta Input/Input}$ for $\Delta Input/Input = 0.001$. It is the sensitivity of the silver concentration in the moving water compartment to variation of various input parameters.
[b] S_{Sed} is the sensitivity of the silver concentration in the top layer of the sediment to variation of parameters.
[c] Based on Kos et al. (2000).
[d] Based on ICPR (2003).
[e] Estimated.
[f] Based on MacLeod et al. (2002) and Omlin et al. (2001).
[g] Based on Reichert and Mieleitner (2006).
[h] Based on MacLeod et al. (2002).
[i] Based on Kramer et al. (2002).

Recent field data for silver in river sediments range from 0.2 to 2 mg ·kg^{-1} (Fig. 5) indicating that model results (2–14 mg kg^{-1}) lie rather on the high side. On the other hand, they are still below concentrations found in early measurements from highly impacted river beds with up to about 150 mg kg^{-1} (Dissanayake et al., 1983; USEPA, 1980; cited in Eisler, 1996).

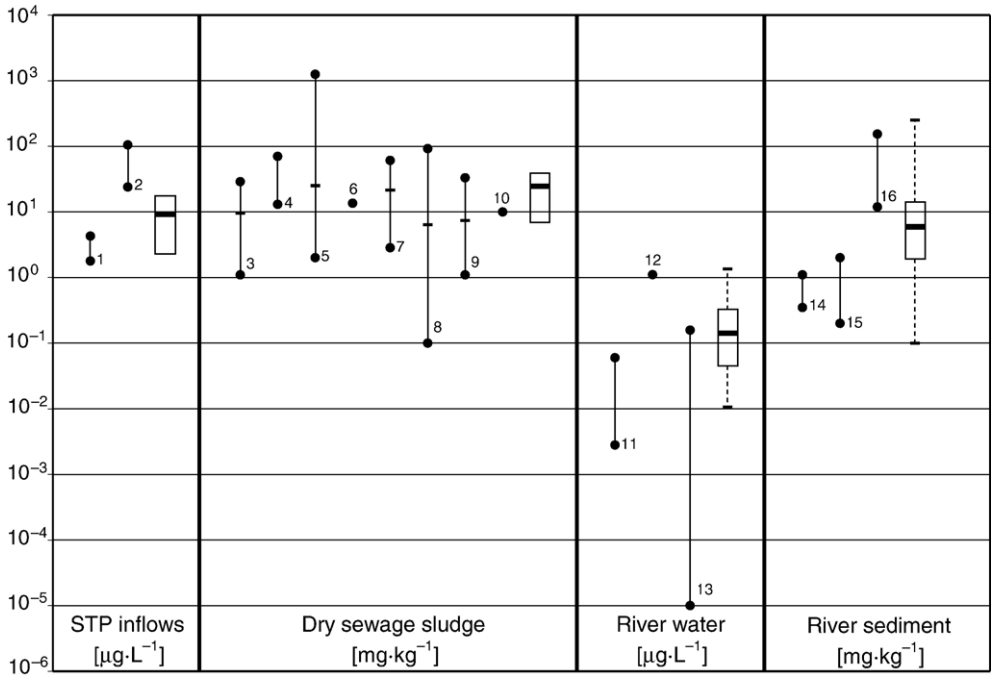

Fig. 5 – Comparison of empirical and model data. Full lines represent measured ranges, means are indicated where available. Boxes represent results for the three emission scenarios, vertical bars represent values for the Intermediate scenario; uncertainty is indicated by dashed line. References: 1 = Unimpacted STP inflows; Wisconsin, USA; Shafer et al., 1998. 2 = Heavily loaded STP inflows; Wisconsin, USA; Shafer et al., 1998. 3 = Sludges from 26 STPs in Germany; Lottermoser, 1992. 4 = Sludges from 8 STPs in Australia; Lottermoser, 1995. 5 = Sludges from STPs in UK; Smith, 1996. 6 = Sludge from one STP in USA; Hirsch, 1998. 7 = Sludges from 14 STPs in Japan; Kawasaki et al., 1998. 8 = Sludges from 29 STPs in Switzerland; Chassot et al., 1999. 9 = Sludges from 48 STPs in Sweden; Eriksson, 2001. 10 = Sludge from one STP in Sweden; Fjällborg et al., 2005. 11 = Downstream from STP effluents; Colorado, USA; Wen et al., 2002. 12 = Downstream from photoindustry discharge point; Colorado, USA; Wen et al., 2002. 13 = Riverwaters; Texas, USA; Wen et al., 1997. 14 = Top sediment of St. Lawrence River; Canada; Gobeil, 1999. 15 = Top sediment of South Platte River Basin in urban and agricultural areas affected by urban sources; USA; Heiny and Tate, 1997. 16 = Sediment of highly industrialized cut-off channel of the Rhine; Germany; Dissanayake et al., 1983.

4. Data for dose–response assessment

Most toxicological studies use silver nitrate ($AgNO_3$), which forms free silver ions in aqueous solution, as test substance. As mentioned before, Ag^+ occurs only in extremely low concentrations in the environment. The effects of the environmentally most relevant forms of silver, organic and inorganic silver sulfide compounds, in contrast, have only recently been studied. Toxicity data of silver species present in the environment are summarized in the following subsections. Predicted no-effect concentrations (PNECs) have been estimated where sufficient data is available (Table 5) and assessment factors (AF) have been determined according to the Technical Guidance Document (TGD) of the EU (ECB, 2003). When no effect was observed in toxicological tests, we report the highest observed no-effect concentrations (HONECs).

4.1. *Silver sulfide compounds*

Silver sulfide clusters, which are expected to be the most common form of silver in the dissolved fraction of natural freshwaters (defined by the silver species passing a 0.45 μm filter), have been tested for toxicity to *Daphnia magna* and *Oncorhynchus mykiss*. In both cases, silver was added as $AgNO_3$ to a solution containing varying concentrations of zinc sulfide clusters. The major part of Ag^+ is expected to bind to ZnS resulting in silver zinc sulfide clusters. In the study with *D. magna* (Bianchini et al., 2002) no acute effect could be detected up to 19 nM silver (with 250 nM sulfide present). When the silver concentration exceeded the sulfide concentration, acute toxicity of the free silver ion was reduced according to the amount of sulfide present. Here we use a HONEC of 19 nM Ag–ZnS, corresponding to 2 μg Ag L^{-1} in combination with an AF of 1000 to evaluate the risk for the freshwater ecosystem, see below. No toxicity data have been found for silver in the particulate fraction (>0.45 μm).

In the interstitial water of strongly polluted sediments silver might be bound to organic sulfides, for example, cysteine and glutathione. Acute and chronic effects of these silver thiolates were studied on *Ceriodaphnia dubia* (Bielmyer et al., 2002). For silver cysteinate an 8d-LOEC of 0.001 μg Ag L^{-1} was found, for silver glutathionate an 8d-NOEC of 0.6 μg Ag L^{-1}. In both cases, we use an AF of 100 to calculate the PNEC. These tests suggest that silver thiolates are even more toxic than the free silver ion if endpoints other than lethality are considered. However, this finding contradicts the free ion activity model of toxicity where the ligand-bound fraction of a metal is considered nonbioavailable (Di Toro et al., 2001). No data are available for silver thiolates tested on benthic organisms.

To address the toxicity of silver to microorganisms in STPs, we use data derived from a simulation study of aerobic degradation of organics in silver-containing photoprocessing wastewaters (Pavlostathis and Maeng, 1998). This kind of study investigates the possible effects of silver on STP microorganisms in the presence of reduced sulfur. Because high levels of reduced sulfur are likely in STPs (Shafer et al., 1998), we assume that the conditions of such a simulation study are similar to the conditions under which STP microorganisms are exposed to silver from various types of wastewaters. Pavlostathis and Maeng (1998) did not find any effect of silver on the performance of the aerobic biodegradation of wastewater for silver influent concentrations up to 1.85 mg/L. For the risk assessment of the microbial community in STPs, we use 1.85 mg/L as HONEC and divide this value by an assessment factor of 10. This factor is suggested in the EU TGD for values from inhibition controls in biodegradation tests (ECB, 2003, p. 109).

4.2. *Free silver ion*

In settings where the silver concentration exceeds the concentration of organic and inorganic sulfides, the toxicity of the free silver ion has to be considered. It is the only form of silver that has been extensively tested on aquatic organisms and plants (Ratte, 1999; Wood et al., 2002). In many studies the various ligands present in the test solution were not monitored. Therefore it is often unclear which form of silver was actually tested. The lowest NOEC was found by Bielmyer et al. (2002) for *C. dubia* with 0.001 μg Ag L^{-1}. We divide this value by an AF of 10 to derive the PNEC in freshwater. Note that NOECs for Ag^+ from other studies with cladocerans are in general 2 to 3 orders of magnitude higher (Nebeker, 1982; Rodgers et al., 1997) than data from Bielmyer et al. (2002). According to Wood et al. (2002) the most sensitive benthic organism is *Hyalella azteca* with an LC50 of 1.9 μg Ag L^{-1}. An AF of 1000 is applied for the derivation of the PNEC in sediment.

Table 5 – Predicted no-effect concentrations (PNECs in μg Ag L^{-1}) or highest observed no-effect concentrations (HONECs) divided by assessment factors (HONEC/AF in μg Ag L^{-1})

Compartment	Form of Ag	Derived from	AF	PNEC[a]	HONEC/AF	Reference
STP	Ag-thiosulfate	HONEC[b]; activated sludge	10		$1.85 \cdot 10^2$	Pavlostathis and Maeng (1998)
Freshwater	Ag–ZnS	HONEC[b]; *Daphnia magna*	1000		$2 \cdot 10^{-3}$	Bianchini et al. (2002)
	Ag^+	NOEC; *Ceriodaphnia dubia*	10	$1 \cdot 10^{-4}$		Bielmyer et al. (2002)
Freshwater sediment	Ag^+	LC50; *Hyalella azteca*	1000	$1.9 \cdot 10^{-3}$		Wood et al. (2002)
	Ag-cysteinate	LOEC; *C. dubia*	100	$1 \cdot 10^{-5}$		Bielmyer et al. (2002)
	Ag-glutathionate	NOEC; *Ceriodaphnia dubia*	100	$6 \cdot 10^{-3}$		Bielmyer et al. (2002)
	Ag–ZnS	HONEC[b]; *D. magna*	1000		$2 \cdot 10^{-3}$	Bianchini et al. (2002)

[a] PNEC is estimated as NOEC/AF or LC50/AF or LOEC/AF. Assessment factors were selected according to ECB (2003).
[b] The highest observed no-effect concentration (HONEC) is used where no toxic effect was observed up to highest concentration tested.

5. Risk characterization

To characterize the environmental risk caused by silver in aquatic systems, we calculate the PEC–PNEC ratio in cases where a PNEC could be derived, see Table 5. In cases where only a HONEC could be derived, we calculate the ratio PEC/(HONEC/AF). This ratio does not directly indicate the presence or absence of a risk but it shows the range in which a NOEC from future measurements would have to lie for a risk to be ruled out.

The $[PEC_{STP}]/[HONEC_{Ag\text{-}thiosulfate}/AF]$ ratios for STPs are below one (0.01 for the Minimum, 0.05 for the Intermediate and 0.1 for the Maximum scenario), which indicates that no adverse effect of silver is expected for the microbial community in STPs.

For freshwater, the concentration of sulfide has to be estimated in order to evaluate whether the HONEC for Ag–ZnS clusters is applicable. In freshwater, sulfide – under oxic conditions most probably stabilized by the formation of metal-sulfide clusters such as CuS clusters – detoxifies silver, at least up to a concentration of 2 μg Ag L^{-1} (Bianchini et al., 2002). Chromium reducible sulfide (CRS) can be used to determine the inorganic sulfide concentration in water (Kramer et al., 2007). No measurements of CRS were found for European freshwaters. Therefore, the relationship reported by Kramer et al. (2007) between organic carbon and CRS is used to estimate sulfide concentrations:

$$\mathrm{CRS\,(nM)} = 14.6\ \mathrm{DOC\ (mg\ L^{-1})} \tag{6}$$

Empirical data reported for DOC in the Rhine range from 2.5 to 3.5 mg L^{-1} (Huebner and Karrenbrock, 2000; Schubert, 2003), which yields CRS values from 37 to 51 nM. This is in agreement with the range of 10–50 nM reported by Kramer et al. (2007) for impacted urban waters. Therefore, the ratio CRS/PEC_{water} exceeds 10 and it can be assumed that all silver is bound to sulfide (in addition, it is assumed that mercury and copper concentrations are low enough so that neither of these metals competes with silver for sulfide). Under these conditions, Ag–ZnS clusters are the relevant silver species and the risk can be evaluated in terms of $PEC_{water}/(HONEC_{Ag\text{–}ZnS}/AF)$.

The $PEC_{water}/(HONEC_{Ag\text{–}ZnS}/AF)$ ratios are 20, 70 and 160, depending on the emission scenario. Therefore risk for the freshwater ecosystem can only be ruled out if the $NOEC_{Ag\text{–}ZnS}$ exceeds the $HONEC_{Ag\text{–}ZnS}$ used here by at least 160 times, i.e. exceeds 320 μg Ag L^{-1}. Current data does not allow a conclusive risk evaluation. A determination of the actual NOEC of silver sulfide clusters and the concentration of sulfide in European rivers would be clarifying.

In the sediment, sulfides also play a key role for the assessment of silver toxicity. For example, Adams and Kramer (1999a) measured sulfide concentrations of 350 to 1000 nM in the colloidal fraction (from 10 kDa to 0.45 μm) of the anoxic sediment of Lake Ontario. In addition, thiols were detected at concentrations between 9.1 and 15.5 nM in 10 to 20 cm depth of the lake sediment. The conditional stability constant of silver zinc sulfide clusters was determined by Bowles et al. (2002) to be 8.9, whereas Adams and Kramer (1999b) found for silver cysteinate and silver glutathionate values of 11.9 and 12.3, respectively. If the Rhine sediment contains thiols at similar levels to the sediment of Lake Ontario, silver would probably be bound to these organic ligands. In this case, the ratios $PEC_{porewater,sediment}/PNEC_{Ag\text{-}cysteinate}$ and $PEC_{porewater,sediment}/PNEC_{Ag\text{-}glutathionate}$ would be relevant for the risk characterization. For silver cysteinate, PEC/PNEC ratios of 940, 2970 and 6800 are obtained, for silver glutathionate the values are 1.6, 5.0, and 11.3, depending on the emission scenario.

However, it has to be kept in mind that no actual measurements of sulfide species have been carried out in the sediment of the Rhine. If silver sulfide is the dominating species in the sediment, the $HONEC_{Ag\text{–}ZnS}$ has to be used, which yields risk quotients of 4.5 (Minimum), 15 (Intermediate) and 35 (Maximum). Uncertainties of the sediment risk assessment for silver sulfide are high because the no-effect concentrations do not refer to a benthic species, and because for silver sulfide clusters only a lower bound of the PNEC can be estimated since no effect was observed at the highest concentration tested. Analysis of prevalent silver and sulfide species in river beds and their toxicological effects on benthic organisms would allow more significant conclusions.

6. Discussion and recommendations

In our analysis we find that up to 15% of the total silver emitted to water in Europe may be released from biocidal plastics and textiles in 2010. Considering that the market for such products is expected to grow until 2015, we conclude that biocidal plastics and textiles will account for a substantial share of total silver emissions in EU25 in the future. However, given the prospective character of our study and the uncertainties in our estimates of emissions and environmental levels of silver and of the toxicological data presently available in the literature, we recommend that our analysis be updated during the course of increasing usage of silver as a biocide.

Relevant uncertainties in our emission estimates include the following:

i. There are additional applications of silver as a biocide, e.g. in exterior paints. These applications were not included in the present study because they are still in an early stage and difficult to quantify. They may contribute to a considerable extent to silver releases to the environment, even beyond our Maximum scenario, and should be quantified as well.
ii. Release rates for silver-containing polymer coatings need to be determined more extensively and overall releases from possible applications of this technology need to be quantified. Assuming that the release rates are not fundamentally different from the ones for silver nanoparticles (Marini et al., 2007), we estimate that releases from this kind of application are covered by our Maximum scenario.
iii. It may be possible that discrete nanoparticles of elemental silver could be released from nano-functionalized polymers. However, this process has not yet been quantified and its contribution to silver releases is difficult to assess.

iv. We did not explicitly consider releases from the stock of silver-containing plastics and textiles. Table 1 shows that the main contribution to silver releases from biocidal applications comes from plastics in continuous contact with water (plastics$_{H_2O}$ in Table 1); this category includes items such as sanitation tubes and medical catheters, and contributes about 95% of the releases from biocidal applications. To estimate the possible emissions of silver contained in the stock of products, we focus on this category. It takes about three years for silver in plastics in continuous contact with water to be completely released. Therefore, the maximum amount of product present as stock and still releasing silver is given by three annual production volumes. However, the useful lifetime of many products in this usage category is shorter than three years; for example, products used in medical applications are likely to be thrown away after one use. We therefore assume that the average lifetime of the products in the category plastics$_{H_2O}$ is one year. Under this assumption, our present estimate completely reflects emissions from this category and there is no significant contribution from the stock. If the average lifetime of the plastics products in contact with water is longer than about one year, we underestimate the emissions by neglecting the stock by a factor of less than 3, probably less than 2.

To evaluate our estimates of the emissions and environmental levels of silver, we compared our results to silver concentrations measured in the field. Here it is important that our results reflect a situation in which silver emissions from biocidal products will be higher than at the times when the measurements were taken (mostly in the 1990s). On the other hand, silver releases from non-biocidal sources such as photographical industry were higher in the last decades and are likely reflected by the measured values. In comparing model results with field data, we make the assumption that emission reductions in the recent years are largely compensated for by new releases from biocidal silver applications. If this assumption holds, the measured concentrations from earlier years can be used to evaluate our calculated results.

Fig. 5 shows that in all cases – STP inflows, sewage sludge, freshwater, and sediment – our model results fall in the range of measured values. Concentrations in STP inflows are slightly higher than in unimpacted inflows but clearly below heavily loaded inflows. Concentrations in sewage sludge are slightly higher than mean values of measurements but within the variability range. This agreement indicates that the silver mass fluxes entering the aquatic system are reasonable estimates and that the emission scenarios provide a useful basis for the exposure assessment. Concentrations in river water and river sediment are in the middle of the variability range of data from field measurements. Unclear remains in the exposure assessment which chemical species of silver is predominant in surface waters, to which extent and in which form sulfide is available for binding silver, and whether the free silver ion plays a role in natural waters.

Uncertainty in the environmental chemistry of silver and scarcity of toxicological studies make a conclusive risk assessment impossible at the present time. The main problem is that the actual effect concentration of silver sulfide clusters remains unclear, although some testing has been carried out. To rule out possible effects from silver to freshwater organisms, toxicological studies on aquatic species should confirm a NOEC$_{Ag-ZnS}$ higher than 320 μg Ag L^{-1}.

However, not only the aquatic exposure to silver from biocidal plastics and textiles should be studied, but also the impact on terrestrial ecosystems. For this purpose, measurements of silver concentrations in soil, a determination of prevalent silver forms, and a better understanding of the toxicological effects on terrestrial organisms is necessary. In the long term, the fate of silver nano-functionalized products in solid waste landfills could also be of concern. Such plastics and textiles might show a delayed degradation as microorganisms are hindered in colonizing the silver-containing polymer to decompose it.

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TABLE 1. Properties of Silver Nanoparticles in the Parent Suspensions Made by Varying Sodium Borohydride Concentrations and Their Corresponding Toxicity to Nitrifying Bacteria

$NaBH_4$ (mM)	0.025	0.05	0.09	0.15	0.3
molar ratio of BH_4^-/Ag^+ ($AgNO_3$, 0.25 mM)	0.1	0.2	0.36	0.6	1.2
average particle size, (nm)	9 ± 5	15 ± 9	14 ± 6	12 ± 4	21 ± 14
Ag nanoparticle concentration (mg/L)	3 (±2.4)	14 (±0.6)	25 (±2.3)	26 (±0.3)	24 (±2.1)
surface plasmon peak (nm)	400	400	395	395	395
degree of inhibition at 0.5 mg/L Ag	53% (±4.2)	63% (±8.6)	6% (±9.8)	17% (±5.2)	23% (±4.0)

2). As the concentrations of $NaBH_4$ increased, the fraction of Ag nanoparticles less than 5 nm was reduced, reaching a minimum at $R = 0.6$ and became increased as more $NaBH_4$was added (Figure 1A). At $R = 0.1$, the relative abundance of the nanoparticles less than 5 nm was the highest among the prepared nanosilver suspensions while the concentration of Ag nanoparticles was low due to incomplete reaction. Because of excess of Ag^+ ions in the suspension, red shifts in the maximum plasmon peak occurred (*29*) when R was less than 0.2. Most added Ag^+ ions were reduced to Ag nanoparticles at R equal to 0.36 or above, possibly because PVA functioned both as a reducing agent and a capping agent (*30*).

A plot of the degree of nitrification inhibition as a function of the fraction of nanoparticles of defined sizes in the suspension showed that the inhibition correlated with the relative abundance of Ag nanoparticles smaller than 5 nm (R^2= 0.738) (Figure 3a). There was no correlation between the inhibition and the average nanoparticle size (see Supporting Information Figure S3). Some relationship existed between the inhibition and the Ag^+ ion concentrations added in the cell suspensions ($R^2 = 0.595$) (Figure 3b). However, Ag nanoparticles were more toxic to nitrifying bacteria than Ag^+ ions at the same total Ag concentrations (Figure 4). Therefore,

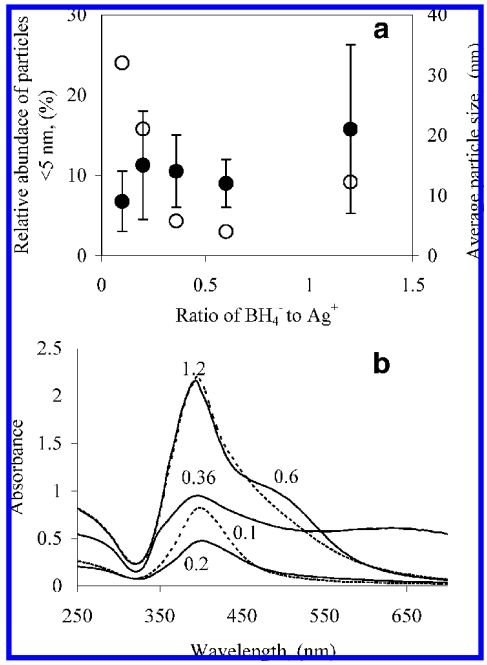

FIGURE 1. (a) Change of relative abundance of Ag nanoparticles less than 5 nm (open circle) and average particle size (filled circle) by varying molar ratio of sodium borohydride to silver nitrate. Error bars indicate the ranges of duplicate determinations. (b) UV−visible spectra of Ag nanoparticle suspensions by varying molar ratios (see values on the figure) of sodium borohydride to silver nitrate.

the high degree of inhibition at low BH_4^-/Ag^+ ratios cannot be attributed to the presence of Ag^+ ions only. We suggested that the Ag nanoparticles less than 5 nm were responsible for inhibiting microbial growth and could be more toxic to bacteria than their counterpart species.

Nanosilver Toxicity. In a first set of experiments, the degrees of inhibition on microbial growth by Ag nanoparticles and their counterpart species were determined. Silver nanoparticles had the highest toxicity to nitrifying bacteria (Figure 4) among the Ag species tested. The effective concentrations of Ag nanoparticles, AgCl colloids, or Ag^+ ions causing 50% inhibition (EC_{50}) were 0.14 mg/L, 0.25 mg/L, and 0.27 mg/L Ag, respectively, using a saturation-type biological toxicity model (*31*).

Toxicity Related to Intracellular ROS Concentration. Exposure of Ag nanoparticles of 15 nm average size at incremental concentrations onto preincubated nitrifying cultures resulted in an overall increase in intracellular ROS concentrations (Figure 5A). It appeared that at the same total Ag concentrations, AgCl colloids generated at least an equivalent amount of ROS compared to that by Ag nanoparticles. In contrast, Ag^+ ions generated less intracellular ROS than that by Ag nanoparticles (Figure 5A). The degree of inhibition by Ag nanoparticles was strongly correlated with intracellular ROS concentration ($R^2 = 0.86$) using a saturation type model (Figure 6 and Supporting Information Figure S5). Similarly, the inhibition was correlated with intracellular ROS levels induced by Ag^+ ions or AgCl colloids ($R^2 = 0.70-0.89$). Taken all Ag species together, however, there was a poor correlation between the inhibition and intracellular ROS concentrations ($R^2 = 0.54$) by a saturation-type model. It appeared that although there was correlation between the nitrification inhibition and the intracellular ROS concentrations, the ROS correlations were different for the different forms of silver (Ag nanoparticles, AgCl colloids and Ag^+ ions).

Toxicity Related to Photocatalytic ROS Concentration. ROS generation in cells may proceed through *exogenous* (extracellular) and *endogenous* (intracellular) processes. We therefore measured the ROS concentrations induced by Ag nanoparticles, AgCl colloids, and Ag^+ions in the absence of nitrifying cultures. Since without light there was no ROS generation in Ag nanoparticle suspensions or bulk solutions (data not shown), the concentrations of photocatalytic ROS was measured after 30 min natural light illumination in the laboratory. It appeared that photocatalytic ROS increased rapidly in the presence of 0.1 mg/L Ag but gradually decreased as the Ag nanoparticle concentrations increased (Figure 5B). A similar trend applied for AgCl colloids. The pattern of photocatalytic ROS generation by Ag^+ ions, however, appeared differently. ROS only increased significantly when Ag concentrations were above 0.4 mg/L. Poor correlation ($R^2 = 0.53-0.72$) was noticed between the observed inhibition and the photocatalytic ROS concentrations by a saturation-type model (see Supporting Information Figure S6). Therefore, photocatalytic ROS concentrations were not a good predictor of the growth inhibition by Ag nanoparticles.

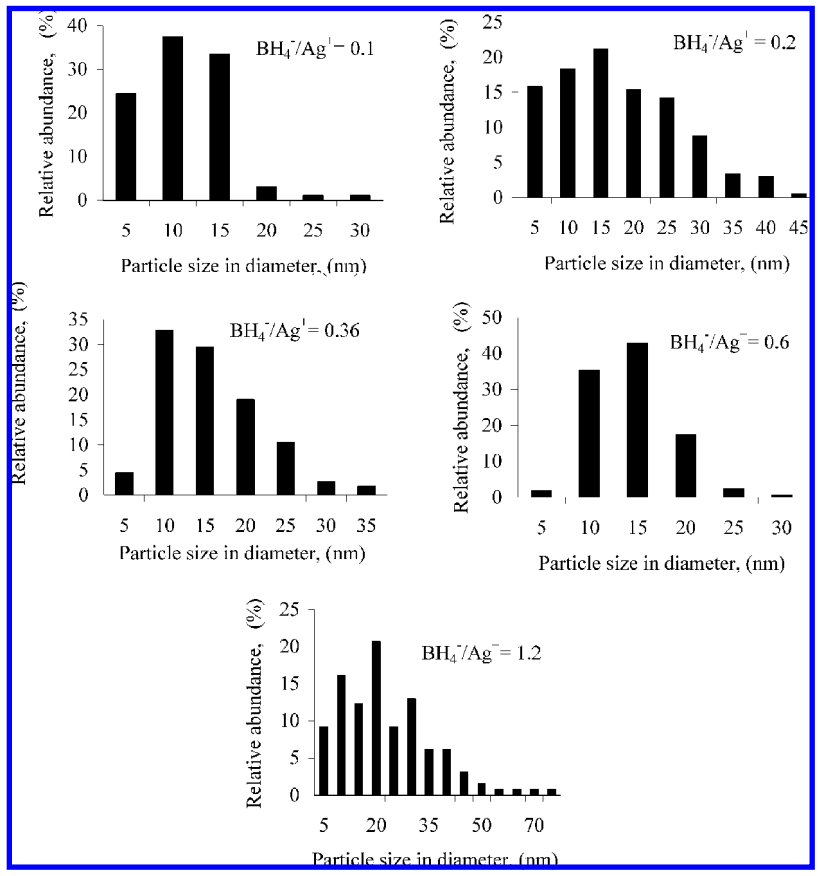

FIGURE 2. Size distribution of Ag nanoparticles with an average size range of 9−21 nm made by varying molar ratio of sodium borohydride to silver nitrate.

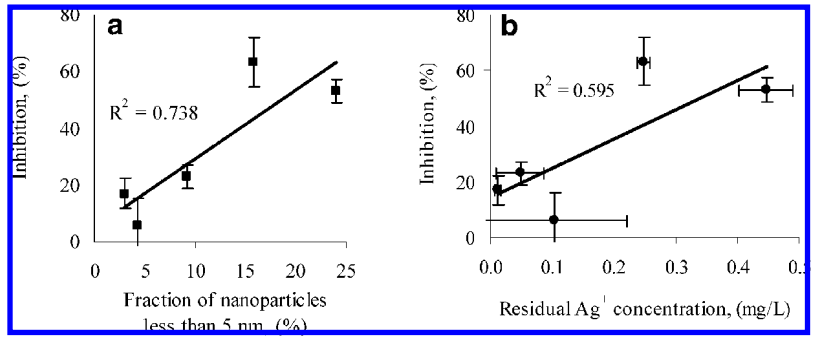

FIGURE 3. The inhibition as a function of (a) the fraction of Ag nanoparticles less than 5 nm and (b) the projected Ag^+ concentrations in the biomass suspensions. Error bars indicate one standard deviation ($n = 3$).

Discussion

Sizes of the nanoparticles play an important role in silver toxicity. Smaller and uncharged Ag nanoparticles with higher surface areas could interference cell membrane function by directly reacting with cell membrane to allow a large number of the Ag atoms to attack or easily enter the cells (*10, 12*). Our results demonstrated that the inhibition correlated well with the fraction of Ag nanoparticles less than 5 nm, but not with the other particle size fractions (e.g., 10, 15, 20 nm, R^2 = 0.04−0.39), suggesting that these smaller size nanoparticles could be more toxic to bacteria than any other fractions of the nanoparticles. This result is consistent with recent findings that Ag nanoparticles of 1−10 nm sizes interacted strongly with HIV-1 after attached to the virus (*32*), and the average size of Ag nanoparticles penetrating into an *E. coli* membrane was 5 (±2) nm even though the average particles size of added nanoparticles was 21 (±18) nm (*12*). Particles must be sufficiently small to pass through transmembrane porins (typical internal pore size in nm) for transport across cell membranes (*33*) to cause the damage of cellular constituents and metabolism.

The results of ROS measurements showed that Ag nanoparticles, AgCl colloids, and Ag^+ ions all induced intracellular ROS generation and accumulation, and the inhibition correlated well with their individual intracellular ROS concentrations. Previous studies in a variety of cell types suggested that ROS generation might damage cell DNA and induce apoptosis (programmed cell death) with no visible membrane damage (*24, 25, 34*). Our earlier results from bacterial live/dead assays indicated that cell membrane integrity was not compromised at 1 mg/L Ag for all of the forms of Ag tested (*27*). This suggested that the toxicity of Ag nanoparticles could be related to intracellular ROS mediated cell death process. At the same level of intracellular ROS or the same total Ag concentrations, however, Ag nanoparticles appeared to be more toxic than Ag^+ ions (Figure 4 and 6),

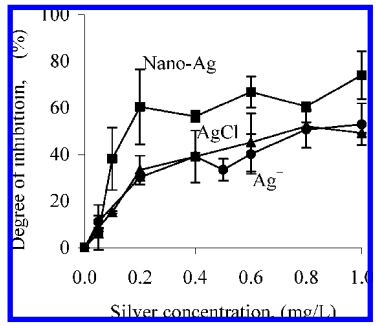

FIGURE 4. The degree of nitrification inhibition by Ag nanoparticle (■), AgCl colloid (▲), and Ag^+ (●). Error bars show one standard deviation ($n = 3$).

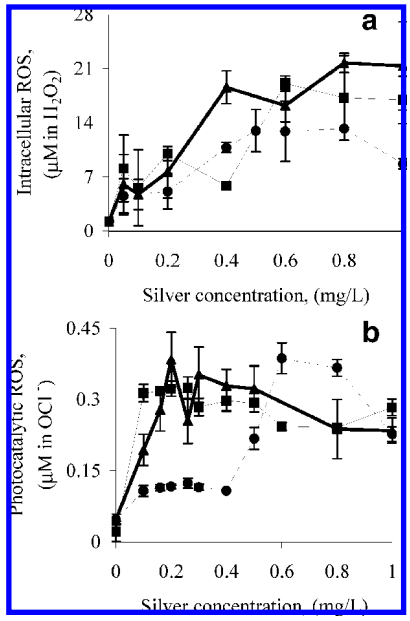

FIGURE 5. The increase of intracellular ROS (a) and photocatalytic ROS concentrations (b) as function of the concentration of Ag nanoparticle (■), AgCl colloid (▲), and Ag^+(●). Error bars indicate one standard deviation ($n = 8$).

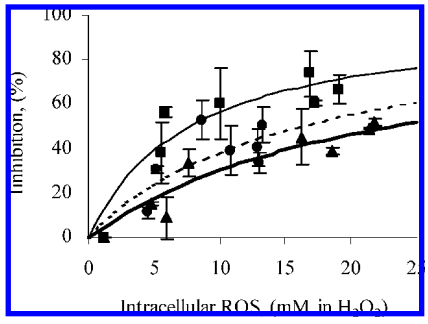

FIGURE 6. The degree of nitrification inhibition as function of the intracellular ROS (in hydrogen peroxide unit) concentration after 30 min exposure of nitrifying bacteria to Ag nanoparticle (■), AgCl colloid (▲), and Ag^+(●). Error bars indicate one standard deviation ($n = 8$). The best-fit lines are also shown using a saturation-type model.

suggesting factors other than intracellular ROS also affect nanosilver toxicity. It is speculated that the uncharged Ag nanoparticles less than 5 nm may more easily transport across the cell membrane than the charged Ag^+ ions. These nanoparticles with high surface areas may directly act on nitrifying cell membranes where key ammonia oxidation enzymes are located.

The results also demonstrated that there was poor correlation between the observed inhibition and photocatalytic ROS concentrations induced by Ag nanoparticles or Ag bulk species. According to previous reports, photocatalytic ROS generated by light can induce intracellular ROS in the cell. For example, the photocatalytic ROS generation by TiO_2 nanoparticles under sunlight illumination was positively correlated with antibacterial activity (*21, 35*). Nanoparticles such as TiO_2, SiO_2, and ZnO are photosensitive to promote ROS generation (*20*). Because of their transition metal characteristics, they can be catalytically active (*9*) to inhibit microbial growth under dark conditions (*20*). Our study, however, showed that Ag nanoparticles did not generate ROS in the absence of light. Silver nanoparticles produced photocatalytic ROS under natural light, but the photocatalytic ROS generation had no relationship (or more precisely negative correlation) with the intracellular ROS concentrations. Therefore, there was no direct correlation of photocatalytic ROS with nitrification inhibition.

The mechanism of ROS production by Ag nanoparticles remains to be investigated. It appeared to be involved with photocatalysis as ROS production was observed only in the presence of light. Since both $OCl^-$and $OH\cdot$ can be detected in photocatalytic ROS production using APF, it is not safe to say that the photocatalytic ROS was attributed to $OH\cdot$ production only. It is possible to monitor $OH\cdot$ production separately using another fluorochrome called hydroxyphenyl fluorescein (HPF, Invitrogen, OR), but with less sensitivity. Overall, fluorescence-based detection using APF, HPF or H_2DCFDA allows to detect only a few reactive oxygen species, mainly hydroxyl radicals.

It should be noticed that a direct proof of ROS affecting inhibition was not made in this work. ROS form as a natural byproduct of the normal aerobic metabolism although its levels can increase dramatically under environmental stress (*10*), as was shown that intracellular ROS correlated with Ag concentration (Figure 5) and with inhibition (Figure 6). The ROS correlations were different for the different forms of silver (nanoparticles, AgCl colloid, and Ag^+) indicating that factors other than ROS are also important in determining nanosilver toxicity.

Nitrification involves the conversion of ammonium-nitrogen (NH_4^+-N) to nitrite (NO_2^-) by ammonia oxidizing bacteria and the conversion of nitrite to nitrate (NO_3^-) by nitrite oxidizing bacteria. It is generally agreed that inhibition to ammonia oxidation is more severe than inhibition to nitrite oxidation in the presence of chemical toxins (*36, 37*). It is, therefore, not desired to differentiate the inhibition on the two-step ammonia oxidation reactions in this study. High nitrate concentrations (NO_3^--$N = \sim$280 mg/L) in the feed did not inhibit nitrification (data not shown), as was also reported before (*37*). To delineate meaningful inhibition results, complexation of Ag^+ ions or Ag nanoparticles with other anions such as SO_4^{2-}, Cl^- was minimized. Work is underway to reduce or remove nanosilver toxicity by complexation.

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Supporting Information Available

The ROS standard curves, correlation analyses between the average nanoparticle sizes, Ag^+ concentrations and the degree of inhibition, and relationship between observed inhibition

and ROS concentrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth

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ABSTRACT

Emerging nanomaterials are of great concern to wastewater treatment utilities and the environment. The inhibitory effects of silver nanoparticles (Ag NPs) and other important Ag species on microbial growth were evaluated using extant respirometry and an automatic microtiter fluorescence assay. Using autotrophic nitrifying organisms from a well-controlled continuously operated bioreactor, Ag NPs (average size = 14±6 nm), Ag^+ ions ($AgNO_3$), and AgCl colloids (average size = 0.25 μm), all at 1 mg/L Ag, inhibited respiration by 86±3%, 42±7%, and 46±4%, respectively. Based on a prolonged microtiter assay, at about 0.5 mg/L Ag, the inhibitions on the growth of *Escherichia coli* PHL628-gfp by Ag NPs, Ag^+ ions, and AgCl colloids were 55±8%, 100%, and 66±6%, respectively. Cell membrane integrity was not compromised under the treatment of test Ag species by using a LIVE/DEAD Baclight™ bacterial viability assay. However, electron micrographs demonstrated that Ag NPs attached to the microbial cells, probably causing cell wall pitting. The results suggest that nitrifying bacteria are especially susceptible to inhibition by Ag NPs, and the accumulation of Ag NPs could have detrimental effects on the microorganisms in wastewater treatment.

1. Introduction

Nanosilver (silver nanoparticle, Ag NP) materials have a wide range of applications including spectrally selective coating for solar energy absorption (Rand et al., 2004; Cole and Halas, 2006), catalysis in chemical reactions (Zhai et al., 2006), surface-enhanced Raman scattering for imaging (Yamamoto and Watarai, 2006), and antimicrobial sterilization (Savage and Diallo, 2005; Sambhy et al., 2006; Pal et al., 2007). Because of their effective antimicrobial properties and low toxicity toward mammalian cells, Ag NPs have become one of the most commonly used nanomaterials in consumer products (104 out of 502 nanoproducts surveyed) (Maynard and Michelson, 2006). These nanoparticles will likely enter the sewage pipes and the wastewater treatment plants (WWTPs). At present, little is known about the adverse effects of Ag NPs on wastewater treatment and the environment.

It is known, however, that free silver ion (Ag^+) is highly toxic to a wide variety of organisms including bacteria. Metal toxicity to planktonic species such as algae (Lee et al., 2005) and bacteria (Hu et al., 2002, 2003) is often governed by the concentrations of aqueous free metal species (i.e., Ag^+). The inhibitory effect of Ag^+ is believed to be due to its sorption to the negatively charged bacterial cell wall, deactivating

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cellular enzymes, disrupting membrane permeability, and ultimately leading to cell lysis and death (Ratte, 1999; Sambhy et al., 2006). The aqueous concentrations of Ag^+ are typically low in wastewater treatment systems or in the natural environment because of its strong complexation with various ligands such as chloride ($K_{sp} = 10^{-9.75}$), sulfide ($K_{sp} = 10^{-49}$), thiosulfate, and dissolved organic carbon (Shafer et al., 1998; Wang, 2003). As a result, silver toxicity to microorganisms is generally not observed.

Nanosilver, a particle of Ag element, is a new class of material with remarkably different physiochemical characteristics such as increased optical, electromagnetic and catalytic properties from the bulk materials (Wenseleers et al., 2002; Kelly et al., 2003). Nanoparticles with at least one dimension of 100 nm or less have unique physicochemical properties, such as high catalytic capabilities and ability to generate reactive oxygen species (ROS) (Limbach et al., 2007) (see recent review by Nel et al., 2006). Silver in the form of nanoparticles could be therefore more reactive with its increased catalytic properties and become more toxic than the bulk counterpart. Furthermore, toxicity is presumed to be size- and shape-dependent (Pal et al., 2007), because small size nanoparticles (e.g., <10 nm) (Kloepfer et al., 2005; Morones et al., 2005) may pass through cell membranes and the accumulation of intracellular nanoparticles can lead to cell malfunction.

Little work has been done to evaluate the inhibition of microbial growth by different Ag species, especially Ag NPs in wastewater treatment systems where such information is valuable for operation planning and control. Both autotrophic and heterotrophic microorganisms are important in wastewater treatment. While heterotrophs are responsible for organic and nutrient removal, autotrophs are responsible for nitrification that is considered as the controlling step in biological nitrogen removal because of the slow growth rate of nitrifying organisms and their sensitivity to temperature, pH, dissolved oxygen (DO) concentration, and toxic chemicals (Blum and Speece, 1991; Hu et al., 2002). Consequently, the objective of this research was to evaluate the impact of important Ag species such as Ag NPs, Ag^+ ions, and AgCl colloids on heterotrophic and autotrophic growth.

In this research work, Ag NPs and AgCl colloids with larger sizes were synthesized and characterized by UV–vis spectroscopy and electron microscopy. The inhibitory effects on the autotrophic and heterotrophic growth were determined by a short-term extant respirometric assay and an automatic microtiter assay, respectively. Environmental scanning electron microscopy (ESEM) was applied as a complementary technique to examine the microbial/nanoparticle interactions. The mode of action of nanosilver toxicity was finally discussed based on the results of membrane integrity using a LIVE/DEAD Baclight™ bacterial viability kit.

2. Materials and methods

2.1. Silver materials

2.1.1. Silver nanoparticles

Ag NPs were synthesized by reducing silver nitrate with sodium borohydrate ($NaBH_4$) and adding polyvinyl alcohol (PVA) (Aldrich) as the capping agent to control the growth of nanocrystals and agglomeration of nanoparticles. To dissolve PVA, a solution containing 0.06% (wt) PVA was heated to 100 °C and cooled down to room temperature before use. Silver particles were prepared by rapidly injecting 0.5 mL of 10 mM $NaBH_4$ into 20 mL PVA solution containing 0.25 mM silver nitrate at room temperature. After 5 min of stirring, the reaction mixture was stored at 4 °C before use.

2.1.2. Silver ions

A silver nitrate standard solution (14 mM, Fisher Scientific) was used as a source of Ag^+ ions.

2.1.3. Silver chloride colloids

Aliquots of 100 mg/L AgCl colloids were prepared freshly by vigorously mixing (700 rpm) 1 mL of 14 mM silver nitrate standard solution and 1 mL of 28 mM sodium chloride with 18 mL of distilled water. Twice as much sodium chloride as silver nitrate was added to ensure complete complexation with no residual Ag^+ ions in the colloidal solution (confirmed by Ag^+ measurements with an ion-selective electrode).

2.2. Microbial cultures

2.2.1. Autotrophic bacteria

The mixed and enriched nitrifying bacteria were cultivated in a continuously stirred tank reactor (14 L) operated at solids retention time of 20 d and hydraulic retention time of 1 d using seed from a local nitrifying activated sludge plant in Missouri, USA. The reactor was fed with an inorganic medium containing ammonium (8.3 mM, NH_4NO_3) as the sole energy source and requisite macro- and micronutrients (Table 1). Low concentrations of anions such as chloride and sulfate were present in the reactor to minimize their complexation potential with Ag^+ ions. Sodium carbonate (0.5 M) was intermittently added to maintain the reactor pH at 7.5 ± 0.1 and fulfilled both carbon and alkalinity requirements. After a few months of operation, mixed liquor was periodically withdrawn from the nitrifying reactor for batch respirometric studies.

2.2.2. Heterotrophic bacteria

The test heterotrophic bacterium was *Escherichia coli* PHL628-gfp, a gift from Dr. Anthony Hay at Cornell University. This strain tagged with a green fluorescence protein (GFP) was a derivative of the *E. coli* K12 that forms biofilms as a consequence of the over-expression of curli (Junker et al., 2006). The test strain was grown overnight on a mechanical shaker (200 rpm) at room temperature in a nutrient-rich medium (BBL™ containing 5 g/L Gelysate™ peptone and 3 g/L beef extract, pH 6.9 ± 0.2).

2.3. Silver species characterization

Aliquots of the prepared Ag NP suspensions were periodically scanned from 250 to 700 nm to obtain absorption spectra using a UV–vis spectrophotometer (Cary 50, Varian, CA). Additional aliquots were used to determine the stability of the Ag NPs by measuring the concentrations of Ag^+ ions in the

Table 1 – Composition of the growth nutrients in reactor influent

Compound	Concentrations in reactor influent		
	mg/L	Cations (mM)	Anions (mM)
$Mg(NO_3)_2$	61	$0.41Mg^{2+}$	$0.82NO_3^-$
$Ca(NO_3)_2$	41	$0.25Ca^{2+}$	$0.25NO_3^-$
$NaNO_3$	879	$10.34Na^+$	$10.34NO_3^-$
NH_4NO_3	667	$8.33NH_4^+$[a]	$8.33NO_3^-$
K_2HPO_4	3.9	$0.04K^+$	$0.02HPO_4^-$
$FeCl_2 \cdot 4H_2O$	2	$0.01Fe^{2+}$	$0.02Cl^-$[b]
$MnSO_4 \cdot H_2O$	3.4	$0.02\ n^{2+}$	$0.02SO_4^{2-}$[c]
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ $0.001Mo_7O_{24}^{6-}$	1.2	$0.006NH_4^+$[a]	
$CuSO_4$	0.8	$0.01Cu^{2+}$	$0.01SO_4^{2-}$[c]
$Zn(NO_3)_2 \cdot 6H_2O$	1.8	$0.01Zn^{2+}$	$0.02NO_3^-$
$Ni(NO_3)_2 \cdot 6H_2O$	0.3	$0.001Ni^{2+}$	$0.002NO_3^-$

[a] Total $NH_4^+ = 8.336$ mM.
[b] Total $Cl^- = 0.02$ mM.
[c] Total $SO_4^{2-} = 0.03$ mM.

Ag NP suspensions using a silver ion/sulfide selective electrode (Denver instrument, Denver, CO).

The sizes of Ag NPs and AgCl colloids were characterized by an FEI Quanta 600F ESEM (resolution: 3 nm at 30 kV, FEI Company, OR) equipped with a scanning transmission electron microscopy (STEM) detector. The Ag NP suspension was added to standard carbon-coated TEM grid. Images of the samples were taken at an accelerating voltage of 30 keV.

2.4. Autotrophic growth determined by extant respirometry

Autotrophic growth inferred from oxygen uptake rates due to ammonia oxidation was measured in triplicate using a batch extant respirometric assay (Hu et al., 2002). Aliquots (60 mL) of nitrifying bacteria were collected from the nitrifying reactor. MOPS[3-(*N*-morpholino) propanesulfonic acid, pH adjusted to 7.5] at a final concentration of 20 mM was added to maintain relatively constant pH of 7.5 during ammonium oxidation. The nitrifying bacterial suspensions were amended with Ag NPs, Ag^+ ions and AgCl colloids individually at the final concentration range of 0.1–1 mg/L Ag, filled into the respirometric bottles with no headspace, and then tightly capped. Every batch respirometric test was accompanied by a positive control (e.g., untreated nitrifying bacteria only) at room temperature ($25 \pm 2\ ^\circ C$). The nitrifying bacterial suspensions were aerated with pure oxygen gas before aliquots of NH_4^+-N (10 mg/L N as NH_4NO_3) were injected. Magnetic stirring at ca. 100 rpm was provided in the bottles to ensure complete mixing. A decrease in the DO level in the respirometric vessel was measured by a DO probe (YSI model 5300A, Yellow Springs, OH) and continuously monitored at 4 Hz by an interfaced personal computer. The degree of inhibition of autotrophic microbial growth was inferred from the difference between the measured specific oxygen uptake rate in the absence and presence of the Ag species (Hu et al., 2002).

2.5. Heterotrophic growth determined by an automated microtiter assay

To evaluate the inhibitory effects of Ag species on heterotrophic growth, *E. coli PHL628-gfp* was grown in nutrient broth (BBL) at room temperature overnight. For the microtiter fluorescence assay, aliquots of the fresh medium (190 μL) were pipetted into eight parallel wells of a 96-well microplate (i.e., 8 replicates), and aliquots (10 μL) of overnight *E. coli cells* were inoculated in each well. Aliquots of the Ag NP suspension, Ag^+ or AgCl colloidal solution were added individually to each well to reach predetermined Ag concentrations. The cells were exposed to ambient air and mixed intermittently to support their growth on the plate. A program was made to incubate the samples with vigorous mixing for 10 s per hour before the fluorescence intensities (535 nm) excited at 485 nm were recorded automatically every hour for 24 h. The plate was pre-equilibrated at room temperature ($25 \pm 2\ ^\circ C$) for 0.5 h and the fluorescence (in relative fluorescence unit, RFU) of microbial suspensions was measured with a fluorescence microreader (VICTOR^3, PerkinElmer, Shelton, USA).

The time-dependent microbial growth associated with organic substrate oxidation in the 96-microwells was simulated using an exponential growth model:

$$X = X_0 e^{\mu t} \quad (1)$$

where X and X_0 are final and initial biomass concentrations, respectively, as reflected by the fluorescence intensity. The parameters of the specific microbial growth rate, μ, were determined via least-squares error analysis using the SOLVER routine in Microsoft Excel.

2.6. Microbial/nanoparticle interaction

The microbial/nanoparticle interaction was visualized using the FEI Quanta 600F SEM in the environmental (ESEM) mode that allows organic samples to be examined without applying a conductive coating prior to imaging. The enriched nitrifying culture amended with commercially available Ag NPs (10 nm, Nanostructured & Amorphous Materials, Inc., Houston, TX) was placed in an Al cup on a cold stage (10 °C) and imaged at ca. 7 Torr and 80% relative humidity. In order to obtain higher-resolution images, bacteria amended with our own Ag NPs were examined under high-vacuum conditions utilizing a back-scattered electron (BSE) detector. The nanoparticle samples synthesized in our laboratory were prepared using a standard protocol described above, critically point dried, and coated with ~10 nm of Pt.

2.7. LIVE/DEAD bacterial viability assays

Experiments were carried out in the presence and absence of nanoparticles to determine the cell viability of heterotrophic (*E. coli* PHL628 without GFP tagged) and autotrophic cultures by using a LIVE/DEAD Baclight^{TM} bacterial viability kit (Molecular Probes, Eugene, OR) (Hu et al., 2003). Viable and dead cells were detected by differential staining with a mixture of a green fluorochrome, SYTO 9 (stains all cells, live or dead), and a red fluorochrome, propidium iodide (stains

only bacteria with damaged membranes). A reduction in the SYTO 9 fluorescent emission results when both dyes are present in the cell. Dead cells subject to 75% ethanol killing for 1 h were provided as a positive control. To reduce background fluorescence, the microbial suspension was washed with 0.85% $NaNO_3$ after centrifuging at 10,000*g* for 15 min. After adding aliquots of microbial suspensions and stain solution to each well of a 96-well microplate, the plate was incubated at room temperature in the dark for 15 min, and the relative fluorescence intensity was measured by the VICTOR fluorescence microreader. Enumeration of stained cells was facilitated by excitation at 485 nm and detection at 642 nm (red) and 535 nm (green), for propidium iodide and SYTO 9, respectively.

3. Results and discussion

3.1. *Characterization of Ag NPs and AgCl colloids*

The absorption spectrum (Fig. 1) of dark brown Ag NPs prepared by chemical reduction showed a surface plasmon absorption band with a maximum of about 400 nm, a characteristic peak of Ag NPs (Petit et al., 1993; Kong and Jang, 2006), indicating the presence of Ag NPs in the solution. Due to the excitation of plasma resonances or interband transitions, some metallic nanoparticle dispersions exhibit unique bands/peaks (Creighton and Eadont, 1991). The broadness of the peak is a good indicator of the size of nanoparticles. As the particle size increases, the peak becomes narrower with a decreased bandwidth and an increased band intensity (Petit et al., 1993; Kong and Jang, 2006). Furthermore, there is an inverse linear relationship between the full-width at half-maximum (FWHM) and the diameter of particles (Petit et al., 1993):

$$\mathrm{FWHM} = 50 + \frac{230}{D} \quad (2)$$

where both FWHM and the particle diameter (*D*) are in nanometers. The size of the Ag NPs was estimated as approximately 16 nm based on Eq. (2). This result is consistent with the STEM results, which showed a size distribution between 10 and 40 nm (Fig. 2) of the Ag NPs with an average of 14 ± 6 nm.

A shoulder at approximately 425 nm was noticed in UV–vis absorption spectra, indicating a broad distribution of particle sizes and shapes in the solution because of crystallization, as was confirmed by STEM imaging. The position and the number of peaks in the absorption spectra are dependent on the shape of the particles: for an ellipsoidal particle there are two peaks whereas for spherical silver particles there is only one peak centered at about 400 nm (Creighton and Eadont, 1991; Petit et al., 1993).

The concentrations of Ag^+ ions were measured simultaneously to evaluate the stability of Ag NPs in the suspension. The beginning Ag^+ concentration to make the Ag NP suspensions was 27 mg/L (0.25 mM). At the completion of the reaction, the residual Ag^+ concentration was 0.6 ± 0.1 mg/L. The Ag^+ concentration remained largely unchanged at the end of 1 day of resting at room temperature. Afterward, the Ag^+ concentrations increased gradually (data not shown), as also indicated from the changes of solution color.

During a week of Ag^+ monitoring at room temperature, the color of Ag NP suspensions changed from dark brown to

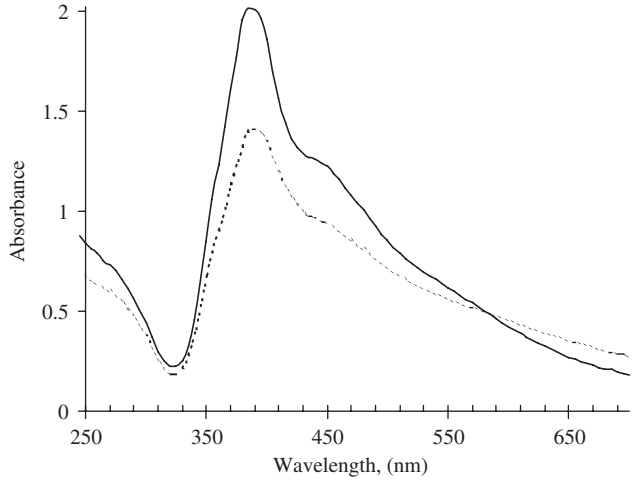

Fig. 1 – UV–vis absorption spectra of an Ag NP suspension recorded immediately after chemical reduction (solid line) and after 1 week (dash line) at room temperature.

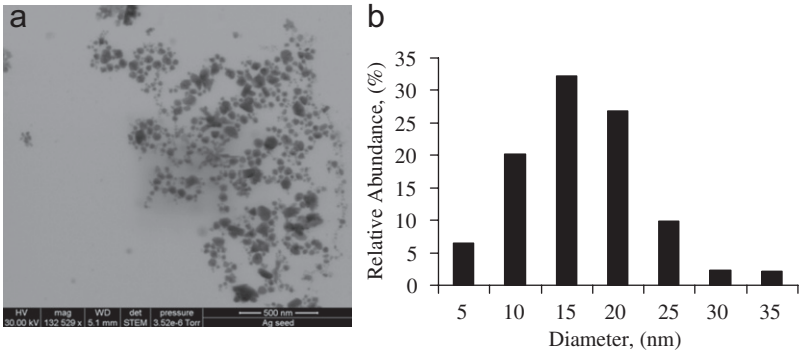

Fig. 2 – STEM image of Ag NPs prepared by chemical reduction (a) and the particle size distribution (b). The average particle size was 14 nm. Bar size: 500 nm.

yellow, presumably due to oxidative dissolution of the Ag NPs

$$4Ag + O_2 + 2H_2O = 4Ag^+ + 4OH^- \quad (3)$$

The color change associated with particle dissolution and the presence of multiple UV–vis absorption bands indicate the existence of Ag NPs of various shapes and sizes, as was confirmed by STEM imaging (Fig. 2). To minimize the interference of dynamic changes of Ag NPs, we used the freshly prepared Ag NP suspensions that were stored shortly (a few days) at 4 °C before use, during which no significant changes of Ag^+ concentrations were observed in the suspension (data not shown).

Silver chloride colloids (100 mg/L Ag) were prepared with an average size of ca. 0.25 μm. The particle sizes ranged from 0.1 to 2 μm. A constant low Ag^+ concentration was detected because of the overdose of chloride. The fraction of Ag^+ was measured to be less than 0.1% of the total Ag in the AgCl colloidal solution.

3.2. *Effect of Ag species on autotrophic growth*

An extant respirometric technique was developed to determine biokinetic parameters from small pulses of substrate (e.g., NH_4^+) while minimizing changes in the microbial physiological state (Chandran and Smets, 2000; Hu et al., 2002). Fig. 3 shows a representative respirograph of ammonia oxidation after an aliquot of ammonium was injected at ~100 s in the enriched nitrifying microbial suspension. The lack of change in DO illustrates nitrification inhibition in the presence of Ag NPs. There was no significant pH change before and after the test because of the addition of MOPS.

As shown in Fig. 4, at 1 mg/L Ag in the nitrifying suspension, the inhibitions by Ag NPs, Ag^+ ions, and AgCl colloids were 86 ± 3%, 42 ± 7%, and 46 ± 4%, respectively. Of all the Ag species tested, Ag NPs presented the highest inhibition on nitrifying bacterial growth. Interestingly, the freshly prepared AgCl colloids with an average size of 0.25 μm also inhibited nitrification. At the same level of Ag dose, there was no statistical difference ($p > 0.05$) of inhibition between AgCl colloids and Ag^+ ions. At this small size, silver chloride colloids appeared to reduce the bacterial growth as effectively as Ag^+.

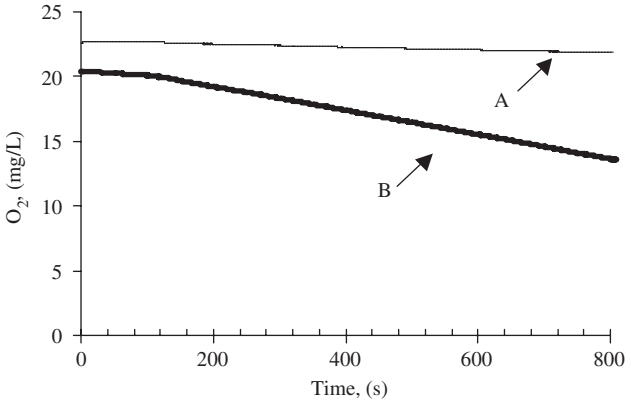

Fig. 3 – Nitrification inhibition inferred from the decrease of specific oxygen uptake rate (slope of curve A) in the presence of Ag NPs, as compared with control (curve B) after an aliquot of ammonium nitrate was injected individually at approximately 100 s.

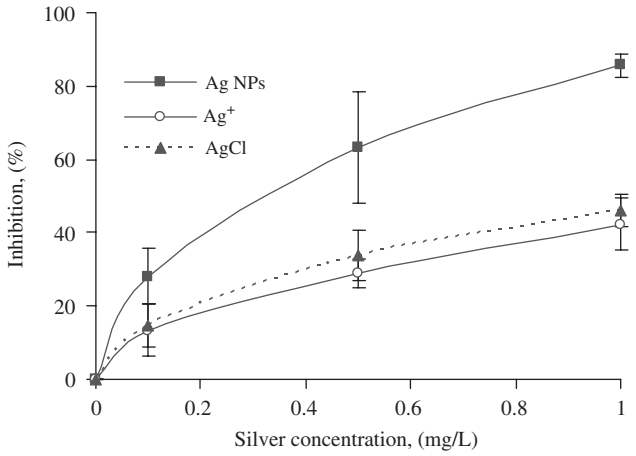

Fig. 4 – Nitrification inhibition as a function of the concentrations of silver in the form of Ag NPs, Ag^+ ions, and AgCl colloids. Error bars indicate one standard deviation.

3.3. *Effect of Ag species on heterotrophic growth*

Consistent with the results from autotrophic growth study, Ag NPs inhibited *E. Coli* growth. While no inhibition was observed at Ag NP concentrations below 1.0 μM, the heterotrophic growth rate was reduced significantly by 55% as the Ag NP concentrations increased to 4.2 μM (Table 2). The IC 50 inhibition by the Ag NP suspension, or the half maximal inhibitory concentration, was estimated to be 4.0 μM ($n = 8$). Surprisingly, silver ion was the most toxic species to inhibit heterotrophic growth. At 4.2 μM (~0.5 mg/L Ag), the inhibitions on the growth of *E. coli* PHL628-gfp were 55 ± 8%, 100%, and 66 ± 6% by Ag NPs, Ag^+ ions, and AgCl colloids, respectively. *E. Coli* treated with 1 mg/L Ag (or 9.3 μM) in the forms of Ag NPs, Ag^+ ions, or AgCl did not exhibit signs of growth (data not shown). The inhibition on heterotrophic growth appeared to be more severe from the long-term microtiter fluorescence assays, as we reported earlier that inhibition on microbial growth with longer period of metal exposure tends to be more significant (Hu et al., 2004).

A slight lag phase of *E. coli* growth (~1.5 h) was observed during the automatic microtiter assays (Fig. 5). Stationary phase was reached after incubation of the heterotrophic strain for approximately 12 h at room temperature (25 ± 1 °C). Upon the addition of Ag NPs in the microbial suspension, a slight decrease of fluorescence efficiency (i.e., fluorescence quenching) with increasing Ag NP concentrations was observed. In the case of AgCl colloids, the quenching effect was less significant (data not shown). The results are consistent with the existing experimental data (Sabatini et al., 2007; Yamaguchi et al., 2007), indicating that the overlap between the GFP-tagged microbial fluorescence and the plasmon absorption of Ag nanoparticles may slightly

Table 2 – Specific growth rates (μ) of *E. coli PHL628-gfp* and the inhibitions by silver species at different concentrations

Concentration (μM)	Ag NP		Ag^+		AgCl colloid	
	μ (d^{-1})	Inhibition (%)	μ (d^{-1})	Inhibition (%)	μ (d^{-1})	Inhibition (%)
1.4	0.40 (±0.03)	17 (±5)	0.41 (±0.02)	11 (±4)	0.43 (±0.01)	7 (±4)
2.8	0.34 (±0.03)	30 (±6)	0.14 (±0.03)	69 (±6)	0.35 (±0.02)	24 (±5)
4.2	0.22 (±0.04)	55 (±8)	0	100	0.16 (±0.03)	66 (±6)

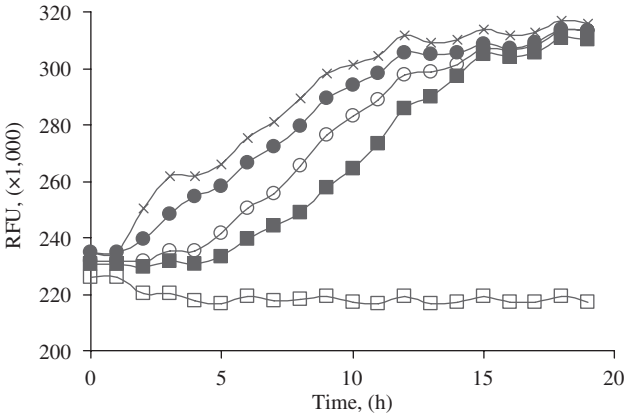

Fig. 5 – Effect of Ag NP concentrations (×, 0 μM; ●, 1.4 μM; ○, 2.8 μM; ■, 4.2 μM; □, 9.3 μM) on the growth of *E. coli* PHL628-gfp, as measured by relative fluorescence units.

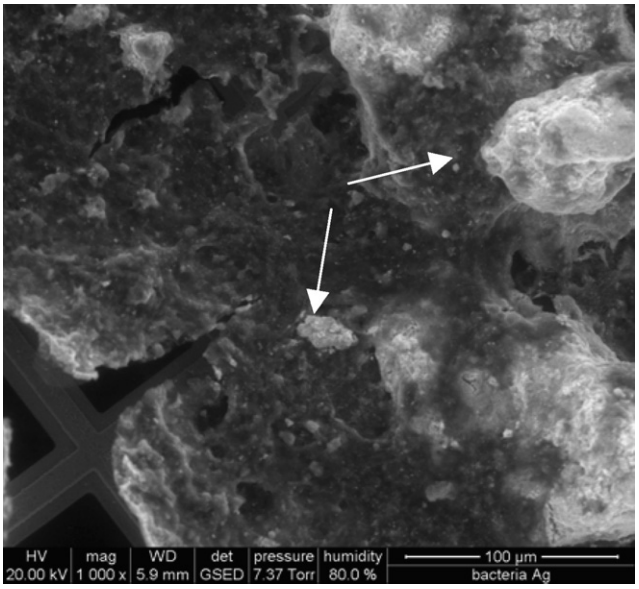

Fig. 6 – Silver nanoparticles adsorbed to the enriched nitrifying culture on a copper grid using ESEM. Arrows show aggregated AgNPs that attached to microbial cells or embedded in microbial extracellular polymeric substances. Bar size: 100 μm.

cause the quenching of the excited-state of GFP molecules on the Ag NPs.

3.4. *Microscopic observation of microbial/nanoparticle interaction*

The microbial–nanoparticle interaction was visualized by ESEM, a specialized technique capable of imaging hydrous samples without the need of pretreatment for conductive coating (Redwood et al., 2005; Priester et al., 2007). Commercially available Ag NPs (Nanostructured & Amorphous Materials, Inc., advertised powder size of 10 nm) aggregated in water and the nitrifying bacterial suspension. It appeared that the particles were embedded in microbial extracellular polymeric substances (Fig. 6). The true size (from 200 nm to a few μm) of Ag NPs in water suspension was significantly different from the claimed size of commercial nanopowders, consistent with the results reported by others (Adams et al., 2006).

Higher-resolution electron micrographs were obtained using BSE mode. After mixing a freshly prepared Ag NP suspension with the mixed and enriched nitrifying cultures, it appeared that Ag NPs were adsorbed to the microbial surfaces, probably causing cell wall pitting (Fig. 7). Additional work is underway to take higher-resolution images in order to better understand the microbial–nanoparticle interactions.

3.5. *Cell membrane integrity inferred from LIVE/DEAD assays*

The fluorescence intensities of the stained microbial cells at 535 nm (green) and 642 nm (red) represent live and dead cells, respectively. The green/red fluorescence ratio, obtained by dividing the green and red intensities, was applied to compare the difference among various treatments by Ag species. At 1 mg/L Ag, the ratio obtained from the microbial suspensions treated with Ag NPs showed no significant difference compared to controls ($P > 0.05$), indicating that there is no evidence of cell membrane leakage caused by Ag NPs. Similar results were observed in samples treated with Ag^+ ions or AgCl colloids.

3.6. *Inhibition comparison and mode of antimicrobial action*

Among the Ag species tested, the freshly prepared Ag NPs presented the highest inhibition to autotrophic nitrifying organisms. In contrast, silver ion appeared to be most toxic to heterotrophic growth. Different experimental assays chosen

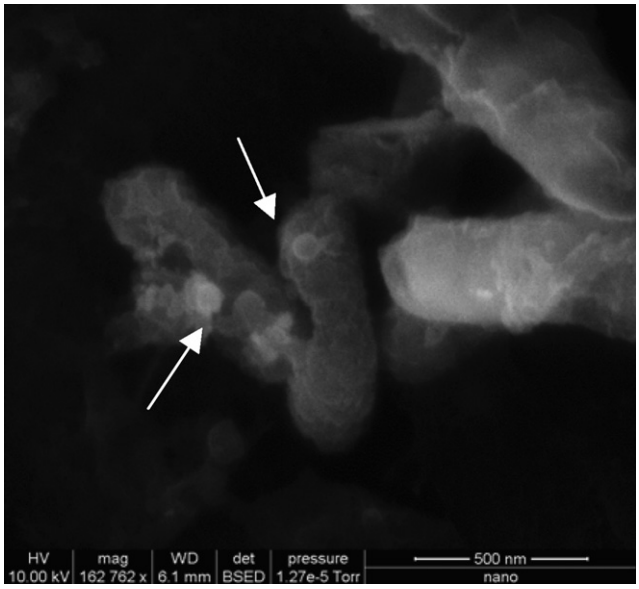

Fig. 7 – Silver nanoparticles adsorbed to the enriched nitrifying culture using a high-speed BSE detector. Arrows show spherical or hexagon types of Ag NPs that attached to the microbial cells, probably causing cell wall pitting. Bar size: 500 nm.

for the enriched nitrifying bacteria and heterotrophic *E. coli* cells made it difficult to compare the toxicity of Ag NPs to the two different bacterial species. The difference in toxicity may be attributed to different growth conditions and cell properties. The nitrifying bacteria were completely mixed in the respirometric bottles, aerated with pure oxygen and their activities were monitored by oxygen uptake rate measurements. Conversely, the *E. coli* cells were mixed intermittently in microwells, aerated with ambient air and their activities were inferred from fluorescence measured over a prolonged period of time (~1 d). Because they have a faster growth rate than nitrifying bacteria, *E. coli* cells may have stronger oxidizing/reducing power and interact with Ag species to form cell-particle aggregates (Sondi and Salopek-Sondi, 2004; Kahraman et al., 2007), as visible from Figs. 6 and 7, or produce extracellular or intracellular Ag NPs (Efrima and Bronk, 1998), causing more complex growth problems. Nitrifying bacteria have remarkably complex internal membrane systems where ammonia monooxygenase (AMO, responsible for ammonia oxidation to produce hydroxylamine, NH_2OH) is located, whereas hydroxylamine oxidoreductase (HMO) is located in the periplasm (Madigan et al., 2000). Therefore, we speculate that Ag NPs may have a direct impact on nitrifying cell membranes where key ammonia oxidation enzymes are located.

The use of PVA during the synthesis of Ag NPs to control the nanoparticles size could affect the antibacterial activity by the coverage of PVA on AgNPs to prevent the direct contact with bacteria. Due to the significant dilution (1:25 or higher) of the Ag NP suspension and solvent competition in cell cultures, the effect of PVA on nanosilver toxicity, however, would be minimal.

Nanoparticles have substantially different physiochemical properties from those of bulk materials of the same composition, possibly resulting in different toxicity mechanisms to biological systems (Nel et al., 2006). The mode of antimicrobial action by Ag NPs could be the inhibition of the microbial processes on the cell surface and in the cell. Previous research demonstrated that Ag NPs attach to the surface of cell membrane, causing the change of membrane permeability, dissipation of the ATP pool and proton motive force, and finally cell death (Sondi and Salopek-Sondi, 2004; Morones et al., 2005; Lok et al., 2006). The results from our bacterial viability tests indicated that there is no evidence of the cell membrane leakage caused by any Ag species at 1 mg/L Ag. The size of the Ag NPs used in this study was 14 ± 6 nm. These particles would be too large to diffuse into the cell, as only the smaller particles mainly in the range of 1–10 nm could enter the cell based on indirect microscopic evidences (Morones et al., 2005). Although electrophoresis studies indicated no direct effect of Ag NPs on intracellular DNA or protein expression (Gogoi et al., 2006), our recent results demonstrated that inhibition by the Ag NPs might be attributed to the accumulation of intracellular ROS (Choi and Hu, 2008).

Bulk silver toxicity is generally governed by the total concentration of labile dissolved intracellular Ag species (Lee et al., 2005). In the cell, silver ions may deactivate cellular enzymes and DNA by reacting with electron-donating groups such as thiol (–SH) groups and generate ROS (Matsumura et al., 2003; Sambhy et al., 2006). Because of its cationic nature and its strong association with various ligands in natural waters, the toxicity of Ag^+ ions depends largely on the strength and amount of the ligands present (Ratte, 1999). The freshly prepared AgCl colloids can be viewed as one of the labile species with respect to their small size and low stability constant ($\log K_1 = 3.3$) in solution (Stumm and Morgan, 1996). Depending on their size and bioavailability, the inhibition caused by AgCl colloids can be as significant as that of Ag^+ ions.

3.7. *Ag NP dissolution*

The time-dependent increases of Ag^+ concentrations and associated color changes of the Ag NP suspension demonstrated the complexity of various processes such as oxidation, crystallization, dissolution and aggregation involved in microbial–nanoparticle interactions. Previous research showed that Ag NPs were susceptible to oxidation by oxygen, and the partially oxidized particles appeared more toxic than the freshly prepared nanoparticles (Lok et al., 2007). Others found, however, that the concentration of Ag^+ decreased by 80% after 24 h, possibly due to Ag^0 cluster formation (Morones et al., 2005). When the Ag NPs were added into a liquid medium, the antimicrobial effectiveness appeared to decrease when compared to that on the agar plates, presumably because of microbial-induced coagulation of nanoparticles (Sondi and Salopek-Sondi, 2004). Experiments involving synthetic zinc sulfide nanoparticles and representative amino acids also indicated a driving role of microbially derived extracellular proteins in rapid nanoparticle aggregation (Moreau et al., 2007). Further research is required to investigate nanoparticle properties such as size, shape, dissolution/aggregation,

surface coating, and solubility that may affect the specific physicochemical and transport properties, which could exert a significantly different impact on microbial growth (Nel et al., 2006).

3.8. *Environmental application and implication*

The numerous engineered nanomaterials with different sizes, shapes, compositions, and coatings require high-throughput benchmarked protocols to screen for potential hazards in the environment (Maynard et al., 2006). The developed extant respirometric assay and the automatic microtiter assay employed in this research are suitable for toxicity assessment of nanomanterials to microorganisms. The bacteria selected for each assay, however, are generally not exchangeable between the two assays. Because of the intrinsic slow growth rate (about an order of magnitude lower than that of heterotrophs) of autotrophic nitrifying bacteria and their high oxygen uptake (4.3 mg O_2/mg of NH_4^+-N oxidized to nitriate) (Grady et al., 1999), the enriched nitrifying cultures are particularly useful in respirometric assays, but failed to show significant growth in the cell-enumeration-based microtiter assay in this study. In contrast, *E. coli* cells were easily determined with the automatic microtiter assay because of their fast growth rate, but failed to produce meaningful oxygen profiles from the extant respiorometric assay because of the low biomass concentrations from overnight batch cultivation and their low oxygen uptake constants (~0.5 mg O_2/mg COD removed) (Grady et al., 1999).

The results of nanosilver toxicity to environmentally sensitive nitrifying microorganisms suggest that stringent regulations of Ag NPs entering WWTPs are necessary. Nitrifying microorganisms involved in nitrification are critical to biological nutrient removal in modern wastewater treatment. Research is underway to evaluate the fate and impact of Ag NPs in wastewater treatment systems.

4. Conclusions

The nature of the cell growth and oxygen uptake behavior allowed us to determine nanosilver toxicity by applying two independent microbial growth assays—extant respirometric assay and automatic microtiter assay—for nitrifying organisms and *E. coli* cells, respectively. The following conclusions were drawn from this work:

(1) Silver nanoparticles (Ag NPs) strongly inhibited microbial growth. Based on a short-term batch respirometric assay, at 9.3 μM Ag (i.e., 1 mg/L Ag), the inhibitions on nitrifying bacterial growth by Ag NPs, Ag^+ ions, and AgCl colloids were 86±3%, 42±7%, and 46±4%, respectively. Based on a prolonged microtiter assay, at 4.2 μM Ag, the inhibitions on the growth of *E. coli* PHL628-gfp by Ag NPs, Ag^+ ions, and AgCl colloids were 55±8%, 100%, and 66±6%, respectively.
(2) Silver chloride colloids inhibited microbial growth. Depending on their particle size and bioavailability, the inhibition by AgCl colloids can be as significant as that by Ag^+ ions.
(3) There was no evidence of change in cell membrane integrity at 1 mg/L Ag for all of the Ag species tested based on the results from the bacterial LIVE/DEAD assays.

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