SILVER NANOTECHNOLOGIES AND THE ENVIRONMENT:

OLD PROBLEMS OR NEW CHALLENGES?

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PEN 15
SEPTEMBER 2008

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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.

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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
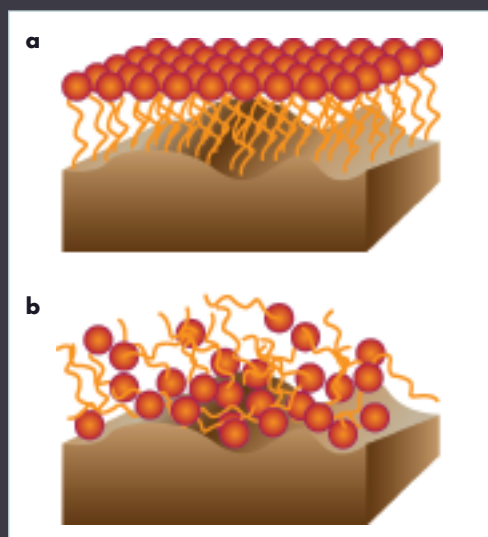
TEXT BOX 1. Nanoparticles, nanomaterials and nanotechnology

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 (nanopar-

ticles). 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).
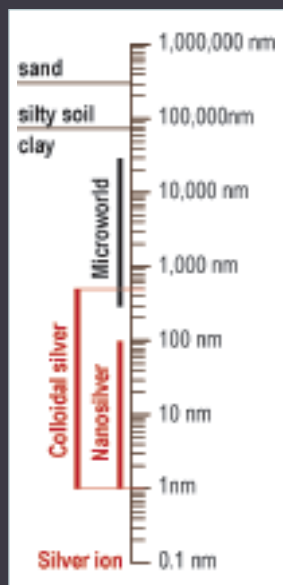
FIGURE 1

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, *silver* 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."

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.

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/

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 bene-fits 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). Concen-

trations 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).
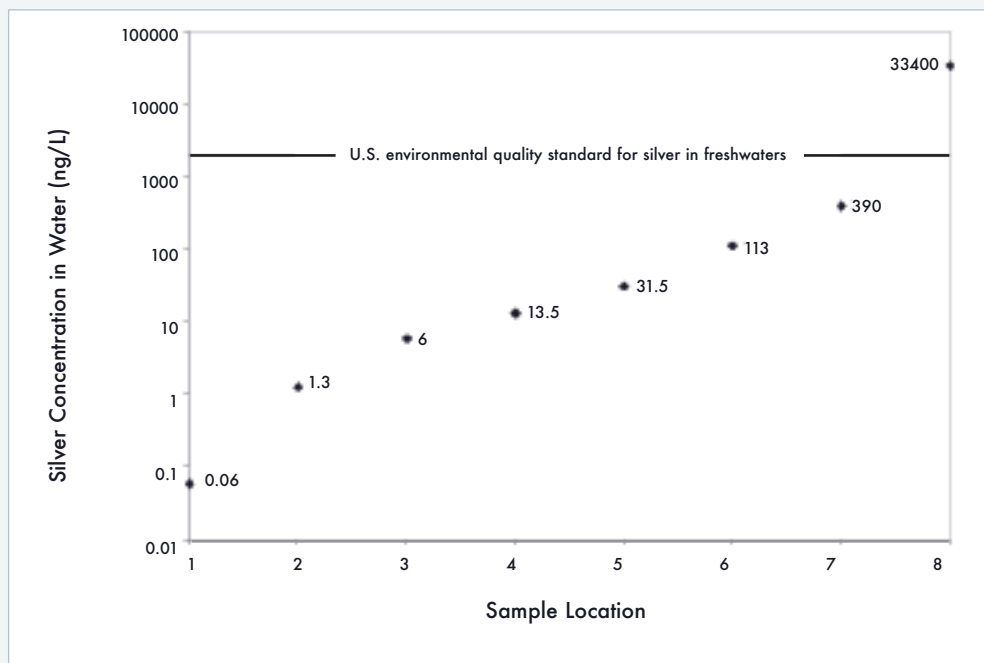
Environmental water quality standards provide guidelines for the upper limits for acceptable metal concentrations in water bodies. These regulatory limits are based on data from toxicity tests and on assumptions about dilution after discharge into the water body. They are enforced by comparing observations 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).
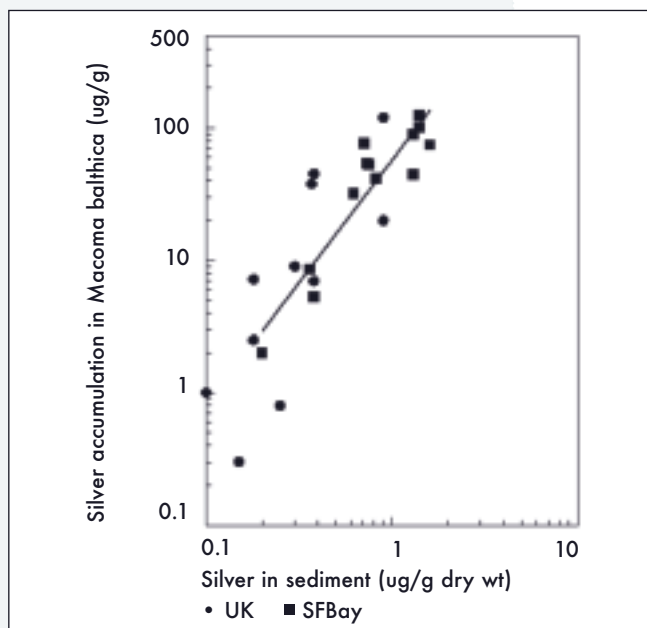
The form of the silver in sediments is important to bioavailability (Text box 3). Bioaccum-

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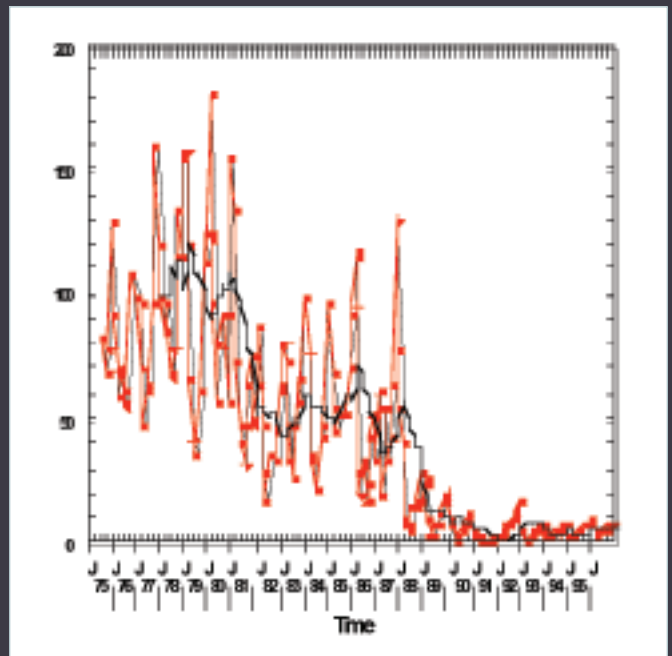
Text box 8. Effects of silver on invertebrates as observed in San Francisco Bay

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 uni-directionally 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-
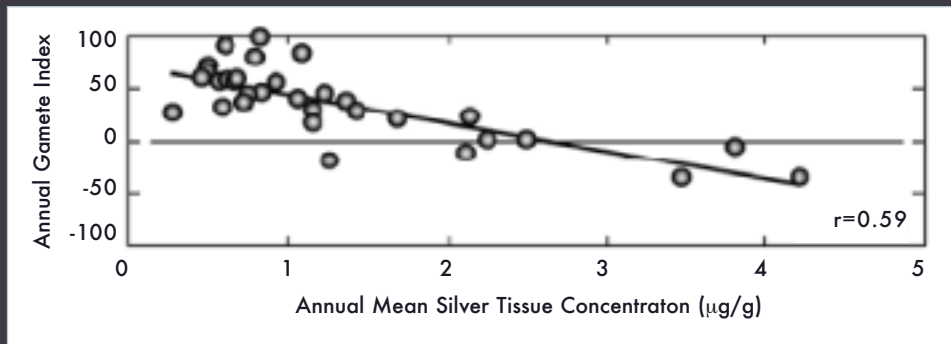
FIGURE 6.

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

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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/silver nano/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

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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.

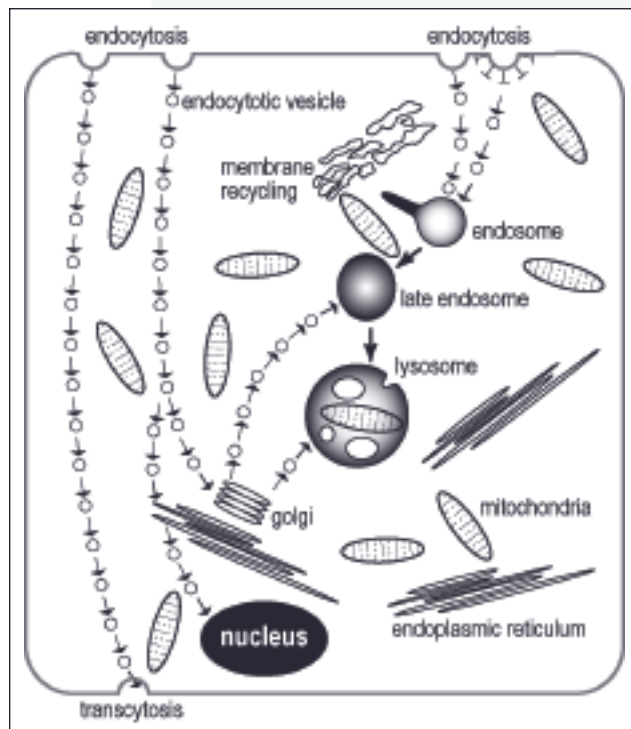Monitoring silver itself might be an interim step in environmental surveillance for which very sensitive methodologies already exist. If inputs of silver nanotechnologies reach problematic levels, the baseline silver metal concentrations are likely to reflect that change. The environment is sensitive 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 impli- cations 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 envi- ronment; 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 mem- branes that surround internal cells, but they are typical- ly 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 nanopar- ticles 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

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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 pri-

vate 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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**The Project on
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