Nanosilver: Should the precautionary principle form the basis for regulatory policy?

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Abstract

Biotechnology companies and manufacturing leaders producing consumer and medical products are using engineered nanosilver so prolifically and at such a fast rate that scientists and regulatory agencies have not been able to appropriately assess their safety. Of the nano materials currently in use, silver is used in the most products. Nanosilver has much higher reactivity than ionic or metallic silver. Its nano form enables it to pass through cell membranes to reach tissues and the organs at a faster rate and more completely. It may cause chromosomal aberration and DNA damage. Its widespread use can lead to development of microbes that exhibit various forms of antimicrobial resistance. Nanosilver is persistent in the environment and toxic to some aquatic species and can bioaccumulate in some species. It affects plant growth and biological treatment of wastewater by inhibiting nitrification. The thesis evaluated potential human health and environmental risks associated with expanded use of nanosilver based on review of current scientific data obtained through the literature and by conducting interviews of nanosilver researchers and other experts. This thesis also assessed current initiatives for nanosilver oversight and regulations in the US and internationally. This thesis identified research and policy needs and recommended initiatives and oversight that would provide a precautionary approach for nanosilver use.

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1.0 INTRODUCTION

Biotechnologists and industry leaders in consumer and medical products are using engineered silver nanoparticles so prolifically and at such a fast rate that regulatory agencies have not been able to appropriately assess their safety. In particular, there is (1) a lack of standardized nomenclature and scarcity of information regarding the physical and chemical properties of nanosilver needed to evaluate sources, pathways, and receptors; and (2) no standardized dose metric needed to evaluate toxicity in order to assess the need to mitigate human and environmental exposure to the nanosilver.

The risk to humans and the environment caused by introducing engineered nanosilver to consumers without thorough evaluation has been demonstrated by the premature introduction of polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), and bisphenol-A (BPA). Based on the currently available toxicity data, widespread use of nanosilver may pose a risk to health and the environment. It is therefore critical that the hazards of nanosilver be thoroughly evaluated before the associated damage becomes irrevocable.

The purpose of this study was to gather relevant information in order (1) to make a determination about whether or not the EPA should invoke the precautionary principle with regard to the use and regulation of nanosilver and (2) to make recommendations for implementing the precautionary principle as appropriate based on this determination. Specifically, I gathered information about separate but related concepts: (1) nanosilver properties and toxicity, (2) regulations and challenges associated with its use, and (3) current researcher

perspectives related to risk. With regard to nanosilver properties and toxicity, I gathered information about the physical and chemical properties of nanosilver to evaluate sources and pathways of the particle in the environment and what influences its toxicity as well as identify the currently established toxicity of the nanosilver. I used this information to assess risk. With regard to regulations associated with nanosilver use, I explored what regulations are in place both in the United States and internationally as well as the challenges to implementing those regulations. With regard to current researcher perspectives related to risk, I explored perceptions of current researchers with regard to identified risks to health and the environment based on nanosilver properties and toxicity. I used this information to describe currently used regulatory practices and challenges to the regulation of nanosilver.

This study is significant because information I collected indicated the need for regulation of nanosilver. More importantly, results from this study could become a catalyst for positioning the United States, a global leader, as a role model for other countries that use nanosilver without restraint, such as India and China. Invoking the precautionary principle with regard to nanosilver use and regulation ultimately could reduce the potential for exposure of humans and the environment to its dangerous effects.

2.0 LITERATURE REVIEW

One purpose of this thesis was to assess whether the United States Environmental Protection Agency (EPA) should invoke the precautionary principle with regard to the use and regulation of nanosilver. In order to make that determination, it was necessary to have a thorough understanding of (1) the chemical and physical properties of nanosilver; (2) its human health effects and toxicity in other organisms, including in the environment; and (3) regulations at present or planned that govern its use in the US and worldwide. I first provide a discussion of the material properties of the parent compound, silver, as well as information on acute and chronic toxicity and the regulations in place with regards to its use.

2.1 Silver

Silver (Ag) is a rare and precious metal; it averages approximately 0.1 ppm in the earth's crust (Lansdown 2010). Most silver comes from mining silver ores, but some of it is a byproduct of mining other metals like gold, copper, lead, zinc, and sometimes bismuth and antimony (Butterman 2005). According to the United States Geological Survey (USGS), the largest producers of silver in 2012 were Mexico, Peru, Australia, and the United States.

Silver in the environment mostly exists in the elemental state, although it also can exist in the environment as silver halides, silver fulminate, silver nitrate, and silver oxide (Smith and Carson 1977). Silver in the environment is present in

four oxidative states, Ag^0, Ag^+, Ag^{+2}, Ag^{+3}, of which Ag^0 and Ag^+ are the most common (Christensen et al. 2010; Smith and Carson 1977).

2.1.1 Properties

The atomic weight of silver is 107.868; its atomic number is 47. Silver melts at 961.93^o C, boils at 2212^{o}C; has a density of 10.5, is highly malleable, and is the most ductile of all the metals (Smith and Carson 1977). Silver also has the highest thermal and electrical conductivity of all the metals (Butterman 2005). It reacts with light and is highly toxic to bacteria, fungi, and viruses but only moderately toxic to humans (Luoma 2008).

2.1.2 Applications

Because silver is malleable and ductile, for centuries it has been used to make jewelry, coins, utensils, and tableware, and because it reacts with light, it has been used to make materials for developing traditional photographic film. Historically, perhaps indirectly aware of silver's bactericidal properties and moderately low levels of toxicity to humans, Egyptians used silver vessels for keeping their water clean, and Romans used it for treating wounds and infections (Luoma 2008). In particular, for over a century, colloidal silver, a mixture of fluid and silver particles, has been used for medicinal purposes as a natural antibiotic and for treating wounds and burns. In the twentieth century, silver's bactericidal properties have been exploited to purify drinking and recreational swimming water. Silver has also been used in healthcare products such as implants,

catheters, and wound dressings. Because silver is highly conductive, it also is used in appliances such as washing machines, as a component in most circuit boards, in an array of electrical goods, and in electronics, including computers, cell phones, televisions, and radios.

2.1.3 Toxicity

Because silver is toxic to fresh water crustaceans, and both fresh water and marine fish, the EPA regulates the concentration of silver in surface water. Silver induces toxicity in fresh water fish when its positively charged ions bind to the fishes' negatively charged gills and reduce the uptake of Na^+ and Cl^-, inhibiting Na^+ and K^--ATPase activity (Bianchini et al. 2005). The subsequent loss of cell energy results in the loss of ions from blood plasma, which in turn disrupts fluid volume regulation and leads to circulatory failure and death. The same mechanism appears to occur in ocean fish except that the target organ is the intestine rather than the gills (Hogstrand and Wood 1998).

Silver is less toxic to mammalian species than to fish. The United States Agency for Toxic Substances and Disease Registry (ATSDR) and the World Health Organization (WHO) have developed toxicological profiles for silver. The studies referenced in these agencies' reports span from 1928 to 1988. Silver has been found in the skin, liver, spleen, and adrenal glands of mammals that have ingested silver; traces also have been found in muscle and brain tissue (Lansdown 2010).

The EPA's reference dose for silver is 0.005 mg/kg/day (IRIS 2013). Studies cited in the ATSDR report indicated that chronic exposure to silver by inhalation or ingestion results in argyria, a condition where the skin turns bluish–grey. Argyria does not result in any adverse health effects other than the cosmetic discoloration of skin. Silver doses in the range of 50 mg injected as a natural alternative to antibiotics and as an anti-inflammatory drug have been found to result in acute effects, including pulmonary edema, hemorrhage, and cellular necrosis in bone marrow, the liver, and the kidney. Administration of about 7% silver nitrate as an abortifacient in a German woman resulted in rapid fatality due to congestive changes in her lungs, brain, and kidney (Fowler et al. 2007).

2.1.4 Regulations

Silver is included as a toxic chemical subject to Section 313 of the Emergency Planning and Community Right-to-Know Act of 1986 (EPCRA). In 2008, the allowable concentration was 0.01 mg/m^3 in air and 0.05 mg/L in water (Luoma 2008). At the time of this study, the Unites States Occupational Safety and Health Administration (OSHA) and the National Institute of Occupational Safety and Health (NIOSH) specify an inhalation exposure limit of 0.01mg/m^3 in an 8-hour day per 5-day work-week.

Due to the advances in nanotechnology silver is being manufactured at nanoscale. Because silver's conductivity and antimicrobial properties are greatly enhanced at this scale, it is being increasingly used in commercial products (Wijnhoven et al. 2009). Nanosilver is included with elemental and ionic silver in

environmental regulations. There currently are no separate regulations for concentrations of nanosilver that can be present in environmental media including air and water. Nanosilver is included as silver under CASRN 7440-22-4.

2.2 Nanosilver Properties

Nanosilver, as shown in figure 2.1, consists of silver particles that have at least one dimension of 1 to 100 nm (Song et al. 2007). At this scale, the quantum effects associated with particle behavior at atomic and subatomic scale become dominant and affect the particles' electrical, optical, and magnetic behaviors. At nanoscale, materials have a larger surface area than materials of the same mass in their elemental or ionic forms. This makes the nanoscale materials more chemically reactive, which affects their strength and electrical properties (Nanowrek). Silver in nano form has more available free radicals and, therefore, has increased anti-microbial properties when compared to silver in its ionic form (Wijnhoven et al. 2009).

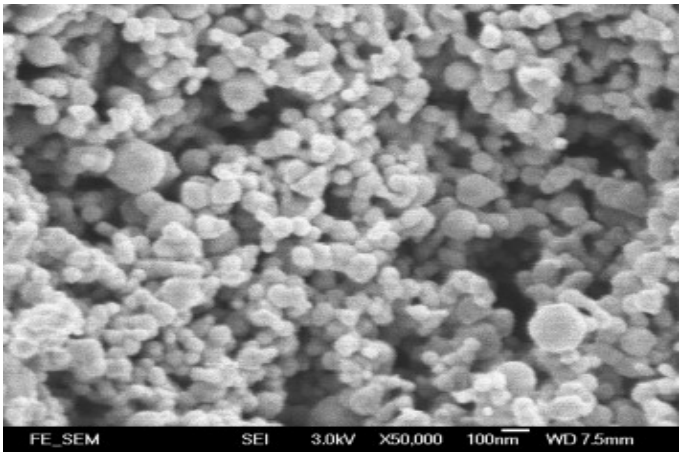

Figure 2.1. Scanning electron microscope image of nanosilver. (Song et al. 2007)

Silver in nano form not only has unique biological properties but also physical and optical properties that are not present in its elemental or ionic forms:

- Antibacterial: Nanosilver is known to effectively kill gram-negative and gram-positive bacteria, including antibiotic-resistant strains (Sondi 2004; Wijnhoven et al. 2009).
- Antibacterial mechanism: The toxicity of nanosilver is mainly attributed to the release of silver ions, but it is also thought to be due to generation of the reactive oxygen species (ROS; Foldbjerg et al. 2011). The antibacterial properties of nanosilver are dependent on its particle size (Morones et al. 2005). The antibacterial properties decrease as particle size increases (Martínez-Castañón et al. 2008). Antibacterial properties also are dependent on the shape of the particles (Sadeghi et al. 2012). Truncated nanosilver has been found to have stronger antibacterial properties than other shapes, a finding that can be attributed to the higher surface area to volume ratio (Wijnhoven et al. 2009).
- Antifungal: Nanosilver has been found to have a strong inhibitory effect on Aspergillum Niger sporulation due to the disruptive effect it has on the spores cells (Pinto et al. 2012). Nanosilver is a quick-acting and effective fungicide agent (Kim 2008).
- Antiviral: Nanosilver inhibits the HIV-1 virus by binding to the host cell. This binding process, however, is dependent on particle size and

has been found to be effective only with particles less than 10 nm (Elechiguerra et al. 2005).

- Anti-inflammatory: Nanosilver alters the expression of proteolytic enzymes that are important in various inflammatory and repair processes (Bhol and Schechter 2007).
- Anti-glycoprotein film: Nanosilver can diffuse through the glycoprotein film (Furno et al. 2004).
- Anti-biofilm property: Nanosilver is known to prevent the formation of biofilms (Percival et al. 2007).
- Surface plasmon resonance: Nanosilver coupled with light electromagnetic wave, enhances a wide range of useful optical phenomena (Kooij et al. 2011), this is helpful in determining target molecules of organic compounds.(Liang et al. 2012).
- Plasmonic heating: This nanosilver property allows for localized heating with very high selectivity similar to laser treatment (Skirtach et al. 2004).
- Metal-enhanced fluorescence: Nanosilver in sizes ranging from 30 to 80 nm alters the intrinsic spectral properties of fluorophores. It is used in detection of DNA, RNA and immunoassays (Wijnhoven et al. 2009).
- Biosynthesis promotion: Nanosilver can be synthesized using bacteria, fungi, and plant extracts (Percival et al. 2005).

It is because of all these properties of nanosilver that it is increasingly used not only in consumer and medical goods but also in biotechnology.

2.3 Characterization and Measurements of Nanosilver

It is important to characterize and measure nanosilver because the size, coating, shape, and charge affect the fate, transport, and toxicity of nanosilver (Venkatapathy 2010). For this reason, Liu et al. (2012) summarized the current technology available for identifying and characterizing nanosilver by its properties and concentrations. The researchers concluded that the most efficient way to quantify nanosilver is with inductively coupled plasma-mass spectrometry (ICP-MS) and inductively coupled plasma-atomic emission spectrometry (Liu et al. 2012).

2.3.1 Quantification

ICP-MS has element-specific detection and high sensitivity that make it ideal for quantifying nanosilver. However, because the samples must be digested before they are analyzed, it is difficult for the instruments to differentiate between silver ions and nanosilver (Mitrano et al. 2012).

2.3.2 Separation

Currently, a variety of methods are being used to separate nanosilver: cloud-point extraction; field-flow fractionation (FFF), hydrodynamic, counter-current, and size-exclusion chromatography, electrophoresis, capillary

electrophoresis; and density-gradient centrifugation. Of the available separation methods, FFF is probably the most powerful and versatile for separating nanosilver in undigested samples. It is, however, time consuming and laborious (Liu et al. 2012).

2.3.3 Identification and Characterization

The most common methods for examining the size, distribution, and shape of nanosilver are scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The downside of using electron microscopy is that only a small amount of sample can be imaged and analyzed at a time and so it is difficult to validate the whole sample. Therefore, there is a need for technology capable of characterizing the whole sample at one time since nanosilver tends to be non-homogeneous (Zanker and Schierz 2012).

Despite significant development in identification, separation, characterization, and quantification of nanosilver, different techniques are used for each process and each process is complex. There is a need for one single technique to identify, separate, characterize, and quantify nanosilver so that its environmental pathways and its impact on the environment and humans can be accurately and effectively evaluated (Liu et al. 2012).

2.4 Nanosilver Applications

Nanosilver acquires various optical, electrical, and magnetic properties at nanoscale. It also has increased chemical reactivity and, therefore, has enhanced

antimicrobial properties. Due to these properties, its use is widespread with many different applications. According to data from The Project on Emerging Nanotechnologies (TPEN, 2011), a partnership between the Woodrow Wilson International Center for Scholars and the Pew Charitable Trusts, of the nano products available in 2011, nanosilver was used in the most (fig. 2.2).

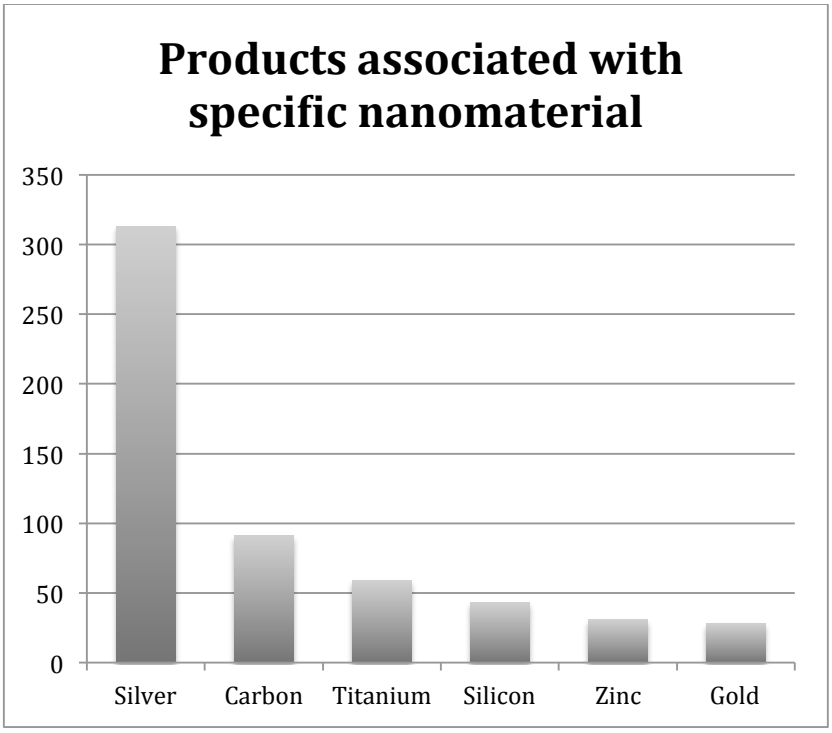

Figure 2.2. Numbers of products associated with specific nanomaterial. Data obtained from TPEN (The Project on Emerging Nanotechnologies 2013).

2.4.1 Consumer Products

TPEN's database, last updated on January 24, 2011, includes 346 consumer products on the market that contain nanosilver. It is very likely the list has doubled in the two years since the last database update. The next database

update will be released in September of 2013.[1] In 2008, nanosilver was most used in polymers, as spun silver, in powder form, or as ionic silver (Luoma 2008). Figure 2.3 illustrates the different forms of nanosilver found in consumer products in 2011.

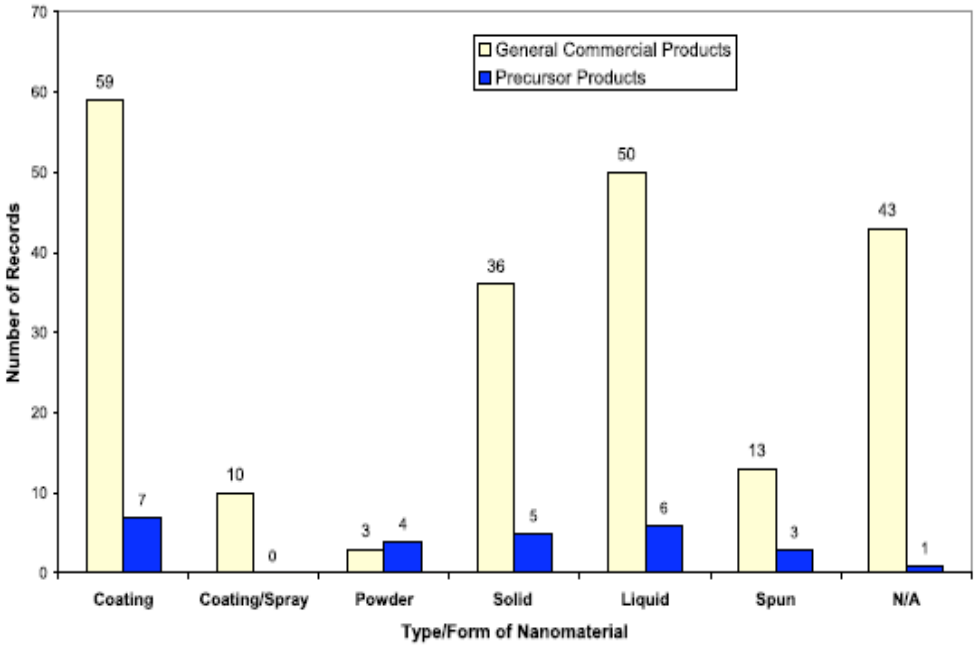

Figure 2.3. Forms of nanosilver incorporated in consumer products. Graph Obtained from "The Silver Nanotechnology Commercial Inventory" By Emma Fauss (2008)

Because of its increased conductivity, nanosilver is used in circuit boards, solar panels (Lim et al. 2012), batteries, and printer inks (Kirby et al. 2011). Because of its antimicrobial, antifungal, and antiviral properties, nanosilver is used in textiles, food, and cosmetics as well as in water purification systems

[1] Telephone conversation with Todd Kuiken of the Wilson Center, April 17, 2013.

(Heidarpour et al. 2010). Other examples of products manufactured with nanosilver to exploit these protective properties are coatings for handrails and refrigerators, socks, shoe insoles/inserts, detergents, toothpastes, shampoos, air filters, paints (to prevent mildew), vacuum cleaners, personal grooming kits, mattress covers, sportswear, and under garments (Luoma 2008). In particular, nanosilver is used in the food industry to prevent the growth of mildew, fungus, and other microbes.[2] Because nanosilver prevents bacterial growth, it is used as a coating in food processing, storage, and packaging as a means of extending the shelf life of the food. Proponents of alternative medicine use nanosilver for medicinal purposes and in supplements, claiming the nanosilver particles have anti-inflammatory properties as well as additional antimicrobial, antifungal, and antiviral properties (Silver 2003).

2.4.2 Medical Goods and Research

Nanosilver is used for diagnostics (ultrasensitive biomedical detection) because of its plasmon resonance property, which helps determine target molecule of organic compounds (Kooij et al. 2011), and in biotechnology (drug delivery systems) because of its plasmonic heating properties (Skirtach et al. 2004). Because of its antiviral, antibacterial, antifungal, and anti-inflammatory properties, it also is used in wound care and coatings for medical tools, in devices such as catheters, implants, and heart valves, and in medical equipment. It also is used in medical implants because it prevents the formation of biofilms and has

[2] The first nanosilver product to be registered as a pesticide was in the early 1950s (EPA).

anti-glycoprotein properties (Wijnhoven et al. 2009). Because nanosilver enhances metal fluorescence, it alters emission intensity and photostability of fluorophores; therefore, it is used for DNA/RNA detection (Wijnhoven et al. 2009). Table 2.1 shows a list of devices containing nanosilver that are currently being used.

Table 2.1. Medical devices containing nanosilver

Medical domains	Examples
Anesthesiology	Catheter for administration of local anesthetic
Cardiology	Battery used in implantable cardioverter-defibrillator
Nephrology	Hemodialysis catheter
Urology	Urinary catheter, battery used in implantable electrical pulse generator
Wound care	Professional use and over-the-counter burn and wound dressings (e.g., tubular stretch knit, burn glove, burn sock), over-the-counter burn gels and compresses, and IV/catheter dressings

Note. Source: Wijnhoven et al. 2009 .

2.5. Environmental Pathways of Nanosilver Exposure

Nanosilver is released into air, water, and soil during manufacturing and when consumers use and dispose of products containing nanosilver. As seen in figure 2.4, the primary pathways for nanosilver are air, water, and soil. Once the nanosilver is in the environment via secondary pathways, it has the potential to find its way into humans.

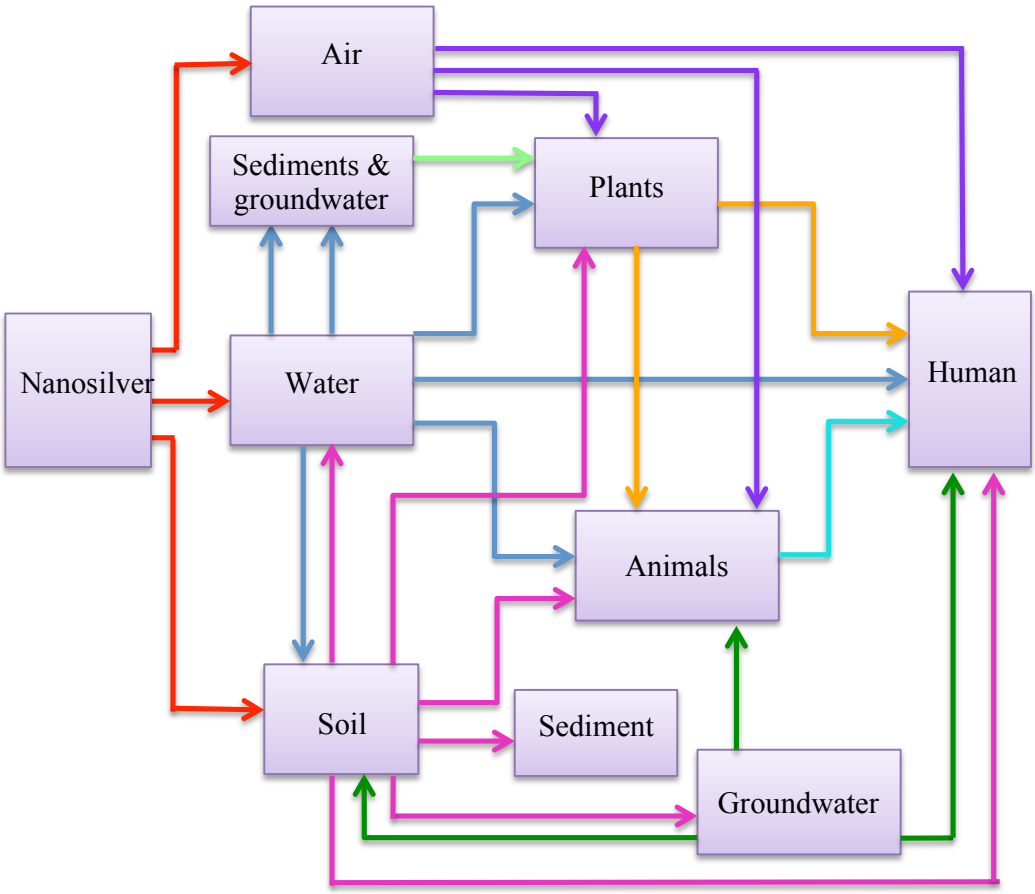

Figure 2.4 Environmental pathways of nanosilver. Fig by Vinita Bose

2.5.1 Ecological Pathways

Nanosilver is released into water, soil, and air when it leaches out of manufactured goods. Leaching is the result of the deposition of suspended nanosilver, which can occur during the manufacturing process or as runoff after the nanosilver has been introduced into the environment. The fate of nanosilver in the environment is largely dependent on the nature of the nanosilver and the environment into which it is released. Factors such as (1) the size of the nanosilver's charge, (2) the capping agent used to manufacture the particles, (3)

the pH, electrolyte compositions, and ionic strength of the medium into which the nanosilver is released, and (4) the level of natural organic matter in the medium into which the nanosilver is released. These parameters will determine if the nanosilver will agglomerate or disperse, how far it will travel, and whether it will bioaccumulate in the environment (Venkatapathy 2010).

Because silver is known to bind to sulfur that contains oxygen, the presence of dissolved organic carbon and particulate matter affect the behavior of nanosilver in solid media (Wijnhoven et al. 2009). Similarly, the suspension's pH, composition, and ionic strength also are important in determining the stability of nanosilver. High ionic strength results in aggregation, and pH influences the surface potential of the nanosilver and thereby the aggregation (Yu, Yin, and Liu 2013).

Some of the leached nanosilver is likely to enter wastewater in treatment plants. A study sanctioned by the British Department of Environment, Food, and Rural Affairs found no evidence of increasing nanosilver concentrations in the rivers of England and Wales and therefore concluded that the wastewater treatment plants were effectively able to remove all the nanosilver leached from consumer products (Johnson et al. 2011). Therefore, the majority of nanosilver removed from wastewater is deposited into the soil through the sludge bi-product of the treatment process. Sludge from wastewater treatment plants is used as an agricultural fertilizer in the United States and the United Kingdom. For this reason, nanosilver may pose risks to soil and groundwater over time (Blaser et al. 2008).

2.5.2 Aquatic Environments

Lowry et al. determined the long-term behavior of coated nanosilver in fresh water mesocosms of a wetland (2012). (Coated nanosilver is nanosilver to which a layer of organic molecules has been applied to stabilize the particles; mesocosms replicate the natural environment of a wetland on a small scale under controlled conditions.) The researchers concluded that nanosilver could enter the waterways from both erosion and runoff from sediments and soil. The researchers also found residues of nanosilver in terrestrial plant biomass and relatively high body burdens of silver in mosquitoes and fish (Lowry et al. 2012; Yin et al. 2012).

2.5.3 Plants

In a recent study by the Duke University's Centre for Environmental Implications of Nano Technology (CEINT), researchers found that low concentrations of nanosilver in bio-solids applied under realistic field conditions led to adverse effects in the ecosystem by increasing the N_2O fluxes, which leads to changes in microbial community composition, biomass, and extracellular enzyme activity. The study also demonstrated the potential for plants to take up nanosilver from the soil. CEINT also found that the effect of nanosilver treatment on soil was as large as the effect of silver nitrate treatment, which was applied at four times the concentration of nanosilver (Colman et al. 2013). These results suggest that even when nanosilver is transformed in biosolids due to oxidation and sulfidation, it still has an impact on plants and microbes.

2.5.4 Soil

In wastewater treatment, a large amount of nanosilver from wastewater partitions into the sludge, which is a commonly used fertilizer in agriculture in the United States and the United Kingdom (Fabrega et al. 2011). In addition to controlling injurious organisms, nanosilver also kills the beneficial bacteria responsible for aiding the de-nitrification process in soils. Environmental de-nitrification is important because excess nitrates in the soil can reduce plant growth, which in turn can lead to the eutrophication of rivers, lakes, and marine ecosystems. Hampering de-nitrification could potentially cause disruption of ecosystems. Excess nitrates also are a drinking water pollutant (Panyala et al. 2012).

2.5.5 Aquatic Organisms

Silver in any form is toxic to aquatic organisms. Silver induces toxicity in fish when its positively charged ions bind to the negatively charged gills and reduce the uptake of Na^+ and Cl^-, inhibiting Na^+, K^-, -ATPase activity (Bianchini et al. 2005). At high concentrations, this inhibition leads to blood acidosis, resulting in circulatory collapse and death (Hogstrand and Wood 1998). In their toxicity assessment of gold and nanosilver in zebrafish embryos, Bar-llan et al. concluded that the toxicity is caused by either the nanoparticles themselves or the Ag^+ that is formed during *in vivo* nanoparticle destabilization (2009). In another study conducted on zebrafish embryos, Cunningham et al. found that surface

charge and ionic dissolution were the key factors in nanosilver exposure outcome (2013).

During my review of the literature, I found several studies on nanosilver toxicity on aquatic organisms. In those studies, the researchers concluded:

- Citrate-capped nanosilver can pass through the pores and accumulate in zebrafish embryos causing abnormalities and death (Lee et al. 2007).
- Nanosilver induces a dose-dependent toxicity in the embryos of zebrafish, which hinders normal development (Asharani et al. 2008).
- Nanosilver increases the rate of operculum movement (increase in the movement of the flaps covering the gills of a fish) and surface respiration, thus suggesting respiratory toxicity to zebrafish (Bilberg et al. 2012).
- Nanosilver is toxic to the liver; when the adult zebrafish were exposed to nanosilver, it resulted in oxidative stress and apoptosis of liver cells (Choi et al. 2010).

These findings are summarized in table 2.2.

2.6 Human Risk Assessment

There are few studies evaluating the risk to human health from exposure to nanosilver nor is there enough data on the kinetics of nanosilver to fully understand the body burden imposed by this material (Hansen and Baun 2012b) Results from current studies have indicated that nanosilver is absorbed and

distributed to target organs (Christensen et al. 2010). The next section features a discussion of the human health effects associated with exposure to nanosilver.

Table 2.2. Summary of environmental findings

Environmental concern	Findings
Environmental pathways	Pathways are dependent on capping agent used, size, shape and the pH, organic matter content and the pollutants in the medium released. It is also persistent.
Aquatic organisms	Toxicity studies show liver damage, developmental defects, death of embryos, and respiratory toxicity.
Terrestrial biomass	Accumulation observed.
Wastewater treatment plants	Hampers nitrification and affects the biological treatment.
Soil	Sludge used in fertilizer could potentially have nanosilver, which can get into soil and can eventually reach surface water and groundwater.
Plants	Affects plant growth.

2.6.1 Exposure

Human exposure to nanosilver occurs mainly through consumer products, food, and medical products (Wijnhoven et al. 2009). However, the evaluation of exposure is challenging because there is little information available on the release of silver ions from these applications (Duncan 2011) and no information available on the characteristics, size, or shape of the materials in their different forms as used in consumer products (Wijnhoven et al. 2009). Although TPEN manages a periodically updated inventory of consumer goods containing nanosilver, in this

study, the data provide limited use for determining human exposure for two specific reasons. First, because new products are flooding the market at a rapid rate, it is difficult for the TPEN database to remain current. Second, it is difficult for TPEN to ensure the accuracy of this data because there is no government requirement to list nanosilver content in consumer products. As a result, some companies may indicate nanosilver as a product ingredient to boost sales even though the product may not actually contain nanosilver, while other companies whose products do contain nanosilver may choose not to list it as an ingredient.

2.6.2 Toxicokinetics of Nanosilver

The toxicokinetics of nanosilver are complicated by the difficulty of detecting nanosilver but also because of the challenges of measuring transformed or metabolized by-products so as to evaluate absorption, distribution, metabolism, and excretion.

Absorption

The absorption of nanosilver can take place via the respiratory system when products containing nanosilver are inhaled. The deposition and distribution of the inhaled nanosilver is dependent on the size of the nanosilver particles and the rate and force of inhalation. The smaller the nanosilver particle, the deeper it will penetrate into the lungs (Wijnhoven et al. 2009).

The olfactory nerves are another means by which the nanosilver can enter the central nervous system. Nanosilver entering through olfactory nerves appear

to penetrate the blood brain barrier and deposit in the brain (Oberdörster et al. 2005).

Absorption through the gastrointestinal tract occurs from oral exposure to nanosilver by ingestion of products containing nanosilver and from colloidal silver. Colloidal silver is a liquid containing suspended silver particles ranging in size from micro to nano and ultrafine. The suspension often is used as a natural alternative to antibiotic or anti-inflammatory drugs. Using colloidal silver containing only nanosilver, Kim et al. found that dose-dependent accumulation in all tissues occurred following oral exposure in a 13-week study with F344 rats (2008).

Dermal absorption occurs the most due to prolific use of nanosilver as an antibacterial agent in consumer and medical products, including gels, clothing, and dressings. The Danish Study, conducted to observe the effects of exposure to nanosilver from clothing, showed that dissolved silver was absorbed more from dermal exposure than from any other forms of exposure (Tonning 2012).

Distribution.

Lankveld et al. found that when nanosilver was injected into the body of rats, it was widely distributed to all organs but most notably to the liver, lungs, and spleen (2010). Orally ingested nanosilver has been found to cause argyria, a bluish hyperpigmentation of the skin, and decreased kidney function in humans (Mayr et al. 2009). Takenaka et al. found inhaled nanosilver in rats deposited in lung tissue but also in organs such as the heart. The researchers also found

nanosilver absorbed by the olfactory nerves in brain tissue in rats (Takenaka et al. 2001). Because the researchers also detected a significant amount of nanosilver in the blood, they concluded that systemic distribution occurred (Takenaka et al. 2001). Dermal absorption by skin has been found to lead to an increase in blood serum levels in burn victims treated with nanosilver-coated dressings (Moiemen et al. 2011).

Metabolism

The metabolic fate of nanosilver depends on its composition. Some forms of nanosilver may undergo transformation while others forms may be excreted directly from the body (Scientific Committee on Emerging and Newly Identified Health Risks 2007, [SCENIHR]). Nanosilver absorbed in the body through the gastro-intestinal tract is transported to the liver by the portal vein. Wijnhoven et al. speculated that nanosilver may bind to metallothionein proteins, which have the ability to bind to metals and thereby achieve regulation in the body (2009). However, Wijnhoven et al. indicated that there is no evidence of metabolism of nanosilver by the liver enzymes (2009).

Excretion

Nanosilver particles greater than 5.5 nm in size are eliminated from the body by renal excretion. Some nanosilver can be partially dissolved into silver ions and eliminated through urine (Lankveld et al. 2010). Some of the nanosilver can be absorbed from the digestive tract through biliary action, but there is not

enough information about the extent to which the liver is involved. In studies using intravenous dosing in rats, biliary excretion of nanosilver was found to be high (Loeschner et al. 2011).

2.6.3 Mechanism of Toxicity

There is uncertainty associated with the mechanism of toxicity of nanosilver. Current in vitro studies have shown nanosilver toxicity to be attributed mainly to the increase in the production of ROS. Exposure to higher doses of nanosilver leads to an increase in ROS, which stimulates inflammation and leads to secondary genotoxicity and cell death (Christensen et al. 2010). It also appears that smaller nanoparticles exhibit higher toxicity than larger particles (Park et al. 2011). Absorbed smaller particles also are able to cross biological barriers and cause reproductive toxicity (Christensen et al. 2010). Physicochemical properties of nanosilver such as surface chemistry, charge, composition, solubility, crystal structure, aggregation/agglomeration, size and surface area also influence the toxicity of nanosilver (Colognato et al. 2012). Figure 2.5 illustrates possible interactions involving nanosilver on a cellular level.

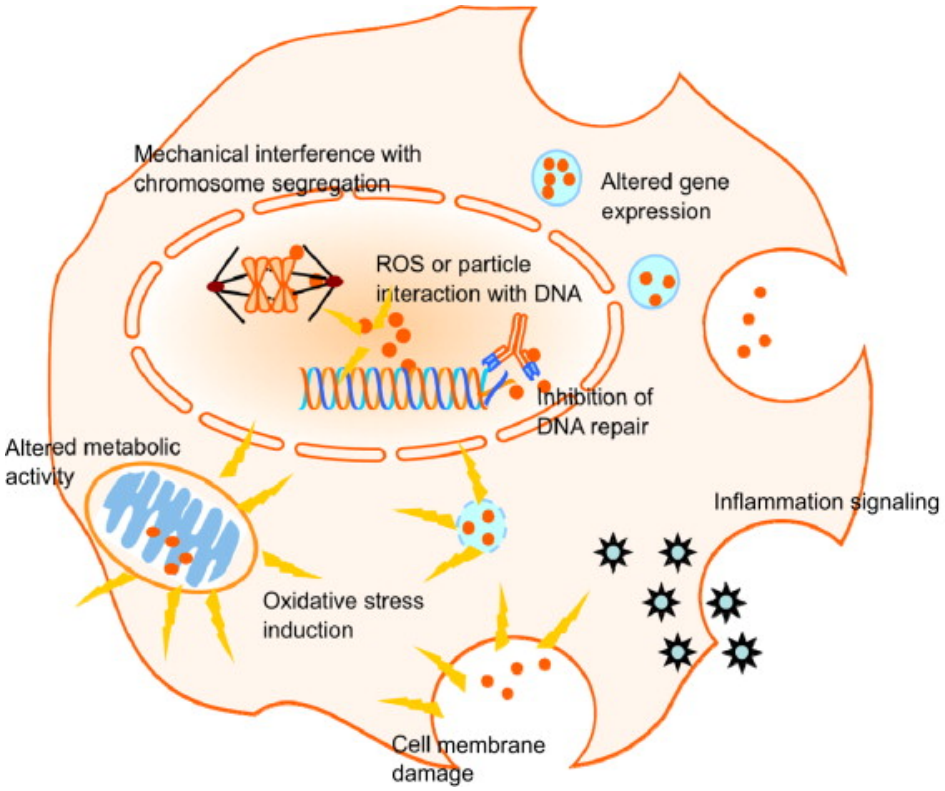

Figure 2.5. Nanosilver interaction with cellular and subcellular structures. Obtained from 'Adverse Effects of Engineered Nanomaterial' (Colognato et al. 2012).

2.6.4 Respiratory System

Deposition of inhaled nanoparticles including nanosilver in the human respiratory tract occurs mainly through diffusion. Once deposited, nanosilver is easily transported to other target organs (Oberdörster et al. 2005). The size of the nanosilver determines where it gets deposited in the respiratory tract. Nanosilver 1 nm in size is deposited mainly in the nasopharyngeal compartment. Nanosilver 5 nm in size gets deposited equally in all three regions of the respiratory tract: the nasopharyngeal, tracheobronchial, and alveolar regions. Nanosilver 20 nm in size is deposited mainly in the alveolar region of the respiratory tract. The clearance of

the deposited nanosilver from the respiratory tract occurs by the processes of physical translocation and chemical clearance (Oberdörster et al. 2005).

In a 2001 study done with female Fisher rats, Takenaka et al. observed that nanosilver accumulated in the rat lungs and remained there for at least 7 days. The researchers also observed that some of the nanosilver also translocated to the liver and other organs. The researchers observed that deposition in the liver was caused by the translocation of particles from the lungs via blood (Takenaka et al. 2001).

Sung et al. published three papers on toxicity following inhalation in Sprague-Dawley rats (Sung et al. 2008; Sung et al. 2011; Sung et al. 2009). The researchers used nanosilver of similar shapes and sizes in all three papers; therefore, the results are comparable. In the 90-day study by Sung et al., the rats were exposed to three different air concentrations of nanosilver, 49 μg/ m^3 133 μg/ m^3 and 515 μg/ m^3 (2008). Sung et al. found an effect at 49 μg/ m^3 (2008). Based on this data, Christensen suggested that 49 μg/ m^3 should be considered the lowest observable adverse effect level (LOAEL, 2010). In the subsequent 2009 study, Sung et al. conducted a subchronic inhalation toxicity study with Sprague-Dawley rats. The animals were divided into four groups; one group was a control group, and the other groups groups were exposed to three different concentrations and three different sizes of nanosilver for 6 hours per day, 5 days per week, for 13 weeks. Observations were made weekly for body weight, food consumption, and pulmonary function. The results indicated dose-dependent increases in lesions in the lungs due to nanosilver exposure. Nanosilver also was found to have

translocated to the liver. The results indicated a no observable adverse effect level (NOAEL) of 100 μg/m^3(Sung et al. 2009).

Sung et al. also conducted an acute inhalation study on Sprague-Dawley rats with similar exposure doses but different sized nanosilver (2011). This time the animals were exposed to a one time four-hour exposure in a whole-body inhalation chamber. They were then evaluated for body weight, food consumption, and pulmonary function on a weekly basis. At the end of the two weeks, no significant change was observed in any of the three groups compared to the control group (Sung et al. 2011).

Ji et al. tested the inhalation toxicity of nanosilver on Sprague-Dawley rats by exposing them to three different concentrations of nanosilver ranging from 1.27 x 10^4 particles/cm^3 to 1.2 x 10^6 particles/cm^3 for 6 hours per day, 5 days per week, over a period of 28 days (2007). The researchers found that exposure to nanosilver at a concentration near the current limit of 100 μg/m^3 indicated by the American Conference of Governmental Industrial Hygienists (ACGIH) did not appear to have any significant health effects (Ji et al. 2007).

Foldbjerg et al. investigated inhalation exposure to nanosilver on human lung cells *in vitro* and found dose-dependent cellular toxicity to silver ions and nanosilver (2011). The researchers also found a strong correlation between the levels of ROS, and both mitochondrial damage and early apoptosis after inhalation exposure (2011). Sung et al.'s 90-day inhalation exposure study to nanosilver showed that prolonged exposure induced lung function changes along with inflammation in rats (2008).

According to Christensen et al., the main target organs affected by exposure after inhalation of nanosilver are the lungs and the liver (2010). The researchers suggested a LOAEL of 49 μg/m^3 based on the lung effect and a NOAEL of 113 μg/m^3 based on the liver effect for the purpose of risk assessment following inhalation (Christensen et al. 2010).

2.6.5 Central Nervous System

Takenaka et al. found traces of nanosilver particles in the brain tissue of rats exposed to nanosilver via inhalation (2001). In a recent in vitro study by Tang et al., the researchers found that at low doses of 100 μg/ml, nanosilver crossed the blood brain barrier (BBB) by the membrane-mobile mechanism (2013). At high doses of 400 μg /ml, the particles crossed the BBB by the cytotoxicity mechanism (Tang et al. 2013). In order to assess the impact of nanosilver on the central nervous system, Liu, Huang, and Gu conducted a study on male mice and found that nanosilver did not affect spatial cognition or hippocampal neurogenesis (Liu et al. 2013). In an *in vivo* and *in vitro* study conducted by Hadrup et al., the researchers found that nanosilver in the of 14 nm size range and ionic silver have neurotoxic effects on female rats (2012).

2.6.6 Gastrointestinal Tract Toxicity

It is difficult to evaluate the uptake of nanosilver after oral exposure because of the variances in diet, mucus secretion, pH, transit time in the gastrointestinal tract (GI), and availability of flora in the GI tract (Frohlich and

Roblegg 2012). However, in a 28-day study conducted to investigate repeated oral exposure to nanosilver, researchers found accumulation of silver in the liver, kidneys, lungs, and brains of rats (Loeschner et al. 2011).

In F344 rats, Kim et al. evaluated toxicity of nanosilver from oral exposure over a period of 90 days (2010). The researchers evaluated four groups of 10 rats each, which were administered with one of four dose levels: control, 30 mg/kg, 125mg/kg, and 500 mg/kg. Significant increases in serum alkaline phosphatase and cholesterol were found in rats that received doses over 125 mg/kg, indicating slight liver damage. Nanosilver also was found in all tissues, and it was noted that the kidneys of the female rats had twice the accumulation of nanosilver when compared to the kidneys from the male rats. The researchers identified a NOAEL of 30 mg/kg and an LOAEL of 125 mg/kg (Kim et al. 2010).

2.6.7 Liver

Because the liver aids metabolism and detoxification, it is potentially a major target organ of systemic toxicity of nanosilver (Christensen et al. 2010) This important role of the liver has been supported in the literature. For example, Gaiser et al. conducted a toxicity study in female Wistar rats following ingestion of nanosilver (2012). The researchers identified evidence of the toxicity and inflammatory potential of nanosilver in the liver following ingestion (Gaiser et al. 2012). Also, Cha et al. found that mice fed nanosilver developed phenotypical changes in the liver, reflecting increased apoptosis and inflammation (2008). In addition, Kim et al. found that when nanosilver was fed to rats, it resulted in slight

liver damage (2008). Lankveld et al. intravenously administered 20, 80, and 110 nm nanosilver to rats and found that nanosilver rapidly disappeared from the blood and was distributed to the liver, lungs, kidneys, spleen, brain, heart, and testes (2010)

2.6.8 Skin

Skin is an important uptake for nanosilver and many studies have been conducted to explore dermal absorption. Trop et al. studied the potential toxicity of silver on a burn victim treated with Acticoat, a nanosilver-coated wound dressing (2006). The researchers found hepatoxicity and argyria-like symptoms. In addition, the burn victim had elevated silver levels in his plasma and urine as well as elevated liver enzymes in serum, which returned to normal as soon as the application was stopped (Trop et al. 2006). Like Trop et al.'s study, most studies on dermal exposure have been focused on the benefits of nanosilver in wound dressings (Christensen et al. 2010). However, researchers also have explored nanosilver uptake via the skin under other conditions.

For example, Korani et al. exposed male guinea pigs to two different concentrations of colloidal nanosilver (1000 and 10,000 μg/ml) in an acute study and three different concentrations (100, 1000 and 10,000 μg /ml) in a subchronic study lasting less than 90 days (2011). The researchers did not notice significant changes in the weight of the target organs or any other effects in the guinea pigs in the acute study. The researchers, however, did detect dose-dependent histopathology abnormalities in the skin, liver, and spleen in the subchronic

sample (Korani et al. 2011). Also, Koohi et al. found greater biological impact relative to particle size in that dermal exposure to 10 nm nanosilver was more toxic than 20 or 30 nm nanosilver (2011).

The Danish Environmental Protection Agency conducted an assessment of nanosilver textiles available in the Danish market (Tonning 2012). The agency calculated the dermal exposure for adults in contact with nanosilver-containing insoles 8 hours per day and for children in contact with nanosilver-containing tank tops for 16 hours per day. The dermal scenarios were based on 0.1% dermal absorption. The Risk Characterization Ratio (RCR) for insoles was 0.0000145. The RCR for tank tops was 0.00414. The agency found that absorption through intact and abraded skin was lower than expected and that the risk from dermal exposure was low (Tonning 2012).

2.6.9 Mutagenicity and Genotoxicity

Kim et al. investigated toxicity and genotoxicity in a 28-day oral study in Sprague-Dawley rats and concluded that nanosilver does induce genotoxicity in male and female rat bone marrow *in vivo* (2008). Ghosh et al. studied the activity of nanosilver *in vitro* and *in vivo* and found DNA damage and chromosomal aberrations in Swiss albino male mice (2012). The researchers also found that when human lymphocytes were exposed *in vitro* to nanosilver at a concentration of 25 μg/ml, the nanosilver caused apoptosis and DNA strand breaks. Therefore, the researchers concluded that genotoxicity from nanosilver exposure cannot be ruled out (Ghosh et al. 2012). Song et al. found that when mice were exposed to

nanosilver, they showed genotoxicity, specifically mitochondrial damage, due to oxidative stress (2012).

AshaRani et al. studied the toxicity of starch-coated nanosilver on normal human fibroblast cells and human glioblastoma cells (2009). The TEM analysis indicated the presence of nanosilver inside the nucleolus and nucleus, which indicated involvement in mitochondrial activity; specifically, the nanosilver led to production of ROS, resulting in reduction in ATP synthesis, which in turn caused DNA damage (AshaRani et al. 2009).. Kermanizadeh et al. conducted an *in vitro* assessment of engineered nanomaterial including silver using human renal cell and detected oxidative stress and genotoxicity (2013).

2.6.10 Mutagenicity and Carcinogenicity

Silver is classified by the EPA under category D as "Not classifiable as a Human Carcinogen" (USEPA 1989). No human cancers have been reported as associated with exposure to silver, its alloys, or compounds (Lansdown 2010). In a study to investigate in vivo genotoxicity in rats exposed to 60 nm nanosilver, Nymark found no evidence of genotoxicity (2012); however, Song et al. found that nanosilver caused genotoxicity in mice (2012). At present, no studies addressing the carcinogenicity of nanosilver were identified for this thesis. However according to Christensen, because signs of genotoxicity have been observed by researchers, carcinogenicity resulting from nanosilver exposure cannot be ruled out (2010).

2.6.11 Reproductive Toxicity

Studies on zebrafish showed that citrate-capped nanosilver can pass through the chorion pore canals and accumulate in zebrafish embryos, causing abnormalities and death (Lee et al. 2007). Asharani et al. demonstrated that nanosilver induced a dose-dependent toxicity in the embryos of zebrafish, which hindered normal development (2008). However, it is still unclear as to how much relevance this result has for humans (Christensen et al. 2010)

2.6.12 No-effect Levels

Lung, liver, and skin appear to be potential target organs. Christensen et al. established critical end points for human health assessment (2010, see table 2.3).

Table 2.3. Toxicity data for risk assessment

Exposure route	LOAEL	NOAEL
Inhalation	49 μg/m^3	
Inhalation		133 μg/m^3
Oral	125 mg/kg/day	
Oral		30 mg/kg/day
Oral		0.5 mg/kg/day

Note: Table obtained from the Danish Report on Assessment of nanosilver in textiles (Tonning 2012).

A summary of summary of findings on the health effects of nanosilver is presented in table 2.4.

Table 2.4. Summary of the health effects of nanosilver

Topic	Findings
Inhalation	Accumulation in the liver and lungs. Inflammation in the lungs.
Ingestion	Accumulation in the liver, lungs and kidneys. Inflammation in the liver.
Dermal	Abnormalities in skin, liver, and spleen. Elevated liver enzymes in serum. Elevated silver levels in plasma and urine.
Mutagenicity and Genotoxicity	Chromosomal aberration and DNA damage.
Carcinogenicity	Cannot be ruled out

2.7 Precautionary Principle

The precautionary principle was first introduced in the early 1980s in Germany as *Vorsorgeprinzip,* meaning the *foresight principle* (Martuzzi 2007). The precautionary principle is a policy that guides regulatory decisions when there is scientific uncertainty concerning risk (Warshaw 2012). Specifically, "when an activity raises threat of harm to human health or the environment, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically" (Kriebel et al. 2001, 871). After the European Union formed, it presented its first environmental principles, including the prevention principle, in the European Commission Treaty, signed in 1987 (Douma 2000). "The prevention principle applies in situations when there is a certainty that a particular human activity might result in damage to human health and environment" (Douma 2000, 132-33). The precautionary principle was

added later in the Maastricht Treaty in February, 1992, and was enacted on November 11, 1993 (Douma 2000).

The European Commission first invoked the precautionary principle in 1996 when the first case of mad cow disease, or bovine spongiform encephalopathy (BSE), was reported (Douma 2000). Although there had been no direct evidence linking BSE and a new disease variant, Creutzfeld-Jacob disease, the European Commission temporarily banned export of beef and veal from the United Kingdom as well as products derived from beef and veal (Douma 2000). Since that initial first ruling, the European Commission has invoked the principle to ban genetically modified maize and antibiotics in animal feed and has also taken measures to protect the ozone layer (Douma 2000) .

Although not under the direction of the EPA, for the last 30 years, the United States has applied an approach to decision making about health safety and the environment that has been precautionary (Ashford 2006, 352). “Because the United States regulatory community balances precaution with costs, precaution has functioned more as a preference than a principle” (Ashford 2006, 354). Precautionary measures were first introduced in the United States by the participants of the Wingspread Conference in Racine, Wisconsin in 1998 (Patrice Sutton 2009). The conference, sponsored by the Johnson Foundation, involved 32 American, Canadian, and European participants from the science, law, policy, and environmental fields. “It was called to define and discuss implementing the precautionary principle, which has been used as a basis for a growing number of international agreements” (National Nanotechnology Initiative 2013).

2.8 Regulatory Approaches to Nanosilver Use

Regulators and policy makers across the world can respond to nanosilver exposure in two basic ways. One is the wait-and-see approach, which promotes delaying the regulatory action until risks are established, and the other is the precautionary approach, which promotes regulatory research but also allows for regulatory action in the absence of perfect information (Falkner and Jaspers 2012). No action has yet been taken to limit human and environmental exposure to nanosilver(Hansen and Baun 2012b). Although a number of reviews have been commissioned, there has been limited progress on oversight of this material. In the next section of this chapter, I discuss some of the policy instruments available to countries interested in advancing regulation and oversight of nanomaterial.

2.8.1 United States

Of the significant regulatory agencies in the United States, three in particular are associated with nanosilver regulation: the EPA, the Food and Drug Administration (FDA), and the Occupational Safety and Health Administration (OSHA). The United States, at the national, state, and local levels, also has implemented various initiatives to raise awareness about nanosilver as an emerging contaminant.

Environmental Protection Agency

The EPA regulates all chemicals and pesticides containing nanosilver within five statutory frameworks:

- Toxic Substances Control Act (TSCA), which regulates all new chemicals and new uses of existing chemicals;
- Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), which regulates all pesticides and biocides;
- Clean Air Act (CAA) which regulates air emissions;
- Clean Water Act (CWA) established to regulate pollutants in surface water; and
- Resource Conservation and Recovery Act (RCRA) which sets standards for waste disposal.

Toxic Substance Control Act

TSCA governs chemical substances, including mixtures, broadly defined as "any organic or inorganic substance of a particular molecular identity" (TSCA sec. 3(2A)). TSCA includes a provision called the Significant New Use Rule (SNUR), which the EPA could use to regulate nanosilver. However, TSCA exempts chemicals produced in quantities of less than 11 tons per year. Eleven tons of nano material is a very large amount of nanosilver, and at present, estimates could be exempt from this regulation. Also, TSCA does not cover pesticides, drugs, cosmetics, and medical devices, all applications in which nanosilver is commonly used. Because TSCA assumes that "no knowledge about a chemical means that there is no risk" (Section 5(e)), the burden to prove harm rests with the EPA. This arrangement renders TSCA, in the assessment of some analysts, to be a weak regulatory instrument (Davies 2009). In its regulatory

agenda for 2013, the EPA announced plans for developing an SNUR under TSCA Section 5(a)(2) for nanoscale materials. It is also plans to develop an SNUR rule under TSCA Section 8(a) rule to require reporting and record keeping (Bergeson 2010).

Federal Insecticide, Fungicide and Rodenticide Act

FIFRA was established to allow for Federal regulation of pesticide distribution, sales, and use. FIFRA requires that all pesticides distributed and sold in the United States be registered (licensed) by the EPA. FIFRA defines pesticides as materials intended to destroy or repel pests, including bacteria and viruses. Because nanosilver is used primarily as an antimicrobial, FIFRA could serve as an appropriate instrument for regulating nanosilver in pesticides (Faunce and Watal 2010). Unlike the other environmental laws that are broad, FIFRA is narrow and places with the manufacturer the burden to prove a product safe (Luoma 2008). Currently, the EPA uses FIFRA to regulate the use of nanosilver as a antimicrobial (pesticide; Faunce and Watal 2010).

For example, in 2003, when Samsung introduced washing machines that used silver ions to disinfect clothes, the EPA required it to be registered under FIFRA. Because FIFRA requires extensive testing before use of all products claiming insecticidal properties, the following year Samsung stopped claiming the disinfection properties of nanosilver and therefore was exempted from extensive testing (Fauss 2008). Also, when IOGEAR made claims that its products, including their wireless laser mouse and their wireless keyboard and mouse

combinations, had a nanosilver coating that would protect against microbes, the EPA fined IOGEAR $208,000 for selling an unregistered pesticide. IOGEAR stopped making claims about their antibacterial products (EPA 2013) .

In December 2011, the EPA announced the conditional registration for HeiQ, a nanosilver-based pesticide for use in the manufacture of textiles, thereby giving the manufacturer four years to provide the product's toxicity data and continue to manufacture and sell the product during that time. The Natural Resources Defense Council (NRDC) challenged this decision in January 2012 because the NRDC believed it to be potentially harmful to human health and the environment. The NRCD filed a federal law suit to block nanosilver from market access (NRDC, 2013). Based on this and all the ongoing concern over the widespread use of nanosilver, the EPA established a Registration Review Docket for nanosilver, which will begin in July 2016. The registration review process will be a means by which the EPA can periodically review pesticide registrations to ensure that each pesticide continues to satisfy the statutory standard for registration, that is, the pesticide can perform its intended function without unreasonable adverse effects on human health or the environment (EPA 2013).

Clean Air Act

The CAA's standards for particulates less than 2.5 micrometer ($PM_{2.5}$) in size in ambient air could be applied to nanosilver emissions in ambient air under the National Ambient Air Quality Standards. However, the CAA's Hazardous Air Pollutant Monitoring standards are not applied to particulate matter. Therefore, in

order to monitor nanosilver, the EPA would have to revise its existing rules. Also, these standards are based on a correlation between volume/concentration and risk and may not be appropriate for measuring nanosilver (Davies 2009). There also is a lack of effective methods for monitoring nanosilver in air because of the complex physical and chemical dynamics associated with nanoparticles (Faunce and Watal 2010).

Clean Water Act

The CWA mandated standards for pollutant discharges to surface waters and authorized the EPA to enforce them. Specifically, Section 304(a) of the CWA directs the EPA to establish water quality criteria that reflects the latest scientific information. As a result, in 1980, the EPA established the aquatic life Ambient Water Quality Criteria (AWQC) for silver, which the agency enforces via the CWA. The EPA's current acute dissolved freshwater criterion for silver is 3.4 µg/liter and the saltwater acute criterion is 1.9 µg/liter. However, there still is a lack of chronic toxicity data for silver, especially for aquatic species other than fish such as crustaceans (Ford 2001)

Resource Conservation and Recovery Act

The RCRA identifies technology standards for disposal sites and establishes a cradle-to–grave reporting system for hazardous wastes. Because RCRA sets standards only for disposal sites rather than regulating sources, the legislation is not a very good tool for regulating nanosilver (Davies 2009).

Food and Drug Administration

The FDA regulates food, drugs, medical devices, therapeutic goods, and cosmetics under the Federal Food, Drug, and Cosmetic Act (FDCA). For food, the FDA mainly regulates food additives and packaging (Davies 2009). Because nanosilver is used as a preservative in food and for packaging, the FDA plays a crucial role in regulating its use in the food industry.

Although the FDA approval process for new drugs as well as medical and therapeutic goods is long and rigorous, it works in favor of public safety (Davies 2009). The problem with regulating medical and therapeutic goods containing nanosilver is that the FDA still considers nanosilver as having the same properties as ionic or elemental silver and regulates products containing nanosilver accordingly (Davies 2009).

The manufacture of cosmetics in the United States is largely unregulated. Manufacturers are not required to disclose product ingredients or report injuries related to the use of their products. Although manufacturers are not required to list ingredients, some of them do, and for those manufacturers that list their ingredients, the FDCA requires that the information be accurate. However, if the FDA were to find adulterated or misbranded cosmetics, it has no authority to recall it or make the manufacturer liable (Davies 2009).

Occupational Safety and Health Administration

OSHA oversees all workplace safety for manufacturers of nanosilver and products made with nanosilver. Specifically, OSHA is responsible for setting

standards for work place safety and monitoring for noncompliance of these standards. However, OSHA does not have enough funding or resources to monitor nanosilver. There also is not enough data available on toxicity to set standards for exposure (Davies 2009).

Other Initiatives

The Unites States government has tried to create a coordinated nano technology strategy through the National Nanotechnology Initiative (NNI), which was established in the United States in 2000 to oversee research, development, and policy (Falkner and Jaspers 2012). NNI is committed to issues pertaining to environmental health and safety of nanomaterial. In 2012, the Federal government awarded NNI approximately $1.7 billion for research and development of which $102 million was allocated for environmental health and safety research (National Nanotechnology Initiative 2013).

Various states and local authorities also have taken steps to include nanomaterials, including nanosilver, as emerging contaminants of interest. Massachusetts, Pennsylvania, South Carolina, and Washington, for example, have identified nanomaterials as contaminants of concern. Maine included particulate matter from nanotechnology in its Air Toxics Priority List of 2007. Wisconsin has called for the creation of a nanomaterial registry and the development of legislation to address risk issues pertaining to its exposure. Also, the Berkley, California City Council introduced the nanomaterial disclosure law, and

Cambridge, Massachusetts has introduced the nanomaterial information collection program (National Nanotechnology Initiative 2013)

2.8.2 European Union

In the first European Commission Treaty of 1987, under the Single European Act, the commission introduced the prevention principle, the polluter pay principle, the rectification at source principle, and the integration principle (Douma 2000). The precautionary principle was added by the Treaty of Maastricht in 1992 (enacted in 1993) and stated that community policies on the environment "shall be based on the precautionary principle and on the principles that preventive action should be taken, that environmental damage should as a priority be rectified at the source and that the polluter should pay" (Douma 2000).

The European Union operates on the precautionary principle as stated in the Integrated Pollution Prevention and Control (IPPC) Directive 96/61, which requires all regulatory agencies to take into account the precautionary principle when issuing permits for production facilities. The IPPC Directive includes a discussion of considerations to be taken into account, generally or in specific cases, when determining the best available techniques for pollution prevention during production, bearing in mind the likely costs and benefits of a measure in adhering to the principles of prevention and precaution (Douma 2000). Therefore, the onus of consumer safety rests on the industry rather than the government.

On the topic of nanotechnology, the European Union has adopted an incremental approach for regulation. This approach focuses on amending the

current nanotechnology regulations to address nanomaterials. The European Union enforces the regulations for nanomaterial under the Registration, Authorization, and Restriction of Chemicals (REACH) program. REACH consists of four elements:

- data collection on chemical use and toxicity,
- examination by governments of the need for additional testing and regulation,
- requirements that firms seek permission to use chemicals of high concern; and
- restrictions or the complete ban of certain chemicals that cannot be used safely (Hansen and Baun 2012a).

In October 2011, REACH defined nanomaterial as "a manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm (Agency 2013). One of the limitations of REACH is the lack of clarity on whether the nano form of a substance with completely different toxicity and properties than the bulk form of a substance should be considered the same or different. The second limitation of REACH is that substances manufactured or imported into the European Union in volumes of less than 1 ton/year do not need to be registered, thus producers or importers of such substances are not required to provide toxicological data or access the risks of environmental exposure to those substances (Hansen and Baun 2012a).

Three additional European Union regulations cover nanomaterials: pharmaceutical regulation, regulations for waste management of products containing nanoparticles, and nanofood regulation. Another concern for the European Union is the use of nanomaterial in cosmetics. The European Union amended its cosmetics legislation in 2009. Currently, the legislation requires manufactures of existing cosmetics to inform the European Union of the types and amounts of nanomaterial used in the product's container and include nanomaterial in the list of product ingredients. For new products yet to be introduced for consumer use, manufacturers are required to notify the commission of the size, properties, and quantities of nanomaterial used along with the toxicological profile and safety data on the material. Under REACH regulations, nanosilver is preregistered as a nanomaterial.

Major developments towards regulating nanosilver also have been taken by individual countries in the European Union. For example, in 2008, Germany's Bundesinstitut für Risikobewertung (BfR; Federal Institute for Risk Assessment) requested that manufacturers not use nanosilver in food and household products (Hansen and Baun 2012b). In addition, in November 2009, the United Kingdom's Department of Environment, Food, and Rural Affairs (DEFRA) commissioned a report on nanosilver. In the report, DEFRA recommended the application of the precautionary principle. Also, in December 2011, the European Commission asked the European Union's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR, 2007) to provide scientific information on nanosilver (Risks). In June of 2012, the Danish Ministry of the Environment

published a paper on the project "Survey, Sampling, Analysis and Assessment of Nanosilver in Textiles on the Danish Market." By 2014, the Netherlands is scheduled to conclude a complete evaluation on nanosilver (European Chemical Agency 2013).

2.8.3 Australia and New Zealand

Australia and New Zealand also have demonstrated concern about the regulation of nanosilver. For example, in February of 2006, Australia and New Zealand requested voluntary information on the uses and quantities of nanomaterials that were manufactured and imported for use in cosmetics and personal care products under the National Industrial and Chemical Assessment Scheme (Faunce and Watal 2010). Australia and New Zealand also have conducted their own review of existing regulations for nanomaterials (R. and N. 2012). In addition, in September 2011, Friends of Earth Australia issued a report titled "Nano-silver Policy Failure Puts Public Health at Risk," which attracted wide attention in the Australian media.

2.8.4 Canada

Nanomaterials are regulated in Canada under the Canadian Environmental Protection Act of 1999, The Pest Control Product Acts, the Fertilizers Act, the Feeds Act, and the Food and Drugs Act (NanoPortal 2013). Although Canada has not adapted any nano-specific rules beyond the safety framework, it has introduced a voluntary reporting scheme for nanomaterials used for

manufacturing and is considering making it mandatory (Falkner and Jaspers 2012). Also, in 2011, Canada's Natural Sciences and Engineering Research Council (NSERC) was awarded a three-year grant to study the environmental effects of nanosilver on fresh water eco-systems (Canadian Broadcast 2013; Lowry et al. 2012).

2.8.5 China and India

Many of the nanosilver products on the market today are manufactured in countries considered emerging economies, which are keen to capitalize on the new technology for manufacturing commercial products. For example, early in 2013, one company in India introduced a nanosilver-containing water filter for distribution to the general public. Although the Chinese government recently initiated a research program on the properties and toxicity of nanosilver, and policy makers in India have begun to identify challenges to regulating nanosilver; these countries have not adequately invested in a capacity necessary to adequately evaluate the risks associated with such technologies. This condition was cited in a recent report on the life cycle of a nanosilver-based candle filter by The Energy and Resource Institute (India) in which the authors concluded that the "development and use of nanosilver candle filter cannot be termed risk free" (Sarma 2011).

2.9 Conclusion

Based on the environmental and health evidence presented in this chapter, it appears that nanosilver is toxic to human health and the environment, particularly to the aquatic organisms. It can build resistance to microbes, breed super bugs, and compromise immunity. Governments across the globe are slowly introducing initiatives for oversight and regulations. To further evaluate the risk from nanosilver use, I interviewed several experts in the field of science and policy. In subsequent chapters, I discuss the methods I used to conduct the interviews and the results of my interviews as well as make recommendations for oversight of nanosilver use.

3.0 METHODS

Due to the prolific and often unregulated use of nanosilver, regulatory agencies have not been able to adequately assess the safety of this materials using the particles. In particular, (1) information regarding the physical and chemical properties of nanosilver is insufficient to evaluate sources, pathways, and receptors; and (2) no standardized dose metric exists that agencies can use to evaluate toxicity and assess the need to possibly mitigate human and environmental exposure to the nanosilver. For these reasons, the purpose of this study was to gather relevant information in order (1) to make a determination about whether or not the EPA should invoke the precautionary principle with regard to the use and regulation of nanosilver and (2) to make recommendations for implementing the precautionary principle as appropriate based on this determination. In this section, I describe the research model I used to achieve the goals of my study. In particular, I discuss the literature I reviewed, the Tufts Institutional Review Board (IRB) process, and the processes for identifying and recruiting participants as well as conducting interviews with those participants.

3.1 Research Model

The research model for this study was composed of a risk assessment and the description of (1) current regulatory practices and challenges posed by nano silver and (2) the perspectives of active researchers related to the possible need for implementation of the precautionary principle by the EPA with regard to nanosilver and its use (see fig. 3.1). The model also encompasses

recommendations for the implementation of the principle if appropriate based on the study results. Currently, not enough data exist on (1) effect levels of nanosilver to calculate the acute and chronic reference dosages needed to evaluate non-carcinogenic effects or (2) the additional unit risk and slope factor needed to evaluate carcinogenic effects of nanosilver. (The International Agency for Research on Cancer [IARC] has published studies on carcinogenicity of carbon based nanoparticles). For this reason, I could not conduct a traditional risk assessment. Instead, my risk model was based on the data available in the current literature. During my review of the literature, I gathered information about the physical and chemical properties of nanosilver to evaluate sources and pathways of the particle in the environment and what influenced its toxicity as well as identified the currently established toxicity of the nanosilver. I used this information to characterize risk.

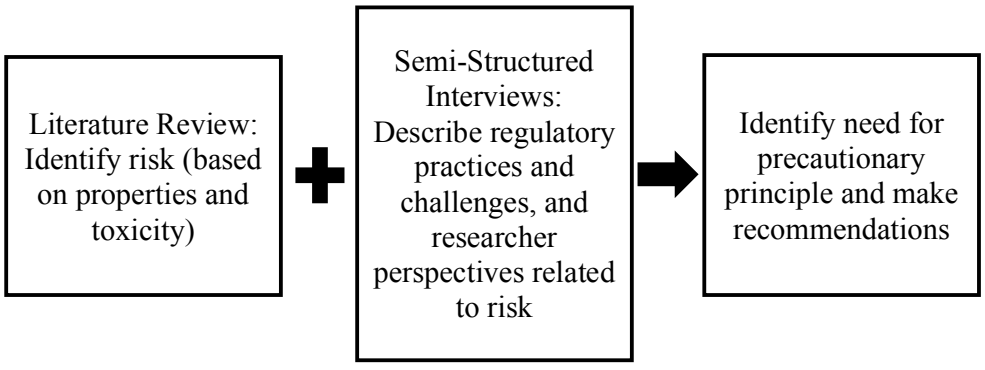

Figure 3.1. Research Model.

Semi-structured interviews were conducted to collect data about current regulatory practices and challenges as well as to allow current researchers to express views on the risks of nanosilver. In this thesis, I also (1) explored what regulations are in place both in the United States and internationally and (2) identified the challenges found in implementing such regulatory approaches. Through the interviews, I also explored identified risks to health and the environment based on nanosilver properties and toxicity.

3.2 Literature Review

To complete the characterization of risk, I conducted an extensive literature review regarding the characterization and properties of nanosilver as well as its applications in order to evaluate sources and pathways of nanosilver in the environment and what influenced its toxicity. With regard to applications in particular, I placed emphasis on identifying as complete as possible an inventory of consumer products already available in the retail market that contain nanosilver, identifying the uses of nanosilver in medical diagnostics and treatment modalities, and describing how nanosilver is employed in the manufacturing sector.

The literature review also encompassed studies and reports documenting human health effects of nanosilver and global initiatives for oversight and regulations concerning its use. Specifically, I identified and reviewed information on the use of the precautionary principle in shaping oversight and usage guidelines for nanosilver in the European Union, Canada, Australia, and New

Zealand. I also gathered information on how India and China, countries that do not currently regulate nanosilver use, are responding to the rapid increase in the domestic use of nanosilver in consumer products, medical goods, and food. I researched how the regulatory authorities in the United States, like the EPA, the FDA, and NIOSH are addressing the exponential increase in the use of nanosilver. Information on local-level initiatives for oversight and the regulation of nanosilver were also sought.

The literature review was conducted by employing the following databases: Scopus, PubMed, ProQuest, Engineering Village, Environmental Complete, Science Direct, Springer Link, Biomed Central, Google Scholar, Policy File and Toxnet. In addition, documents such as USGS reports on nanosilver, United States federal databases, and reports from non-governmental organizations and industry groups such as the Silver Institute were consulted. Current applications of nanosilver were identified on nanotechnology sites such as Nanowerk, International Council on Nanotechnology, nano.gov, Woodrow Wilson School, and the Pew Charitable Trust's project on nanotechnology.

The literature review yielded information that I used to develop a spreadsheet detailing systemic toxicity, genotoxicity, and carcinogenicity pertaining to nanosilver. Knowledge gaps were identified and quantifiable results and conclusions pertaining to the toxicity of nanosilver were noted. Upon completion of the literature review and risk characterization, the next phase in my research process was to conduct interviews.

3.3 Institutional Review Board Process

Because all the interviews conducted were with professionals in their field of expertise, no approval certification was required from the Social, Behavioral, and Educational Research IRB of Tufts University. However, in order to obtain the exemption, I completed the Collaborative Institutional Training Initiative (CITI) training for research on human subjects and I filed a protocol application for exempt status with the Tufts IRB (see Appendix 1). The interview process began once the exempt certificate was granted.

3.4 Identifying and Recruiting Participants

The literature review helped identify potential participants, experts in the fields of regulations, policy planning, biotechnology, science, medicine, and Academia. Potential participants were contacted via email and telephone as appropriate and invited to be interviewed and participate in my study. I contacted four or five people per field (between 24-30 people in all) so that I could obtain a yield of at least two people from each field for a total participant count of 12.

3.5 Interviews

An interview instrument was developed to collect information about (1) regulatory practices and challenges and (2) perceptions of current researchers with regard to identified risks to health and the environment based on nanosilver properties and toxicity. The choice of a semi-structured format afforded me the flexibility to ask questions that became apparent only after I began interviewing a

participant. It also allowed me to follow up on participant statements to elicit more in-depth responses. Having the ability to ask unplanned questions allowed me to conduct more thorough interviews.

Three lists of questions were developed and organized according to topic: toxicity, environment, and regulation (see Appendix 2). Prior to conducting interviews in the field, I conducted pilot interviews with two of my peers who have some background in nanosilver as well as with one member of my thesis committee. Based on the feedback I received, I made adjustments to the initial questions.

The interview schedule was deliberately divided by topic so that the sequence could be individualized to each participant. As a general rule, regulators and policy planners were asked questions about regulations, scientists were queried on questions about toxicity, and those in academia were posed with inquiries about the environment—questions that were a mix of both regulatory and toxicity questions. A unique list of questions based on participant background and/or area of research interest was generated.

With participant consent, I digitally recorded the interviews so that I was able to focus on the interviewees' responses and the asking of applicable follow-up questions. Good rapport was established, which allowed for a free exchange of perspectives to be covered during the interview. Given the semi-structured design some participants required redirection back to questions to stay on topic. The interviews lasted in the range of 20 to 30 minutes.

After the interviews, I transcribed the data and provided each interviewee a transcript for review and confirmation to ensure the information in the transcript accurately reflected their thoughts and ideas without any misinterpretations or misquotes. If additional clarification was required, an email was sent to the participant.

Certain truths govern the collection of data via semi-structured survey techniques. In 1992, Collins wrote that, "It is a common, and perhaps one of the most fundamental criticisms of qualitative research that the entire qualitative research process is biased by implicit assumptions, interests, world- views, prejudices, and one-sightedness of the researcher" (Diefenbach 2008, 876). In addition, I also recognized the potential for elite bias also exists. "Elite bias concerns overweighting data from articulate, well-informed, usually high-status informants and, conversely, under-representing data from intractable, less articulate, lower-status ones" (Heiskanen and Newman 1997). There is also likely to be unconscious bias, which occurs when "the interviewee (and perhaps the interviewer, too) is not aware of the influences of the interview situation and his or her internal, unconscious reactions to being asked 'officially' about certain issues" (Diefenbach 2008, , 881). These potential biases were mitigated to the best of my ability. In particular, the tone and manner in which open-ended questions were asked was closely scrutinized for freedom from leading participants towards a particular conclusion.

4.0 RESULTS

In order to characterize risk from exposure to nanosilver, I conducted a literature review and interviewed experts in the field. In this chapter, I present the results from my literature review and the interviews I conducted.

4.1 Literature Review of Toxicity Studies

I tabulated findings from the current literature that I reviewed on toxicity studies related to nanosilver exposure (Appendix 3). The studies are divided into subgroups according to types of dosing (acute, repeated dose, subchronic dose) and types of exposure (oral, dermal, and inhalation). I also included mutagenicity studies in the review. Most of the studies reference nanosilver specifically, but some studies, as noted in the literature review as applicable, reference both nanosilver and other forms of silver. In this section, I summarize the findings of the literature review.

4.1.1 Inhalation Exposure

Some of the literature included effects of nanosilver following inhalation exposure. Sung et al. found an effect at the 49 μg/ m^3 level (Sung et al. 2008). Based on this data, Christensen suggested that 49 μg/ m^3 should be considered the LOAEL (2010). In Sung et al.'s subsequent subchronic study, the researchers established a NOAEL concentration of 100 μg/m^3 (Sung et al. 2009). In the third of their studies on inhalation exposure, Sung et al. concluded that the risk assessment of nanosilver should be based on surface area rather than the mass

(Sung et al. 2011) Christensen et al. suggested that the main target organs affected after inhalation exposure appear to be the lungs and the liver (2010).

4.1.2 Oral Exposure

Some of the literature included effects of nanosilver following oral exposure. Takenaka et al. indicated that liver appears to be the target organ for oral exposure from nanosilver in all acute, subchronic and repeated dose toxicity tests (Takenaka et al. 2001). Also, Kim et al. suggested a NOAEL of 30 mg/kg/day and an LOAEL of 125 mg/kg/day following a 90-day study on rats (2010).

4.1.3 Dermal Exposure

Some of the literature included effects of nanosilver following dermal exposure. Naghsh, Dehkordi, and Aghababa found that nanosilver caused oxidative stress and apoptosis in red blood cells of rats (2013). Koohi et al. found that smaller nanosilver particles were more toxic than larger nanosilver (2011). Korani et al. found dose-dependent and time-dependent abnormalities in skin, the liver, and the spleen in an acute and subchronic dermal exposure study on guinea pigs (2011).

4.1.4 Mutagenicity and Genotoxicity

Some of the literature included mutagenicity and genotoxicity of nanosilver. AshaRani et al. suggested oxidative stress mechanisms due to the

generation of reactive oxygen species (ROS) or free radicals is one of the causes for mutagenicity and genotoxicity (AshaRani et al. 2009). Song et al. found that when mice were exposed to nanosilver, they showed genotoxicity, specifically mitochondrial damage, due to oxidative stress (2012). Ghosh et al. concluded that because nanosilver caused apoptosis and DNA strand breaks, genotoxicity from nanosilver exposure cannot be ruled out (2012).

4.1.5 Carcinogenicity and Reproductive Stress

No studies investigating the carcinogenicity of nanosilver were found. However, because genotoxicity has been observed, carcinogenicity cannot be ruled out (Christensen et al. 2010).

4.1.6 Nanosilver in the Environment

Some of the literature identified the main effects of nanosilver on the environment. Lowry et al. suggested that nanosilver could enter the waterways from both erosion and runoff from sediments and soil (2012). Lowry et al. (2012) and Yin et al. (2012) found residues of nanosilver in the terrestrial plant biomass and body burdens in mosquito and fish. Panyala, Peña-Méndez, and Havel found that nanosilver kills the beneficial bacteria responsible for aiding the de-nitrification process in soils (2012).Hou et al. found that de-nitrification also affects wastewater treatment in biological process treatment facilities (2012). Lee et al. found nanosilver causes abnormalities and death in embryos of zebrafish (2007). Asharani et al. found nanosilver induces a dose-dependent toxicity in

embryos of zebrafish, which hinders normal development (2008). Bilberg et al. found nanosilver causes respiratory toxicity in zebrafish (2012). Choi et al. found nanosilver is toxic to the liver; when the adult zebrafish were exposed to nanosilver, it resulted in oxidative stress and apoptosis of liver tissue (2010).

4.2 Data Gaps

One goal of the literature review was to identify data gaps in the available nanosilver toxicity and exposure data. By doing so, I was able to identify critical data gaps that need to be addressed before sufficient information will be available for making decisions about the use of nanosilver and its potential effects on human health, the ecosystem, and the environment. The current literature on the toxicity of nanosilver is limited. More studies evaluating toxicity are essential in order to evaluate risk.

The studies documented do not have a standardized format because the size, physical characteristics, and the properties of nanosilver used to evaluate toxicity vary from study to study. Because of these differences, there is no basis for comparison between the studies. Because there is no protocol for laboratory testing or regulations for standardized assays, it is difficult to compare toxicity studies.

No chronic studies evaluating toxicity from exposure to nanosilver are available. Engineered nanosilver in consumer and medical goods has been used for only a decade, so there is no reliable data on long-terms effects of nanosilver

exposure to humans. All the *in vivo* studies are short-term; the longest studies were done over 90 days, which is considered subchronic.

There is still no clear knowledge of what causes nanosilver toxicity. Possibilities do include size, the rate of silver ion release, and the production of reactive oxygen species. In addition, most studies speculate on how nanosilver enters the cells without having any clear evidence.

There is ample data on ionic and elemental silver concentrations in the environment but very little information is available on nanosilver concentrations in soil and water. There is no data on the form in which nanosilver is available for uptake in the environment. These data gaps reveal the absence of information for the accurate determination of toxicity and levels of nanosilver in the environment, a critical determination on which risk could be evaluated.

4.3 Expert Interviews

In order to obtain current information on the state of research and regulatory efforts for nanosilver, I conducted twelve interviews with experts from academia, industry, and government. Table 4.1 is a list of experts I interviewed. If the participant did not wish to disclose his or her name, I identified the participant by job title.

Table 4.1. List of expert interviewees and description of qualifications

Interviewee	Description
Academia	
Andrew Maynard, PhD	NSF International Chair of Environmental Health Sciences Director, University of Michigan Risk Science Center
A Lab Director, PhD[a]	Nano particles in Biotechnology
Simon Silver, PhD	Professor, University of Illinois at Chicago Research: Heavy Metal Resistance in Bacteria
Sheila Jasanoff, PhD	Pforzheimer Professor of Science and Technology Studies, Harvard Kennedy School, Harvard University
Sam Luoma, PhD	USGS: Advisor on Environment at University of California Davis
Government	
Michael Ellenbecker, PhD	Director of Toxics Use Reduction Institute, Massachusetts
Carol Rowan West, MSPH	Director, Office of Research and Standards. Massachusetts Department of Environmental Protection
A Researcher, PhD[a]	EPA
Sam Luoma, PhD	USGS: Advisor on Environment at University of California Davis
Industry	
Joanne Shatkin, PhD	CEO, CLF Ventures
Fatima Toor, PhD	Analyst, LUX Research Vice President, Risk Management, Pharmaceutical

[a]Participant requested to remain anonymous

4.5 Results from expert interviews:

In this section, I present the responses to the interview questions. Ten experts were interviewed. Summaries from the individual interviews will be made available upon request.

Question 1: How concerned are you about the prolific use of nanosilver in consumer and medical products? Can you rate it on a scale of 1-10?

Seven experts expressed significant concern regarding the prolific use of nanosilver. Two experts were concerned but believed that the benefits were substantial as well. When asked to rate it on a scale of 1 to 10, with one being not concerned and 10 being extremely concerned, their answers ranged from 5 to 10.

Question 2: Exposures from which products do you consider being the most hazardous? Why?

The majority of the interviewees believe that products like cleaning sprays and aerosol disinfectants had the highest potential to release silver ions into the environment and cause the most damage. Ingestion from products containing colloidal silver also could potentially provide exposure to nanosilver. Clothing embedded with nanosilver was also a concern for some of the experts because it has the potential to release silver ions into the body and the environment.

Question 3: Which exposure scenario concerns you the most, inhalation, ingestion, dermal, through food chain, aquatic release or chronic?

The interviewees expressed concern regarding aquatic release of all forms of silver. They also emphasized that not enough data are available regarding toxicity from other exposure scenarios for nanosilver. When asked for their personal point of view regarding exposure based on their knowledge and experiences, the answers were divided. Because there was a clear evidence of toxicity to lungs due to inhalation exposure from products like nanosilver sprays, the experts expressed the most concern over exposure through inhalation. They were then most concerned about dermal contact due to fact that there are so many nanosilver-embedded fabric products on the market. Finally, they were concerned about toxicity through ingestion via the food chain.

Question 4: What material property of nanosilver concerns you the most: size, catalytic action or silver ion release?

Most experts were concerned about silver ion release but also felt that there was no clear evidence regarding how nanosilver gets into cells or the rate of silver ion release. Some experts believed that there is a lot of speculation on the mechanism that releases silver ions within a cell; however, no studies have established proof of it. Some experts also thought that the size of the particle is a major concern for nanosilver toxicity.

Question 5: What concerns you more, nanosilver toxicity or microbial resistance?

All experts agreed that there were no documented toxic effects of nanosilver use on humans besides argyria following colloidal silver ingestion. All experts were concerned about developing bacterial resistance from the use of products containing nanosilver over a long period of time.

Question 6: If manufacturers were required to list the quantity and characteristics of the nanosilver used, will it help evaluate their environmental pathways? Should they be required to do it?

The experts were equally divided on this question. Some experts believed that requiring manufacturers to list quantities and characteristics of the nanosilver used it their products would not be helpful for evaluating environmental pathways because nanosilver has a shelf life, after which it aggregates or loses its properties. It is impossible to accurately predict what properties will remain or which will change. The other experts believed that requiring the manufacturers to list the quantity of nanosilver and its properties would help in the future for risk-based decision-making purposes. Having accurate data on how much nanosilver is being used and what might happen to it in the ambient environment would help promote awareness among the general public as well as the expert community. The data could be used in developing a beta model for fate and transport of nanosilver in the ambient environment.

Question7: Do you think the presence of nanosilver in wastewater influent will affect biological treatment processes?

Experts were confident that wastewater plants were capable of removing 80 to 95% of the silver from the influent. Some of the experts were concerned that nanosilver might affect biological treatment by hampering de-nitrification. There was concern of nanosilver ending up in the sludge and that it may move through the food chain.

Question 8: What key information or matrix do you think is needed for the safe use of nanosilver?

Experts need more data on dose-response, for example, an evidence-based matrix of biological responses to different sizes, shapes, and forms of silver, information on how nanosilver enters cells, and information on rate of release of silver ions from nanosilver. This information would be useful in evaluating toxicity. In addition, the experts indicated they needed data on the quantity of silver used in manufacturing products containing nanosilver.

Question 9: What approach should the EPA take for nanosilver? Should it wait and see till it has more quantifiable results or should it invoke the precautionary principle to regulate it?

Some experts agreed nanosilver has a lot of beneficial properties, including silver bandages currently in use for burn treatment. Most experts felt that it was premature to invoke the precautionary principle. Several experts

suggested that it is perfectly reasonable to be cautious with nanosilver use. The experts wanted to increase public awareness regarding the widespread use of nanosilver in consumer and medical goods.

4.6 Summary

It is clear from the literature review and the expert interviews that accurate data on nanosilver form and characteristics as well as robust toxicity studies are critical for evaluating the risk from nanosilver use in consumer and medical products. The data available at present indicate prolific nanosilver use is a cause for concern to human health and the environment and a cautious approach to its use as well as public awareness are necessary until more information becomes available.

5.0 DISCUSSION

In this chapter, I discuss the relevance of my findings from the literature and from the expert interviews for evaluating potential risks associated with the widespread use of nanosilver. This chapter is organized based on topics that were discussed with the experts.

5.1 Is nanosilver different from silver?

The Silver Nanotechnology Working Group, which represents manufacturers of nanosilver devices, claims that because silver metal has been in use since ancient times, there is no reason for concern about nanosilver. Metallic silver has been used for centuries in utensils, jewelry, and other personal items; it also has a long history of use in wound care; and prior to the introduction of antibiotics in the mid-twentieth century, silver nitrate was one of the primary antibiotics in use. However, because it is a rare metal, it is expensive and consequently was used sparingly in limited applications. With the advent of nanotechnology, metallic silver is being engineered at nanoscale and its use has increased not only in consumer goods but in electronics, solar energy, medicine, and biotechnology as well.

The information gathered from the literature and the interviews conducted for this thesis indicate that there is a consensus that nanosilver differs from metallic and ionic silver in its physical, chemical, and possibly toxicological properties. Some of the properties of metallic and ionic silver, such as its antimicrobial effects, are greatly enhanced in nanosilver, likely due to increased

surface area and increased ability to cross cell membranes. Currently, nanosilver is widely used in containers that store produce, in food packaging, and in numerous consumer goods and medical products. Nanosilver is not naturally occurring but is engineered from metallic silver so that it is available in shapes and size ranges that further enhance its physical, chemical, and antimicrobial properties. Based on my review of the literature and my interviews with experts, I have concluded that nanosilver should not be included as "silver" in environmental regulations and standards because its unique properties result in behaviors that are not identical to those of metallic or ionic silver. The Chemical Abstract Service Registry Numbers are the same for all forms of a substance; however, in the case of all nanomaterial including nanosilver, this should be revaluated and the nano form should be assigned a different number from that of its metallic form.

5.2 Human Exposure

Human exposure is a concern because nanosilver is widely used in a variety of consumer and medical goods. Widespread use of nanosilver in consumer goods brings it into contact with human receptors through inhalation, dermal contact, and, in some cases, ingestion. Typically the receptor is not consciously coming into contact with nanosilver, but rather most of this exposure is incidental and occurs when people use cosmetics, other personal care items, and medical supplies that contain nanosilver products. Cosmetic companies use nanosilver in powders, lipsticks, and eye shadow (ewg.org ; The Project on

Emerging Nanotechnologies 2013). However such use is not subject to labeling requirements. Nanosilver is also present in burn dressings, clothing including undergarments and socks, and in sunscreen preparations. Use of these products increases the possibility that people will be exposed to nanosilver from multiple sources. The increasing use of nanosilver as a food preservative also raises the potential that people can be exposed through the food chain.

5.2.1 Inhalation Exposure

When the expert respondents were asked about the exposure scenario that concerned them the most, they all emphasized that although there were limited data;, inhalation exposure was potentially the most important for human receptors. The published acute inhalation studies available in the literature do not report toxicity to the lungs from inhalation after a single exposure. However, results from the subchronic studies indicate lung toxicity, including inflammatory response after exposure to nano-sized particles for periods up to 90 days. It is relevant that nanosilver produced inflammation at much lower doses when compared to inhalation exposure to silver in larger particle sizes (Sung et al. 2008). Nanosilver is present in products such as disinfectant sprays like NANOVER (Nanogist of Korea) as well as in cosmetics that may become aerosolized upon use by the consumer. Nanosilver sprays are used as disinfectants and sprayed on walls, floors, and upholstery in houses, hospitals, and public places to render them free of microbes. Children are put at risk of inhalation exposure because particles settle close to ground during spraying. Nanosilver

sprays used in hospitals also present exposure risks for babies and elderly patients.

Teenage girls and pregnant women are also at risk of inhalation exposure over the long periods because of the use of nanosilver in makeup and other cosmetics. Occupational exposures occur for individuals working in the cosmetic industry, such as makeup counters in department stores and beauty salons. These workers rarely wear respiratory protection and use brushes to apply cosmetics to customers. The use of application brushes, produce airborne particles in the breathing zone of the worker and the customer. Workers in occupational settings during manufacturing of sprays and powders for use in cosmetics and fabrics also are at risk of exposure via inhalation (Friends of the Earth May, 2006).

The acquisition of more data and the conducting of in-depth studies on this topic should be made a priority. It would be useful to conduct long-term epidemiological studies for inhalation exposure using data from occupational settings. However, it was apparent from the literature review and expert interviews conducted, there are no clear indications that such initiatives are yet in place.

5.2.2 Ingestion Exposure

The literature indicates that the liver is the target organ following ingestion of nanosilver in all acute, subchronic, and repeated dose toxicity tests. One of the interviewed experts was concerned about ingestion exposure to nanosilver because of the use of colloidal silver as a natural remedy for a variety

of ailments ranging from infections and inflammation to AIDS and cancer. Ingestion exposure data for colloidal silver used for medicinal purposes have been used to evaluate chronic toxicity from oral exposure to nanosilver. However, the relevance of this to nanosilver exposure is questionable because colloidal silver has nanosilver particles suspended in a liquid along with ultrafine and micro-sized silver particles (Wijnhoven et al. 2009). The mixture may not be representative of the distinctive properties of nanosilver when ingested by humans, although the presence of nano-sized silver particles in these colloidal silver preparations, which are available at health food stores and through the Internet, increases the potential for human exposure.

Ingestion exposure occurs not just from these colloidal solutions containing nanosilver, popular in alternative medicine, but also from cosmetics like lipsticks. People ingest small amounts of applied lipstick through various behaviors, including the licking of lips, eating, and drinking. Ingestion of nanosilver can be a concern in babies because all babies put objects in their mouths, especially while they are teething. Parents may be less concerned about this behavior if they believe that toys and other items are “germ free” because they have been embedded with nanosilver. Baby blankets, which babies often suck on, also contain embedded nanosilver. Nanosilver also is used in baby bottles; babies can be exposed as the particles get dislodged while heating milk in a microwave oven (Taylor 2008). Ingestion exposure also can occur from water filters containing nanosilver, from ingestion of food sprayed with nanosilver to preserve it, and from food storage containers. Nanosilver-impregnated water

filters are used widely in developing nations as an effective and inexpensive potable water treatment. Incidental ingestion of nanosilver can occur if particles become dislodged from paint, upholstery, and clothing, and people, particularly children, become exposed through hand-to-mouth behavior.

Ingestion of nanosilver is likely to be frequent and chronic in people with a variety of exposure patterns in the home and in the workplace as well as in department stores and beauty salons. There is limited information on human exposure, and there are no chronic studies in animal models to allow for the evaluation of the overall exposure and risk. It appears that most ingestion exposure is incidental, resulting from the use of products that contain nanosilver. Therefore, it is important that more studies be conducted to assess how nanosilver is released from these products and what form it takes in both the environment and in human systems. If particles aggregate, there may be lower risk, but if particles remain in their ionic form, there is concern that they may be highly bioavailable.

5.2.3 Dermal Exposure

Dermal exposure to nanosilver is an important concern because of the widespread embedding of nanosilver embedded into fabrics, in wound dressings, and in medical equipment. It is sprayed on walls, toilets, handrails in buses and trains, and in other public places to render them free of microbes. Some of the interviewed experts expressed concerns about dermal exposure. However, there are very few laboratory or epidemiological studies evaluating the toxicity or other

effects following dermal exposure to nanosilver, and the available studies are all based on acute exposure to animals. The only human evaluation of dermal exposure was done by Trop et al. on a seventeen year old being treated for burns over 30 percent of his body. Atticoat dressing, which is embedded with nanosilver, in this case was used for treatment of his burns. Trop et al. found elevated silver levels in plasma and urine and also elevated liver enzymes; the levels returned to normal a week after the treatment was stopped (2006).

The Danish Environmental Protection Agency conducted a study assessing the environmental and human health impact of nanosilver-embedded fabrics and concluded that dermal exposure from nanosilver-embedded textiles did not pose a risk to humans (Tonning 2012). It is worth noting that this analysis was conducted considering exposure from one piece of clothing in children and from soles in shoes for adults; for babies the data from soft toys was used. Dermal exposure also occurs as a result of contact with upholstery, paints, and cosmetics used by teenage girls and women. In order for an accurate assessment of risk, exposure from different products as it occurs on a regular basis needs to be evaluated.

5.2.4 What Causes Nanosilver Toxicity?

It is still not clear what is responsible for nanosilver toxicity. It is possible that the toxicity is caused by the nanosilver particles themselves when they penetrate the cell membrane. It is also possible that the toxicity is caused by the release of free ions once the nanosilver gets into the cells. Most of the experts consulted believe that the silver ions released by nanosilver are responsible for the

toxicity. Two experts thought it was the nanosilver particles themselves that contributed to the toxicity. However, there are several studies in the available literature that indicate silver ion release, along with the ROS (free radicals), is responsible for the toxicity. The human kinetics of nanosilver in the body is still speculative. Until this dynamic is better established, it would be difficult to define dose-dependent toxicity. There is limited literature available on the toxicity of nanosilver after exposure from chronic inhalation, dermal contact, and ingestion. Hence, the accuracy of reference doses with the existing data is simply not adequate for quantifying risk.

Considering all the available information it appears that nanosilver is toxic to the target organs in animals. How these toxicity studies apply to humans is not yet clear because of a lack of chronic animal and human toxicity data. Also, obtaining data on exposure to nanosilver (detecting nanosilver particles in target organs) often is tedious and expensive. In addition, it is impossible to accurately predict exposures with the information available. One possible approach may be to consider conducting a toxicity analysis based on the use of individual products. For example, in nanosilver sprays, inhalation exposure occurs during spraying, but oral exposure from accidental ingestion and dermal exposure from contact with a treated wall or upholstery could be considered. If the pharmaco-kinetics of nanosilver in the cell were better understood, one could then be able to accurately quantify dose dependent toxicity in mammals.

5.3 Nanosilver in the Environment

Silver in any form including the nano forms is potentially toxic to a large number of aquatic organisms. As seen in the literature review, nanosilver can causes abnormalities and death in zebrafish embryos (Lee et al. 2007). Nanosilver induces a dose-dependent toxicity in embryos of zebrafish and hinders normal development (Asharani et al. 2008). It is toxic to the respiratory system of the zebrafish (Bilberg et al. 2012) as well as to the liver (Choi et al. 2010). Therefore, it is important to evaluate the environmental pathways of nanosilver from consumer and medical products to investigate what quantity and form of nanosilver reaches surface waters.

Results of The Danish Study on textiles indicated no increase of nanosilver concentrations in wastewater influent from washing of fabrics embedded with nanosilver (Tonning 2012). In a study commissioned by the Centre for Ecology and Hydrology in the United Kingdom, researchers conducted an exposure assessment for engineered nanosilver in the rivers of England and Wales. Specifically, the researchers conducted a measurement and modeling exercise in nine wastewater treatment plants across England and Wales and concluded that the concentrations of nanosilver in British rivers were low (mean was 6 ng/l) and based on the results from the model predicted that the levels were expected to remain below levels that might be of potential harm to wildlife (Johnson et al. 2011). However because the instruments currently available cannot accurately represent the concentration of the whole sample, this study represents extrapolated and not exact data.

These studies validate the claim by Blazer et al. (2008) and Nowack et al. (2010) that wastewater treatment plants are capable of removing nanosilver during treatment. However, it appears that the nanosilver that is removed from the wastewater ends up in the sludge, which in turn often is deposited on soil as a fertilizer. The nanosilver-containing run-off from such an application could eventually be transported to surface and ground water. This would in turn affect aquatic and possibly marine species. Just because nanosilver has not been detected in wastewater effluent, this does not necessarily mean it is sequestered in a way that will not harm aquatic animals and biota. Therefore, it is important not only to model the effluent from the wastewater plants but also the entire porous environmental media in order to accurately model environmental pathways to evaluate environmental pathways and transport.

Based on the literature I reviewed, I agree with the expert consensus that there is a need to be concerned about how nanosilver may affect the environment. Nanosilver can eventually end up in soil, surface water, and groundwater. This would not only affect aquatic organisms but also plants other biomass (Lowry et al. 2012). A major obstacle in accurately evaluating these pathways is found in the time-consuming, difficult, and expensive monitoring that is required to answer such questions. It also is complex due to the physical and chemical dynamics that exist in working with materials at the nano scale. Significant technological progress needs to be made to make accurate instruments in the effort to evaluate environmental impacts from the use of nanosilver. Random monitoring of a few

pathways is not only inaccurate, but it gives a false sense of safety and sets the scientific community back in terms of funding and research

Microbial Resistance

One of the reasons that nanosilver use is so prolific is because of its antimicrobial properties. Silver's antimicrobial properties, which are more enhanced at the nanoscale, are well known. People in the world today seem to have become so "germ-o-phobic" that they use antimicrobials in all too many everyday applications. Introducing babies and children to antimicrobial agents early in life not only keeps them from building immunity but also indiscriminately reduces beneficial microorganisms on the skin and elsewhere in the body. There is a possibility that the expanding use of antimicrobial agents containing nanosilver could potentially give rise to Methicillin-Resistant Staphylococcus Aureus (MRSA) as found in the case of triclosan and antibiotic resistant disease-causing microbes (Brenwald and Fraise 2003). For these important reasons, I was not surprised that the experts interviewed were more worried about microbial resistance than toxicity

5.4 Precautionary Principle

Some regulatory policy makers believe that there is no need for the precautionary principle in regulatory practice. In their view, the precautionary principle is based on making decisions without scientific justification and, therefore, is not scientifically sound. They also believe that the precautionary

principle will stifle innovation because it requires proof of safety before introducing new technology (Kriebel et al. 2001).

Other policy makers who seem to think that the precautionary principle seeks to minimize the limitations of regulatory policies based entirely on risk assessment and promotes the policies that protect human health and environment (Kriebel et al. 2001). It can be argued that scientific uncertainty is even more pronounced in the consideration of nanotechnology applications, including nanosilver. There still is no clear understanding of what types of risks need to be addressed, what testing methodologies are required or should be developed to assess risk, and what exposure pathways should be evaluated during the lifecycle of nanosilver-containing products (Falkner and Jaspers 2012).

In nanotechnology industries and its applications, traditional risk assessment has limitations in describing and dealing with uncertainty. Risk assessment relies heavily on dose response and upon scientific inquiry based on hypothesis generation and testing. This type of risk assessment has high specificity and low sensitivity. False positives are penalized more heavily than false negatives (Martuzzi 2007). There is also the persistent worry caused by errors of biases, exposure misclassification, and measurement. All these factors often move risk estimation towards the null (Martuzzi 2007), therefore, indicating no relationship between cause and effect. This has been true in the areas of climate change as well as the health effects of smoking, polychlorinated biphenyls

(PCBs), asbestos, Bisphenol-A (BPA), and Triclosan[3] Although the EPA and FDA are now reviewing the health risks of Triclosan, waiting for quantitative evidence-based data to formulate environmental policies has taken years. It can be argued that society has paid a price in terms of harm visited upon the environment, ecosystem, and human health. It is possible that we could be making the same mistake with nanosilver.

5.5 Existing Approaches for Nanosilver Oversight Across the World

Many national governments are currently commissioning studies on nanosilver. For example, the European Union asked SCENIHR to provide scientific information on nanosilver. In June 2012, the Danish Ministry of the Environment published a paper on the project "Survey, Sampling, Analysis and Assessment of Nanosilver in Textiles on the Danish Market." By 2014, the Netherlands is expected to conclude a complete evaluation of nanosilver. The EPA already has completed two reports on nanosilver and has established a Registration Review Docket for nanosilver. Government agencies in the United States, Canada, the European Nation, Australia, and New Zealand appear to be stalling and avoiding having to make a decision on nanosilver, as no regulatory measures have yet to be taken (Hansen and Baun 2012b).

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[3] [3]Triclosan is a material that commonly has been used in soaps and toothpastes for the last forty years but is now believed by the EPA and FDA to cause antibiotic resistance. Brenwald, N. P. and Fraise, A. P. 2003. Triclosan resistance in methicillin-resistant staphylococcus aureus (mrsa). *Journal of Hospital Infection* 55, no. 2: 141-44.

However the European Union has taken several precautionary measures, such as amending its cosmetics legislation in 2009 and requiring manufacturers of existing cosmetics to inform the Commission of the types and estimated amounts of nanomaterial used in their products and to include nanomaterial in the list of product ingredients. The Bundesinstitut für Risikobewertung (BfR) in Germany has requested that producers not use nanosilver in food and consumer products, and the United Kingdom has launched a voluntary reporting scheme for manufacturers of products containing nanoparticles, including nanosilver.

Despite the creation of the NNI, the United States has taken a piecemeal and decentralized approach towards nanomaterial oversight, and there is a growing concern whether the current framework provides adequate authority and instruments for oversight (Falkner and Jaspers 2012). Nanosilver, when used as an antimicrobial, comes under the EPA's FIFRA, which requires manufacturers to prove the safety of products containing nanosilver. According to one of the experts interviewed for this thesis, "FIFRA could be a relatively strong instrument to regulate nanosilver use as long as it is used an antimicrobial." Several experts also believed that the EPA could have more flexibility with TSCA but that the SNUR was specific and would involve a scientific approach to defining nanomaterial.

Nanosilver is used in medical equipment, implants, wound dressings, and drug delivery, products that fall under the jurisdiction of the FDA. Currently, the FDA will regulate nanotechnology products under existing statutory authorities, in accordance with the specific legal standards applicable to each type of product

under its jurisdiction. The agency is taking a prudent scientific approach to assess each product on its own merits, and does not make broad, general assumptions about the safety of nanotechnology products (2013).

If FDA truly takes the actions it claims, the agency, along with EPA's FIFRA, could well be the best option for providing oversight to regulate the use of nanosilver in the United States. The FDA would be a good mechanism for government regulation of nanosilver because the burden to prove products safe lies with the manufacturers rather than the government agency. In this scenario, manufacturers would be required to do all the product safety testing before the product was introduced in the market, thus the FDA would not need to spend its resources in order to establish harm.

Canada always has taken a safety first approach for all chemical usage, including BPA, and it is the first country to consider making the voluntary reporting scheme mandatory (Falkner and Jaspers 2012). This would be an important first step in evaluating pathways for nanosilver. Also, Canada, Australia, and New Zealand have initiated a voluntary information requirement on the use of nanomaterial. However, it does not appear to have provided many tangible results.

TPEN's inventory list created by the Woodrow Wilson International School for Scholars and The Pew Charitable Trust includes all the nano products available on the market. This inventory could be a powerful tool for regulating nanosilver. However, it should be noted that only those products that list nanosilver in their contents are included in the inventory. The inventory also

includes a large number of products from China and India, yet very little information on the regulatory approaches of these emerging economies is available.

5.5 Conclusion

It is reasonable to expect that there will be a strong demand for use of nanosilver as a preservative in food, for food storage, and for rendering drinking water safe not only in India and China but in other rapidly developing economies as well. Nanosilver also is increasingly being used as an antimicrobial to prevent the spread of germs in crowded public places. If more people continue to produce and market products containing nanosilver, the environmental impact could be disastrous solely because of the sheer number of people being exposed to these materials in these economies. In addition to people knowingly using these products, there also is a potentially larger number of people who will be unknowingly exposed to nanosilver in public places, including train stations, shopping malls, and public restrooms. There is also the fear of developing large-scale microbial resistance with the increasing use of nanosilver in sprays, fabrics, and other products. Such prolific use of nanosilver could lead to development of super bugs and diseases that cannot be cured by currently available antibiotics. It is possible that the use of nanosilver to ensure the integrity of food and water could come at a significant environmental cost.

Despite the lack of currently available quantitative data on the potential toxicity of nanosilver, considering these concerns coupled with the knowledge that the health and environmental investigative sciences have been unable to keep up with the innovations of nano manufacturing and that there is a lack of available funding for research in this area, nanosilver should be used sparingly and carefully so that we continue to benefit from its useful properties without implications on human health, ecosystem, and the environment. In addition, there is a need to initiate oversight and regulations for the use of nanosilver across the globe. Specifically, the precautionary principle should be considered in regulating the use of nanosilver.

6.0: Conclusion and Recommendations

The purpose of this thesis was to gather relevant information in order (1) to make a determination about whether or not there is a need to use the precautionary principle with regard to the use and regulation of nanosilver and (2) to make recommendations for oversight and regulation as appropriate based on this determination.

I reviewed the existing literature to gather information about the physical and chemical properties of nanosilver as well as to evaluate sources and pathways of transport in the environment and also to characterize the current toxicity of this agent. This thesis incorporated the use of semi-structured interviews to explore with current experts if they perceived risks to human health and the environment based on nanosilver properties and toxicity. I used all this information to determine the potential risk from the use of nanosilver.

I also took note in the peer-reviewed and grey literature of the state of regulatory practice both in the United States and internationally. I identified the specific implementation in these efforts. I conducted the semi-structured interviews to gain expert opinion regarding current regulatory practices.

Based on the review of the literature and responses of the expert interviews, I have reached conclusions regarding the safe current and future use of nanosilver in consumer and other products. Specifically, I present my conclusions and recommendations with regard to research needs, and oversight and future regulations.

6.1 Conclusions

Even though there is no clear and present danger from the widespread use of nanosilver, it can potentially have environmental implications if its use continues to increase. As has been chronicled in this thesis it is difficult to estimate the risks associated with nanosilver in the environment at the present time because of insufficient data regarding the quantity, form of nanosilver used, and the physical and chemical properties of nanosilver released into the environment from various products. In order to be able to evaluate sources, pathways, and receptors, there is a need for additional resources and new technologies to detect nanosilver in environmental media.

Even though there are limited quantifiable data on the sources and pathways of nanosilver in the environment, the available studies in the literature indicate that nanosilver will eventually find its way into soil and water and have an environmental and ecological impact if its use continues to increase at the projected rate.

Studies show that nanosilver is toxic to some aquatic organisms. Nanosilver appears to be persistent and bioaccumulative in the environment. It can negatively impact the biological treatment of wastewater by affecting nitrification. According to some studies, nanosilver can be taken up by plants and enter the food chain.

The toxicity studies performed on a variety of animals lead to the conclusion that there could be a potential risk from the inhalation of nanosilver in occupational settings. It is not possible at present to judge the risk of consumer

exposure from the available data. However, the use of nanosilver in a large variety of consumer products that can become aerosolized, such as cosmetics and disinfectant sprays, leads to the suggestion that inhalation exposure is a possibility in non-occupational settings.

It is not established yet whether the cause of toxicity through ingestion exposure from colloidal silver is because of the silver ions, the size of the nanosilver, or the ROS, and therefore it is difficult to estimate dose response parameters such as reference doses and reference concentrations.

Dermal exposure in patients treated with wound dressings containing nanosilver has been shown to cause elevated liver enzyme serums. Because the toxicokinetics of nanosilver after dermal exposure are not yet understood, it is difficult to establish dose response.

Currently, there is no standardized format for evaluating toxicity of nanosilver. There also is no standardized protocol for laboratory testing or established standard assays and, therefore, toxicity studies are generally not comparable and have not been replicated. Also, there currently are no long-term data on exposure available. If the past experiences with BPA and PBDEs are consulted we should not assume that nanosilver is safe just because there is no quantifiable data for risk assessment.

One major concern for human health is the possible development of antimicrobial resistance due to widespread use of nanosilver. The overuse of antimicrobials such as nanosilver can not only kill the beneficial bacteria required for human health but also preclude the building of immunity in children. This

condition could give rise to allergies. There also is the fear that prolific use of antimicrobials like nanosilver in hospitals could potentiate “superbugs” such as MRSA and other antibiotic-resistant microbes.

With regard to regulation of nanosilver, countries around the world understand the need to distinguish the differences in properties of nanosilver from its metallic form and to establish regulations for its use. However, due to the lack of data regarding source, pathways, exposure, and toxicokinetics have made this impossible to accomplish. Currently, Europe is developing promising initiatives by requiring registration of nanomaterial under REACH; however, it is extremely important to establish some oversight and guidance for nanosilver use, especially in developing populous economies like India and China where the need for providing clean water and food and rendering public places germ free could result in an explosive use of nanosilver, which could come at a large cost to the environment and future health of the people living in those countries.

6.2 Recommendations

Based on the conclusions stated above regarding the safe current and future use of nanosilver in consumer and other products, I present my recommendations with regard to research needs, oversight and future regulations.

6.2.1 Research Needs

All the analyses available point to the need for more data and more research, yet in the United States, of the $1.6 billion awarded to the NNI by the

Federal government, less than 10 % is available for environmental health and safety research on all nanomaterials, including nanosilver. More funds need to be directed toward a variety of research purposes. I discuss these purposes in this section.

Develop new fate and transport models.

Developing models that will predict the movement of nanosilver through the environment will help in determining how it changes its shape and form when released from nanosilver-embedded products, how it is affected by the environmental medium into which it is released, and where it will eventually end up. This will be helpful in evaluating its toxicity because the toxicity of nanosilver will depend on whether it aggregates, disperses as stable nanosilver particles, or continues to release silver ions.

Develop new exposure models

Human exposure to nanosilver can occur during manufacturing, during use of consumer and medical products, when it is dislodged from the product in the environment, and during disposal. As seen in textiles, exposure can be dermal but also via ingestion for children, who have a habit of putting textile material such as blankets in their mouths. The same textile material can result in environmental exposure when the nanosilver-embedded material is washed and the nanosilver ends up in water or sediment. Nanosilver sprayed on produce to prevent mildew can cause exposure by inhalation but also through ingestion during consumption

of the food. There can also be exposure to aquatic organisms when the produce and containers are washed and the nanosilver reached aquatic environments. Therefore, it is important to evaluate exposures based on individual products and their complete lifecycles.

Develop accurate monitoring equipment tailored for measuring nanosilver quantity and characteristics

It is important to develop new instruments with improved resolution and sensitivity that can measure not only nanosilver concentrations but also identify its characteristics such as size and shape at the same time. These instruments should be standardized and made more readily available. This will be helpful in validating the computer models based on manufacturing data and help in establishing accurate environmental fate and transport models. Nano Tracking Analysis is a new and promising technology introduced by Nanosight. It has the ability to simultaneously measure particle size and particle scattering intensity to allow heterogenous particle mixtures to be resolved and can directly estimate particle concentration and zeta potential. Instruments like these could make a large impact on data collection.

Develop standardized assays and laboratory protocols for exposure assessment

Progress has been made in the use of biological assays for toxicity studies. However, there is still no standardization of assays or laboratory protocols for

toxicity studies of nanosilver. Such measures are important when comparing various toxicity studies and in order to replicate the results.

Develop studies to understand the toxicokinetics of nanosilver

More robust studies are required to understand nanosilver's toxic mode of action, such as what happens when nanosilver enters the cell, what properties contribute to the release of silver ions, and what role is played by ROS produced in cells. Unless the cause of toxicity is understood, it will continue to be difficult to create an exposure model.

Create an exposure matrix based on nanosilver characteristics and exposure

Until the uncertainty regarding nanosilver's toxic mode of action is resolved, it would be helpful to develop an exposure matrix that looks at the size and characteristics of nanosilver, exposure pathways, and biological responses in organisms. This will help in the identification of patterns of biological responses to the different types of exposure (Abbott and Maynard 2010). The matrix could also be expanded for different life stages of humans and other organism.

Develop more chronic and subchronic studies

Even though consumer exposure to nanosilver is likely to be long-term and continuing through food and clothing, there are still no chronic toxicity studies available to evaluate nanosilver exposure. There is also a lack of subchronic studies to establish accurate reference doses and reference

concentrations. It would be helpful to use data from occupational settings to obtain inhalation exposure data and establish a human-based NOAEL based on real time data and end points.

Develop studies to understand the relationship between nanosilver and the development of microbial resistance

Researchers and experts in the field have expressed concern regarding the development of microbial resistance due to overuse of nanosilver in consumer and medical products. However, there are few studies that evaluate antimicrobial mechanisms available in the literature. There is a need for standardized methods to measure the antibacterial mechanism.

6.2.2 Oversight and Future Regulations

Current evidence indicates that it is too early to use the precautionary principle to regulate the use of nanosilver. In order to introduce the precautionary principle, some of the current environmental laws would need to be revaluated. In addition, enough data need to be generated in order for that revaluation to be thorough. However, as seen in the case of triclosan, development of microbial resistance is a concern, and the killing of all bacteria can have negative effects on the immune system, particularly in children. Governments across the globe can take preventative measures with regard to nanosilver use.

These preventative measures will help regulate some of the frivolous uses of nanosilver until there is technology to measure the concentrations in the

various mediums and robust data are available on the antimicrobial mechanism of nanosilver to use the precautionary principle. In the remainder of this section, I describe some of the steps that governments can take at the present time.

Require the manufacturers of nanosilver and products containing it to list the quantity and the form of nanosilver used on the product label

Requiring manufacturers to provide information on the estimated quantities and properties of nanosilver used in their products can be helpful in evaluating nanosilver's environmental pathway and routes of exposure. Nanosilver is released in the environment during manufacturing as well as from consumer and medical products at different times and in different forms. Data on quantities, forms, and physical and chemical properties of nanosilver used as well as the medium in which it is released can be helpful in modeling its behavior in different environments over time.

Labeling of products

Require manufacturers to inform consumers regarding the use of nanosilver, especially in the case of food and cosmetics. Informing consumers when nanosilver is used in products is important for consumer health and safety because it can help consumer self-regulate their use of nanomaterials that include nanosilver. Sunscreen lotion (Figure 6.1) sold at upscale super markets like Whole Foods have started labeling some of its products as "Non Nano." This labeling has helped get consumers' attention regarding nanoproducts.

Figure 6.1. Badger brand product labeled as non nano. Photo by Vinita Bose.

Require manufacture to test the safety of sprays

Exposure through inhalation on a chronic basis is of concern. Therefore, the safety of products such as sprays containing nanosilver should be tested for safety. This can be done using FIFRA.

Issue guidance regarding the use of nanosilver in food and storage

Governments should urge manufacturers not to use nanosilver as a preservative in food and food storage containers. Using nanosilver in food products not only kills the good bacteria necessary for human health but also precludes the building of immunity in children giving rise to allergies.

Register a discrete CASRN for nanosilver

Assigning nanosilver a different Chemical Abstract Substance Registry Number than elemental silver would help differentiate it from elemental silver. This distinction could be valuable in consumer product labels and medical goods. It also would be help generate more data on the quantities of nanosilver used.

6.2.3 Public Awareness and Education

It is important to spread awareness of nanosilver's potential impact on health and the risk of developing microbial and antibiotic resistance associated with its use. Despite the proliferation of the use of nanosilver in consumer products, public awareness is low or almost nonexistent. Currently, only nongovernmental organizations and private research organizations are involved in communicating the potential risks associated with nanosilver use. Many of these organizations are discussing risks without communicating the science, and some are creating public concern because of misinformation. Regulators and industry need to take a more active role in engaging the general public in dialogue about nanosilver and establishing transparency. One of the experts interviewed suggested introducing nanosilver or nanomaterial education in primary schools. That would be an effective way to educate and inform children. Because parents typically are more involved in their children's education during this early stage of their children's life, this would be an ideal time to educate adult parents as well.

6.3 Summary

Based on the results of this thesis, it is clear that nanosilver has a number of beneficial applications in food storage, purification of drinking water, medical diagnostics, and treatment modalities and does not present a clear danger at this time. However, studies indicate that nanosilver has the potential to be hazardous to the environment and aquatic organisms if its use continues to grow unregulated. Although the toxicity of nanosilver to human health is not of concern

at this time, there is a possibility of developing bacterial resistance due to overuse. Analysis of the available data does not necessitate the need to invoke the precautionary principle to regulate its use at the present time. However, it is important to use caution and to invoke oversight in introducing frivolous new products to the market such as nanosilver-treated fabrics, paints, toys, and toothbrushes. Such restraint would allow products that provide more tangible benefit to humans like wound dressings for diabetic ulcers and burns to be available without compromising the environment or posing a future health risk. In the meantime, it is important to continue to generate new and robust data on the quantities of nanosilver used in manufacturing, sources of nanosilver, and pathways and exposure to nanosilver so that the meaningful evaluation of the impact, risks and risk management options for nanosilver can be performed.

Appendix 1

Tufts University IRB Exemption

Tufts UNIVERSITY

OFFICE OF THE VICE PROVOST FOR RESEARCH

Social, Behavioral, and Educational Research
Institutional Review Board
FWA00002063

Title: A policy study of current approaches to regulate nanosilver at the global level and evaluate if the EPA should use the precauutionary princople to regulate it

January 28, 2013 | Notice of Action

IRB Study # 1301023 | Status: EXEMPT

PI: Vinita Bose
Faculty Advisor: Anne Marie Desmarais
Review Date: 1/28/2013

The above referenced study has been granted the status of Exempt Category 2 as defined in 45 CFR 46.101 (b). For details please visit the Office for Human Research Protections (OHRP) website at: http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html#46.101(b)

- The Exempt Status does not relieve the investigator of any responsibilities relating to the research participants. Research should be conducted in accordance with the ethical principles, (i) Respect for Persons, (ii) Beneficence, and (iii) Justice, as outlined in the Belmont Report.
- Any changes to the protocol or study materials that might affect the Exempt Status must be referred to the Office of the IRB for guidance. Depending on the changes, you may be required to apply for either expedited or full review.

IRB Administrative Representative Initials: 

20 Professors Row, Medford, MA 02155 | TEL: 617.627.3417 | FAX: 617.627.3673 | EMAIL: SBER@tufts.edu

Appendix 2

Informal Interview Questions

Informational Interview Questions: Toxicity

1. How concerned are you about the prolific use of nanosilver in consumer and medical products?
   - How would you rate it on a scale of 1-10?

2. Exposure from which products would be the most hazardous? Why
   - Which products concern you the least?

3. What do you think contributes most to nanosilver toxicity?
   - Is it size, catalytic action or silver ion release?

4. Which exposure scenario concerns you the most, -inhalation, ingestion, dermal, through food chain, aquatic release or chronic? Which is the second most? Why these?

5. Should manufacturers be required to test their products for health and environmental effects?

6. Do you think the prolific use of nanosilver in consumer and medical products could lead to bacterial resistance?

7. What concerns you the most nanosilver toxicity or bacterial resistance? Why?

8. What approach should the EPA take for nanosilver? Should it wait and see till it has more quantifiable results or should it invoke the precautionary principle to regulate it?

Informational Interview Questions: Environmental

1. How concerned are you about the prolific use of nanosilver in consumer and medical products?
   - How would you rate it on a scale of 1-10?

2. Exposure from which products could contribute the most hazard? Why
   - Which products concern you the least?

3. What do you think contributes most to nanosilver toxicity?
   - Is it size, catalytic action or silver ion release?

4. What is the leading edge technology available to quantify, characterize and separate nanosilver? Can it do all three?
   - Is it readily available?
   - How expensive is it
   - How much time does it to test each sample.

5. If manufacturers were required to list the quantity and characteristics of the nanosilver used, will it help evaluate their environmental pathways? Should they be required to do it?

6. Do you think the presence of nanosilver in wastewater effluent will affect its biological treatment? In what way?

7. What key information or matrix do you think is needed for the safe use of nanosilver?

8. What approach should the EPA take for nanosilver? Should it wait and see till it has more quantifiable results or should it invoke the precautionary principle to regulate it?

Informational Interview Questions: Regulatory

1. How concerned are you about the prolific use of nanosilver in consumer and medical products?
   - How would you rate your concern on a scale of 1-10

2. Exposure from which products would be the most hazardous? why
   - Which products concern you the least?

3. If manufacturers were required to list the quantity and characteristics of the nanosilver used, will it help evaluate their environmental pathways? Should they be required to do it?

4. Should manufacturers be required to test their products for health and environmental effects?

5. What key information or metrics due you think is needed for the safe use of nanosilver?

6. What approach should the EPA take for nanosilver? Should it wait and see till it has more quantifiable results or should it invoke the precautionary principle to regulate it?

7. Since nanosilver is also heavily used in medical products do you see the EPA and the FDA working together moving forward? How or why not?

8. Nanosilver has antibacterial properties, which makes it attractive for food storage and water purification. How do you view the risk and benefits of the use of nanosilver in the resource challenged countries of the world considering the environmental and health impacts associated with its use? How should India and China handle the prolific use of nanosilver in consumer products and in manufacturing?

Appendix 3

Reviewed Toxicity Studies Related to Nanosilver Exposure

Table. Reviewed Toxicity Studies Related to Nanosilver Exposure

Author	Publication	Title	Conclusion	No Effect	Effect
Acute oral toxicity					
Cha et al.	Biotechnology Letters, 2008	Comparison of acute responses of mice livers to short-term exposure to nano-sized or micro-sized silver particles	Gene expression changes in the nanoparticle-treated livers lead to phenotypical changes, reflecting increased apoptosis and inflammation.		x
Pan et al.	Food and Chemical Toxicology, 2012	Toxicological effects of cationic nanobubbles on the liver - kidneys: Biomarkers for predicting the risk	Immunohistochemistry detected liver fibrosis and inflammation with nanobubbles treatment		
Gaiser et al.	Environmental Toxicology and Chemistry, 2012	Interspecies comparisons on the uptake and toxicity of silver and cerium dioxide nanoparticles	Cellular uptake of all materials tested was shown in for Ag,into the intestine, liver, gallbladder and gills of Carp exposed via water		x
Gaiser et al.	Toxicological Sciences, 2013	Effects of silver nanoparticles on the liver and hepatocytes in vitro	Evidence of the potential toxicity and inflammogenic potential of Ag NPs in the liver following ingestion		x
Liu et al.	Ecotoxicology and Environmental Safety	Exposure to silver nanoparticles does not affect cognitive outcome or hippocampal neurogenesis in adult mice	Exposure to Ag-NPs does not affect spatial cognition or hippocampal neurogenesis in mice	x	
Acute inhalation toxicity					
Foldbjerg et al.	Archives of Toxicology, 2011	Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549	AgNPs and Ag+ can induce oxidative stress correlating with cyto and genotoxicity		x

Author	Publication	Title	Conclusion	No Effect	Effect
Acute inhalation toxicity					
Sung et al.	Toxicology & Industrial Health, 2011	Acute inhalation toxicity of silver nanoparticles	No significant body weight changes or clinical changes were found during the 2-week observation period	x	
Takenaka et al.	Environmental Health Perspectives, 2001	Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats	Silver was cleared from the lungs	x	
Acute dermal toxicity					
Korani et al.	International Journal of Nanomedicine, 2011	Acute and subchronic dermal toxicity of nanosilver in guinea pig	Colloidal nanosilver has the potential to provide target organ toxicities in a dose-and time-dependent manner		x
Koohi et al.	Journal of Physics: Conference Series, 2011	Assessment of dermal exposure and histopathologic changes of different sized nano- sized nano-silver in healthy adult rabbits	Research concluded that dermal exposure to lesser sizes of silver nanoparticles is more disastrous than greater ones		
Naghsh et al.	Journal of Mazandaran U of Med Sci, 2013	Effects of silver nanoparticles contact with skin in HGB and MCH changes in male rats in in vivo condition	In 50ppm concentration of nanosilver, possible mechanisms of the change MCH are releasing free radicals, oxidative stress and apoptosis in red blood cells		x
Repeated dose- oral					
Loeschner et al.		Distribution of silver in rats after 28 days of repeated oral exposure to nanosilver or silver acetate	Sulfur and selenium containing silver granules of similar size and shape were found in lysosomes of macrophages		

Author	Publication	Title	Conclusion	No Effect	Effect
Repeated inhalation					
Ji et al.	Inhalation toxicology, 2007	Twenty-eight-day inhalation toxicity study of silver nanoparticles in Sprague-Dawley rats	Exposure to silver nanoparticles at a concentration of 100 microg/m did not appear to have any significant health effects	x	
Sung et al.	Inhalation toxicology, 2008	Lung function changes in Sprague-Dawley rats after prolonged inhalation exposure inhalation exposure to silver nanoparticles	Nanosized particle inhalation exposure can induce lung function changes, along with inflammation, at much lower mass dose concentrations when compared to submicrometer particles		x
Subchronic oral					
Gagne et al.	Chemosphere, 2012	Toxicity of silver nanoparticles to rainbow trout: A toxicogenomic approach	Exposure to nano-Ag involved genes in inflammation and dissolved Ag involved involved oxidative stress and protein stability		x
Kim et al.	Particle and Fibre Toxicology, 2010	Subchronic oral toxicity of silver nanoparticles	The target organ for the silver nanoparticles was found to be the liver in both the male and female rats (NOAEL = 30 mg/kg; LOAEL = 125 mg/kg)		
Park et al.	Environ toxicology and pharm, 2010	Repeated-dose toxicity and inflammatory responses in mice by oral administration of silver nanoparticles	Repeated oral administration of nano-sized AgNPs may cause organ toxicity and inflammatory responses in mice		x
Subchronic inhalation					
Sung et al.	Toxicological Sciences, 2009	Subchronic Inhalation Toxicity of Silver Nanoparticles in Sprague Dawley rats	Histopathological examinations indicated dose-dependent increases in lesions related to silver nanoparticle exposure, including mixed inflammatory cell infiltrate, chronic alveolar inflammation, and small granulomatous lesions (NOAEL = 100 μg/m3)		x

Author	Publication	Title	Conclusion	No Effect	Effect
Subchronic inhalation					
Sung et al.	Inhal Toxicol, 2008	Lung function changes in Sprague-Dawley rats after prolonged inhalation exposure to silver nanoparticles	Nanosized particle inhalation exposure can induce lung function changes, along with inflammation, at much lower mass dose concentrations when compared to submicrometer particles		
Song et al.	Nanotoxicology	Recovery from silver-nanoparticle-exposure-induced lung inflammation and lung function changes in Sprague Dawley rats	Persistence of lung function changes and inflammation induced by silver nanoparticle		x
Mutacenicity and Genotoxicity					
Ghosh et al.	Mutation Research, 2012	In vitro and in vivo genotoxicity of silver nanoparticles	In vitro and in vivo studies demonstrate that Ag-np is genotoxic to plant and animal system, capable of producing ROS and inducing apoptosis and necrosis; observed significant impairment in nuclear DNA and cell function		x
Braydich-Stolle, Laura et al.	Toxicological Sciences, 2010	Silver Nanoparticles Disrupt GDNF/Fyn kinase Signaling in Spermatogonial Stem Cells	Decline in Proliferation		x
Park et al.	Biomaterials 2011	The effect of particle size on the cytotoxicity, inflammation, developmental toxicity and genotoxicity of silver nanoparticles	Silver nanoparticles induced effects in all endpoints studied, but effects on cellular metabolic activity and membrane damage were most pronounced		

Author	Publication	Title	Conclusion	No Effect	Effect
Mutacenicity and Genotoxicity					
Patllolla et al.	International Journal of Environmental Research and Public Health, 2012	Genotoxicity of Silver Nanoparticles in Vicia faba: A Pilot Study on the Environmental Monitoring of Nanoparticles	Results of this study demonstrate that AgNPs are genotoxic to plant cells.		x
Wasowicz et al.	International Journal of Occupational Medicine & Environmental Health, 2011	Evaluation of biological effects of nanomaterials. Part I. Cyto- and genotoxicity of nanosilver composites applied in textile technologies	Silver-coated nanocomposites (both TiO-Ag and Res-Ag) may cause genotoxic effects in murine macrophages J774A.1		x
Nymark et al.	Toxicology, 2012	Genotoxicity of polyvinylpyrrolidone-coated silver nanoparticles in BEAS 2B cells	No induction of chromosomes MN or CAs was observed at any of the doses or time point	x	
Powers et al.	Environmental health perspectives, 2010	Silver impairs neurodevelopment: studies in PC12 cells	Silver has the potential to evoke developmental neurotoxicity		x
Song et al.	Journal of Clinical Biochemistry and Nutrition, 2012	Metal nanoparticle-induced micronuclei and oxidative DNA damage in mice	The metal nanoparticles caused genotoxicity, and oxidative stress		
Chronic					
Choi et al.	Toxicological Sciences, 2011	Physicochemical Characterization and In Vitro Hemolysis Evaluation of Silver Nanoparticles	Higher level of in vitro hemolysis observed with nanoparticles compared with micron-sized particles		x
Powers et al.	Neurotoxicology & Teratology 2011	Silver nanoparticles alter zebrafish development and larval behavior; distinct roles for particle size, coating and composition	Different AgNP formulations are likely to produce distinct patterns of developmental neurotoxicity		x

Author	Publication	Title	Conclusion	No Effect	Effect
Chronic					
Hayashi et al.	Environmental science & technology, 2012	Earthworms and humans in vitro: characterizing evolutionarily conserved stress and immune responses to silver nanoparticles	findings provide mechanistic clues on cellular innate immunity toward AgNPs that is likely to be evolutionarily conserved across the animal kingdom		x

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