Release of Silver from Nanotechnology Consumer Products and Potential for Human Exposure

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ABSTRACT

Silver nanoparticles (nanosilver) are gaining significant attention from the academic and regulatory communities, not only because of their antimicrobial effects and subsequent product applications, but also because of their potential health and environmental impacts. Although some human health effects of silver nanoparticles have been reported, realistic exposure levels from the use of consumer products are still largely unknown. The objective of this work was to characterize the release of silver and silver-containing particles during the normal use of silver nanotechnology consumer products. Specific objectives were to review the environmental and human health risks of airborne, engineered nanoparticles, to characterize aerosol emissions from nanosilver spray products, and to characterize nanosilver that may be released from children's consumer products under conditions of normal use. We identified possible routes of aerosolization of nanosilver from the production, use, and disposal of consumer products and estimated that about 14% of silver nanotechnology products that have been inventoried could potentially release silver particles into the air during use. The spray products investigated emitted 0.24 – 56 ng of silver in aerosols per spray action, and the plurality of aerosols were 1 – 2.5 μm in diameter, easily inhaled, for two products. Both the products' liquid characteristics and the bottles' spraying mechanisms played roles in determining the aerosol size distributions, but the size of silver-containing aerosols was largely independent of the liquid phase size distributions. We compiled an inventory of 82 children's consumer products that claim to contain nanosilver, of which 13 products were examined for presence of silver and tested for release of silver into liquid media and air, and onto skin. All products contained some form of silver, but silver-containing particles were observed in only four products, with sizes ranging from nanoscale up to 10 μm in size. Silver leached preferably into synthetic biological media with higher chloride concentrations, such as sweat and urine. We determined that levels of silver to which children would be exposed during normal use of these products are likely to be low, and bioavailable silver is expected to be in ionic rather than particulate form.

DEDICATION

To my parents, who valued their children's education and safety above all else.

And to my grandmother, Almira, who taught me a great deal about ethics without even realizing it.

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I'm grateful for my family and friends, far and near. Without their unconditional support, I would not have achieved this goal. Thanks to my Blacksburg friends and the Brazilian Association for company and support.

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Chapter 1: Introduction

Silver nanoparticles (nanosilver) are gaining significant attention from the academic and regulatory communities, not only because of their antimicrobial effects and subsequent product applications, but also because of their potential health and environmental impacts. Numerous in vitro studies have shown that silver nanoparticles are toxic to certain organisms, such as phytoplankton, bacteria, and fish[1-5] and also to human cells.[3,6-8] In vivo studies have also been performed with silver nanoparticles, and these were proven toxic to rats and to translocate between organs[9-11]. Luoma[2] reports that silver can be absorbed by the lungs, skin, gastrointestinal, and urogenital tracts, but it is not thought to be toxic to the nervous, cardiovascular, or reproductive systems in humans. So, general knowledge on the potential health impacts of silver nanotechnology is still under dispute.

There is a large gap in knowledge regarding human exposure to nanosilver. The use of silver nanoparticles as an anti-microbial and anti-odor agent in consumer products is growing, and thus the potential for human exposure to nanosilver must be evaluated.[12-14] The mechanisms, forms, and amount of silver released from consumer products containing silver nanoparticles are not completely understood and at present, cannot be predicted on the basis of product type and physical and chemical characteristics.[2,15] Therefore, new data are needed to answer fundamental questions about the physico-chemical properties and dose-metrics of silver that may be released during use of products containing silver nanoparticles.

The current state of knowledge on the toxicity of nanosilver points toward a potential threat via the inhalation exposure route. Soto et al.[8] suggested that some nanomaterials (notably nanosilver) that are considered non-toxic, and even medicinal, for ingestion or in contact with the skin may pose a threat if inhaled. Various authors have affirmed the potential for inhalation exposure to a wide variety of nanoparticles.[16-18] An array of properties, such as size, density, crystal structure, surface charge, and composition may influence their toxicity,[16,18-20] and these properties have not yet been characterized thoroughly for airborne nanoparticles. Many fundamental uncertainties remain about the specific physical and chemical properties of airborne particles that cause known health risks.[10,21]

Children may be especially affected by the normal use of consumer products designed specifically for them, such as milk bottles, pacifiers, and textiles. Guney and Zagury[22] call for the implementation of toy regulations in the US and Canada based on risk assessment and realistic exposure scenarios. Contaminant exposure is different and probably higher for children than adults for multiple reasons:[23]

- Children have a higher surface area to body mass ratio than adults.
- Children have a higher metabolism than adults; exposure is increased since they breathe more air, drink more fluids, and eat more food per body mass than adults.
- Exposure is also increased because children place hands and objects into their mouths more frequently than adults.
- Children's organs and tissues are still under development (e.g., bones and the nervous system).
- Children have more years ahead of them to develop health conditions from chronic exposure to contaminants.

Policymakers must be equipped with information to assess the potential risks associated with consumer exposure to silver nanotechnology consumer products before they can establish regulatory restrictions on these products. In 2005, the Consumer Product Safety Commission (CPSC) issued a statement declaring the exposure to nanotechnology consumer products as a priority preceding the acquisition of toxicological data and that "[the] introduction of consumer products containing nanomaterials into the marketplace may require unique exposure and risk assessment strategies." [24] An expert workshop on developing an environmental assessment research strategy for nanoscale silver was held in 2011 with participants from academia, industry, government, and other sectors. Workshop outcomes called for understanding silver exposure in susceptible groups, such as women of childbearing age and children, as well as determining appropriate metrics for characterizing exposure.[25,26] Nanosilver is currently regulated under EPA's Toxic Substances Control Act (TSCA)[27] as a general nanomaterial, and the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) as a nanomaterial-containing pesticide.[13] However, nanosilver has not yet been categorized as a "new chemical substance" under TSCA. Such categorization would require practical regulatory restrictions, such as reporting requirements and threshold levels.[13]

After reviewing current literature on the environmental implications of nanotechnology, we identified two concerns of high priority related to silver nanotechnology: (1) the potential exposure to airborne nanoparticles released from silver nanotechnology consumer products and (2) the potential exposure specifically of children to silver nanoparticles in consumer products via multiple routes.

Based on the motivation described above, the main objective of this work was to characterize the release of silver and silver-containing particles during the normal use of silver nanotechnology consumer products. Specific research objectives were to:

1. Review the environmental and human health risks of airborne silver nanoparticles.

2. Characterize the emissions of airborne particles produced during the use of spray products that claim to contain silver nanoparticles or ions and identify properties of the products that control emissions.

3. Quantify and characterize nanosilver that may be released/leached from children's consumer products under conditions of normal use.

ORGANIZATION OF THE DISSERTATION

Chapter 2, *Environmental and Human Health Risks of Aerosolized Silver Nanoparticles*, is a literature review that addresses Research Objective 1. The review summarizes the present state of knowledge concerning airborne nanosilver in order to shed light on the possible environmental exposure scenarios that may accompany the production and popularization of silver nanotechnology consumer products. We present possible routes of aerosolization of nanosilver from the production, use, and disposal of existing consumer products and estimate that about 14% of silver nanotechnology products that have been inventoried could potentially release silver particles into the air during use, whether through spraying, dry powder dispersion, or other methods.

Chapter 3, *Silver Nanoparticles and Total Aerosols Emitted by Nanotechnology-Related Consumer Spray Products*, addresses Research Objective 2. In this chapter we characterize the emissions of airborne particles from three consumer spray products that claim to contain silver nanoparticles or ions, determine the relationship between emissions and the products' liquid

characteristics, and assess the potential for inhalation exposure to silver during product use. Results demonstrate that the normal use of silver-containing spray products carries the potential for inhalation exposure to silver-containing aerosols.

Chapter 4, *Exposure Assessment of Silver Nanoparticles in Select Children's Consumer Products*, addresses Research Objective 3 and describes the development of a framework for evaluating nanosilver in children's consumer products through both quantitative analytical methods and qualitative electron microscopy techniques. This chapter presents a comprehensive inventory of 82 consumer products that claim to contain nanosilver and that may be used by or near children. Thirteen of the products were examined for the presence of silver. All products had at least one component containing silver, but only four products contained particulate forms of silver, which ranged in size from nanoscale up to 10 μm.

Chapter 5, *Release of Silver from Children's Nanotechnology Consumer Products*, also addresses Research Objective 3. In this chapter, we assess the release and potential bioavailability of silver from the products described in Chapter 4 under conditions relevant to real-world use (e.g., Figure 1.1). We determined the amount of silver leached into various liquid media, including tap water, orange juice, milk formula, and synthetic formulations of sweat, urine, and saliva. We also investigated how liquid composition could control leaching of silver. We determined that levels of silver to which children would be exposed during normal use of these products are likely to be low, and bioavailable silver is expected to be in ionic rather than particulate form.

Chapter 6 presents conclusions of this dissertation. Major outcomes of this work are discussed, such as contribution of this research to knowledge about the environmental and health implications of nanotechnology as well as a collection of recommendations for future work.

Figure 1.1. Plush toy's leg and a piece of the baby blanket exposed to weathering.

ATTRIBUTIONS

Dr. Linsey Marr (Department of Civil and Environmental Engineering, Virginia Tech) is the research advisor and committee chair. Dr. Marr provided general guidance in the development of experimental design, interpretation of data, and writing of Chapters 2, 3, 4, and 5. She also reviewed other parts of this dissertation. Additionally, Dr. Marr provided much of the financial support for the work presented in this dissertation through research grants (NSF Cooperative Agreement EF-0830093, Center for the Environmental Implications of NanoTechnology (CEINT) and EPA contract number RFQ-RT-10-00249).

Dr. Nicole Tulve, Dr. Robert Willis, and Dr. Kim Rogers (U.S. Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory) provided general guidance during the inception of the research (RFQ-RT-10-00249) described in Chapters 4 and 5 and the discussion of its results. Dr. Tulve is currently using the information presented in Chapter 4, in addition to other data gathered by EPA to prepare a manuscript to be submitted to a peer-reviewed journal.

Dr. Mitsuhiro Murayama (Department of Materials Science and Engineering, Virginia Tech) assisted in the collection of all images from the FEI Titan high-resolution transmission electron microscope which appear in Chapters 3 and 4.

Dr. Jeffrey Parks (Department of Civil and Environmental Engineering, Virginia Tech) performed all inductively-coupled plasma / mass spectrometry (ICP-MS) analyses presented in Chapters 3, 4, and 5.

COMPLEMENTARY WORK

In addition to the manuscripts included in this dissertation, two full research reports (for EPA contract RFQ-RT-10-00249) and six conference presentations complementary to this work were presented on the topics of exposure to silver from consumer products:

- Quadros, M. E.; Marr, L. C. Exposure assessment of silver nanoparticles in select children's consumer products (RFQ-RT-10-00249), Final project report.; Virginia Tech: Blacksburg, VA, 2011.

- Tulve, N. S.; Quadros, M. E.; Marr, L. C. A preliminary assessment of the potential for exposure to silver nanoparticles in children's consumer products (EPA and CPSC interagency agreement: EPA- IA-RW-61-92317001-0), Final project report; US EPA: Research Triangle Park, NC, 2011.

- Quadros, M. E.; Marr, L. C., Emissions from silver nanotechnology consumer products (oral). In *30th Annual Conference of the American Association for Aerosol Research (AAAR)*, Orlando, FL, 2011.

- Quadros, M. E.; Marr, L. C., Aerosol emissions from silver nanotechnology consumer products (oral). In *3rd International Conference on the Environmental Implications of Nanotechnology (ICEIN)*, Durham, NC, 2011.

- Quadros, M. E.; Marr, L. C., Emission of airborne nanoparticles from the use of nanotechnology consumer products (poster). In *Virginia Tech GSA Research Symposium and ICTAS Research Day*, Blacksburg, 2010.

- Quadros, M. E.; Marr, L. C., Characterization of airborne nanoparticles emitted by nanotechnology consumer products (oral). In *II International Conference on the Environmental Implications of Nanotechnology (ICEIN)*, UCLA, 2010.

- Quadros, M. E.; Marr, L. C., Exposure assessment of aerosols emitted by nanotechnology consumer products (poster*). In *29th Annual Conference of the American Association for Aerosol Research (AAAR)*, Portland, OR, 2010. *Winner, Student Poster Competition.

- Quadros, M. E.; Farina, C. T.; Marr, L. C., A thermophoretic sampler for collecting airborne nanoparticles (poster). In *1st International Conference on the Environmental Implications of Nanotechnology (ICEIN)*: Washington, DC, 2009.

During the author's Ph.D. studies, she also participated in the Cal-Mex 2010 field campaign, which aimed to assess emissions, transport, and transformation of air pollutants at the US-Mexico border. At least three papers are expected to result from this effort:

- Shores, C. A.; Klapmeyer, M. E.; Quadros, M. E.; Marr, L. C., Sources and transport of black carbon at the California-Mexico border. *Atmospheric Environment*, **Accepted**; 10.1016/j.atmosenv.2012.04.031.

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Chapter 2: Environmental and Human Health Risks of Aerosolized Silver Nanoparticles

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ABSTRACT

Silver nanoparticles (nanosilver) are gaining attention from the academic and regulatory communities, not only because of their antimicrobial effects and subsequent product applications, but also because of their potential health and environmental risks. While nanosilver in the aqueous phase are under intensive study, those in the atmosphere have been largely overlooked, even though it is well established that inhalation of nanoparticles is associated with adverse health effects. This review summarizes the present state of knowledge concerning airborne nanosilver in order to shed light on the possible environmental exposure scenarios that may accompany the production and popularization of silver nanotechnology consumer products. The current understanding of the toxicity of nanosilver points toward a potential threat via the inhalation exposure route. Nanoparticle size, chemical composition, crystal structure, surface area, and the rate of silver ion release are expected to be important variables in determining toxicity. We present possible routes of aerosolization of nanosilver from the production, use, and disposal of existing consumer products. We estimate that about 14% of silver nanotechnology products that have been inventoried could potentially release silver particles into the air during use, whether through spraying, dry powder dispersion, or other methods. In laboratory and industrial settings, six methods of aerosolization have been used to produce airborne nanosilver: spray atomization, liquid-flame spray, thermal evaporation-condensation, chemical vaporization, dry powder dispersion, and manual handling. Fundamental uncertainties remain

about the fate of nanosilver in the environment, their short- and long-term health effects, and the specific physical and chemical properties of airborne particles that are responsible for health effects. Thus, to better understand the risks associated with silver nanotechnology, it is vital to understand the conditions under which nanosilver could become airborne.

IMPLICATIONS STATEMENT

The increasing popularization of silver nanotechnology will surely lead to the release of silver nanoparticles into the air. Humans and the environment will be exposed to the particles, and thus the public and policymakers must be equipped with information to assess the potential risk associated with such exposures. This paper will help guide policymakers toward defining airborne silver nanoparticle safety guidelines for industrial, residential, and outdoor environments and researchers toward identifying the most pressing questions for future study.

INTRODUCTION

Airborne nanoscale particles pose a threat to human health due to their abilities to deposit in all regions of the respiratory tract, be taken up by cells, and translocate to sensitive organs via the blood or lymph.[1] Particles with at least one dimension smaller than 100 nm are typically described as “ultrafine” when occurring naturally or incidentally, such as secondary aerosol that condenses from gases or soot that forms during combustion, and as “nanoparticles” when purposefully engineered. Because of their antibacterial properties, silver nanoparticles (nanosilver) have become one of the most popular types of nanomaterials today. In terms of the number of consumer products and the volume of annual research investment, only carbonaceous nanomaterials exceed silver.[2] Like all nanomaterials, nanosilver may present an inhalation toxicity hazard should they become airborne,[3-4] a threat that has received inadequate attention and that is the focus of this review.

An important feature of nanoparticles is that, on a mass basis, more atoms are available at the particle’s surface to interact with its surroundings. At this scale, unique physicochemical characteristics appear, and reactivity is largely increased in comparison to the nanoparticles’ bulk counterparts.[4-7] With silver, antiseptic efficacy increases as particle size decreases due to the higher surface area per unit volume and subsequently enhanced surface reactivity.[4, 8-10] As shown in Figure 2.1, a 4-nm particle has 50% of its atoms on the surface, whereas a 30-nm particle has only 5% of its atoms on the surface.[11-12] This order-of-magnitude difference

exemplifies why surface forces are of critical importance in nanoparticles. These novel properties present opportunities for introducing and improving many products.

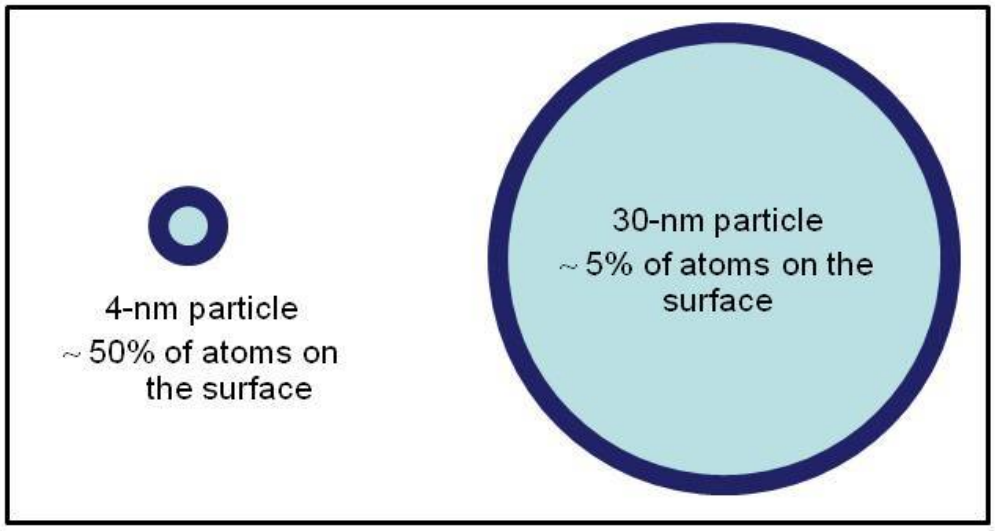

Figure 2.1. Smaller particles have a larger fraction of their atoms on the surface. The lines around the 4- and 30-nm particles represent atoms at the surface and have the same thickness.

Bulk silver has been used in close contact to humans historically, in cutlery, jewelry, and currency. Ancient civilizations knew about silver's antimicrobial potential,[13] and colloidal silver has been used for centuries to heal wounds and preserve materials with no obvious toxic effects to humans. Silver compounds were heavily used as antiseptics in World War I, before the development of modern-day antibiotics.[14] Soluble silver compounds, such as silver salts, have been used for treating mental illness, epilepsy, nicotine addiction, gastroenteritis, and infectious diseases.[13, 15]

Despite the widespread and seemingly safe use of bulk silver, elemental silver is classified as a persistent and toxic pollutant to humans and the environment.[4, 16] This dichotomy is the main source of controversy driving studies on the environmental implications of silver nanoparticles. The toxicity of silver is much lower to human cells than to bacteria,[17] and the mechanism of action may be more closely associated with silver ions (Ag^+), which can be released from bulk silver or more efficiently from nanosilver. However, the specific antiseptic mechanisms and toxicity of nanosilver to humans and the environment still have not been deduced.[3-4, 8, 18-19] Soto et al.[20] suggested that some nanomaterials, notably nanosilver, that is considered non-toxic, and even medicinal, for ingestion or in contact with the skin, may pose a threat if inhaled.

According to the Project on Emerging Nanotechnologies' inventory of nanotechnology-related risk studies,[2] the lungs are the most researched area of the body, comprising about 70% of research projects. Recent reviews of nanosilver applications and toxicity[1, 7, 13-14, 21-25] address the specific effects associated with laboratory-generated inhalation exposures but not the potential for such exposures to occur in real-world situations. Despite the emphasis on the respiratory system in studies of nanosilver toxicity, most research on the fate and transport of engineered nanoparticles has focused on aqueous, rather than gaseous (i.e., atmospheric) systems.[4]

There exists a gap in the body of knowledge between inhalation toxicology studies involving nanosilver and the mass consumption of silver nanotechnology, namely in exposure characterization for airborne nanosilver. The purpose of this study is to shed light on the possible exposure scenarios for airborne nanosilver that may accompany the production and popularization of silver nanotechnology-related consumer products. Specifically, this paper reviews the literature with three objectives: (1) to describe the possible routes of aerosolization of nanosilver from the production, use, and disposal of existing consumer products, (2) to catalog methods of nanosilver aerosolization and characterization for the purpose of guiding future experiments, and (3) to gather published information on the potential toxicity of airborne nanosilver to people and the environment. At every turn, we identify gaps in knowledge where further study is needed.

Silver nanotechnology-related papers have grown from less than a dozen per year in the early 1990s to over 1,500 in 2008 (Figure 2.2). Studies involving airborne nanosilver, rather than aqueous-phase ones, occupy a small niche within publications on silver nanomaterials; they comprise less than 10% of the total. The number of papers published per year on airborne nanosilver has increased from none in the early 1990s to more than 100 in 2008.

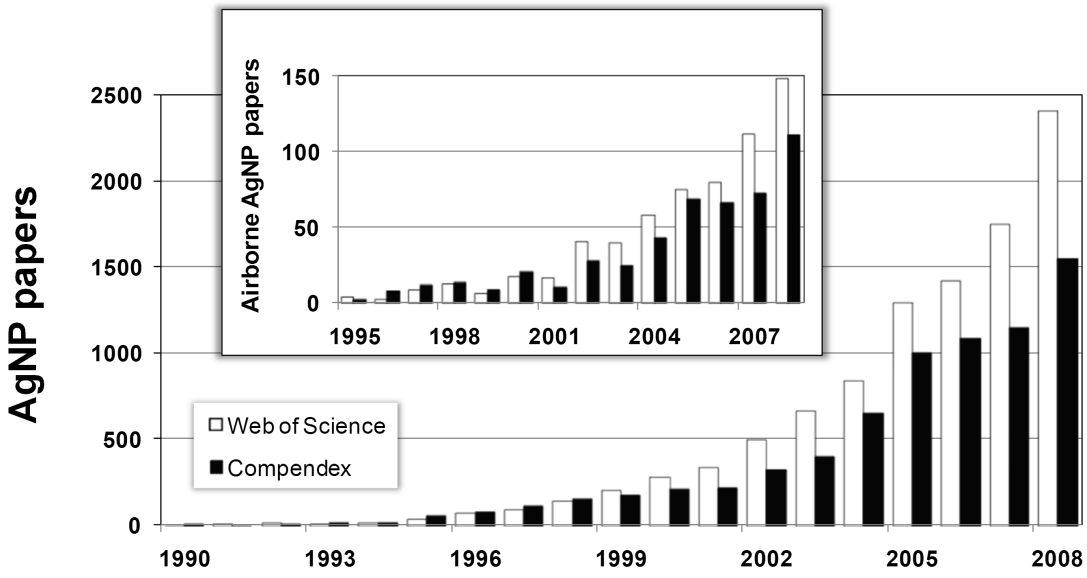

Figure 2.2. Number of papers published on nanosilver in general and specifically in air (inset) in Compendex and Web of Science, from 1990 to 2008.

PROPERTIES AND APPLICATIONS OF SILVER NANOPARTICLES

nanosilver and silver-based compounds are known for their high thermal stability, low solubility, and low volatility.[8, 23] Elemental silver has the highest thermal and electrical conductivity of all metals.[13] There are several oxidation states for silver, namely Ag^0, Ag^+, Ag^{2+}, Ag^{3+}, although the latter two are less common.[15, 26] Ag^+ is a very reactive cation and rapidly binds with available negatively-charged ligands to reach a stable state.[4-5]

Since most naturally occurring colloids have negatively charged surfaces,[27] any silver aerosol originating from natural waters is likely to be charged or to be associated with anions, typically fluoride (F^-), chloride (Cl^-), sulfate (SO_4^{2-}), hydroxide (OH^-), or carbonate (CO_3^{2-}). Organic matter may also act as a ligand. In fresh water, silver is more likely to be associated with sulfide, sulfate, and bicarbonate. Silver may also form a sulfhydrate (AgSH or HS-Ag-S-Ag-HS).[26] Wijnhoven et al.[15] questioned whether nanosilver can be clearly discerned from bulk silver, since there are water-soluble silver compounds, such as silver salts (namely silver nitrate), that may release silver ions just as effectively as nanosilver and cause similar toxicological effects. There are also colloidal dispersions with broad size distributions, in which case not necessarily all silver is nanoscale.

An atmospheric chemist, Grassian,[11] has described nanoparticle properties in terms of seven variables: size, shape, concentration, core composition, surface composition, aggregation, and nanostructure. In the atmosphere, the persistence of particles is mainly dependent on size.[28-29] Those larger than 10 μm are removed by gravitational settling within minutes, while those smaller than 1 μm (1000 nm) may remain suspended for days. Airborne silver is most likely to be in the elemental form, if nanoparticle powders are dispersed, or as Ag^+ in an inorganic salt. Additionally, some nanosilver is coated with polymers and other compounds for stabilization of the particles in water and/or enhanced functionality. Once released, nanosilver should not be considered inert. McMahon et al.[5] demonstrated that nanosilver in contact with ambient air tarnish rapidly through chemisorption of sulfur to particles. The physical and chemical properties of airborne nanosilver are essentially unknown, and characterization is needed to assess the inhalation risk associated with use of nanosilver consumer products.

nanosilver is increasingly being used in emerging products. Silver nanotechnology appears in coatings and is impregnated in materials, such as paints, soaps and laundry detergents, refrigerators, laundry machines, cooking utensils, medical instruments (dressings, catheters, pacemakers) and drug delivery devices, water purifiers, clothing, antibacterial sprays, personal care products (toothpaste, shampoo, cosmetics), electronics, air filters, and humidifiers.[3-4, 8, 17, 22-23, 30-32] Ji et al.[10] have developed an airborne nanosilver generator for disinfection of indoor air. Considering these uses, high concentrations of airborne nanosilver could potentially be found indoors (in industrial and household environments) and outdoors (in the vicinity of smelters, nanotechnology industries, incinerators, wastewater treatment plants, etc).[4, 8, 33]

SILVER NANOPARTICLE AEROSOLIZATION

Production, use, and disposal of consumer products

As consumer products utilizing silver nanotechnology become increasingly popular,[2] environmental releases of nanosilver are expected to escalate. There are multiple potential aerosolization scenarios for silver nanoparticles that can be divided into the same phases that comprise the life cycle of any consumer product: production, use, and disposal (Figure 2.3). Studies are needed to characterize nanosilver emissions across the entire life cycle of nanosilver products; results will facilitate exposure assessments to airborne nanosilver.

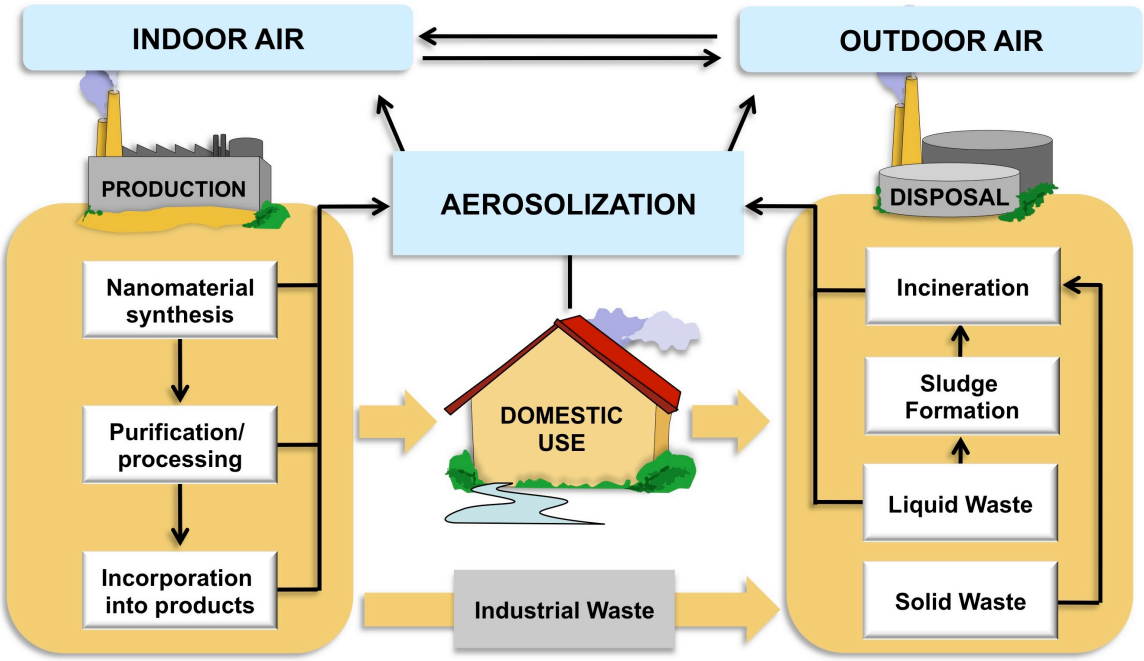

Figure 2.3. Possible aerosolization routes for nanosilver during the life cycle of consumer products. For simplification, the diagram shows only routes that can lead to aerosolization.

Nanoparticle Production

To understand how nanosilver could be aerosolized as a result of manufacturing processes, it is imperative to know how these materials are handled through the industrial line. Specific techniques used to produce nanosilver or to incorporate them into consumer products, as well as the applied air pollution control equipment, are difficult to ascertain, since industrial methods are usually considered privileged information. Most nanosilver is produced using bottom-up methods, in which nanoparticles are synthesized from smaller units, mainly by promoting nucleation from liquid, vapor, or solid precursors.[6]

Aerosolization of nanoscale particles has been detected during production of carbonaceous nanoparticles,[34-36] so the potential for aerosolization may also exist for nanosilver. Some methods used for nanosilver synthesis, especially through bottom-up approaches, are carried out in aqueous media,[26] but even then there may be an opportunity for aerosolization if the particles are dried to form a powder. In addition, some of the aerosolization methods discussed in the following section of this paper, namely spark discharge, could be scaled up to produce large amounts of high-purity nanosilver in powdered form.[37] Fugitive and accidental releases are a potential source of nanosilver emissions to the atmosphere. Production waste could also lead

to airborne particles, through direct emission of aerosol streams, incineration of solid waste, or aerosolization of liquid suspensions. Nanosilver may also become airborne as a byproduct of other industrial methods. Even industries that are not related to nanotechnology, such as photography before the digital age, might be responsible for emitting nanosilver.[15]

Production of nanosilver can result in two different exposure scenarios: occupational exposure of workers to the particles generated inside the industrial environment and ambient exposure of the public to nanosilver emitted to the atmosphere by industries. In occupational exposure, the main issue is designing capture, ventilation, and personal protective equipment that is effective for nanoparticles. In environmental exposures, the main concern is the use of particulate control equipment with high collection efficiencies for nanoparticles and minimization of the volume of nanosilver aerosolized in the first place.

Nanoproduct Use

The domestic use of consumer products that contain nanosilver is a potential source of silver-containing aerosols in the household. Research is needed to determine whether the extended use of these products would pose a long-term threat to consumers. But, first, these products must be identified and characterized.

The Project on Emerging Nanotechnologies (PEN) has compiled an inventory of nanotechnology-based consumer products that were on the market as of 2009, and more than 800 different items are listed, from mouth sprays to computer keyboards.[38] The same organization has also published a database on more than 200 silver nanotechnology products that were commercially available in 2007;[39] many more are likely to be available now. Out of the 240 silver nanotechnology products listed from 65 manufacturers at the time of writing, 214 are in fact commercial products, and the remaining 26 are precursor products (e.g., master batches, colloids, and powders). nanosilver or other nanostructures have been used in these products to promote antimicrobial protection.

These products, among others that we have identified in the marketplace through the internet and, in some instances, e-mail contact with sellers and manufacturers to obtain clarification on how nanoparticles are used in the products, are listed in Table 2.1. Half of the products fall into the categories of fabric applications (29%) and cosmetics or medical dressings (21%). In addition to listing the form of nanosilver in the products, we have also rated each product

category on its potential to produce airborne silver-containing particles. We estimate that about 14% of the products could potentially release silver particles into air during use, whether through spraying (e.g., liquid cleaning products or personal care sprays), dry powder dispersion (e.g., vacuum cleaners and hair dryers), or other unknown methods. Other products containing embedded or coated nanosilver may also act as sources through erosion and suspension of the material, but we expect these to be much less important for inhalation exposures compared to products that intentionally release particles.

Table 2.1. Summary of consumer products claiming to employ silver nanotechnology.[21, 38-39]

product	form of nanosilver	potential for aerosolization
disinfectant sprays, deodorants, oral sprays	liquid	high
hair dryers	solid coating	high
air filters	embedded in solid	medium
vacuum cleaners	solid coating	medium
humidifier	colloid	high
fabrics (shirts, pants, hats, socks)	incorporated into fibers	medium
medical instruments, milk bottles, teether, toothbrush	embedded in solid	none
hair straightening or curling irons	solid coating	none
cosmetics / dressings	powder or cream	low
hardware (computer, mobile phone, handles, etc)	embedded in solid	none
mineral supplements	liquid	none
food containers, cooking utensils	embedded in solid	none
refrigerators, washing machines, pet products, algaecide, laundry soaps	solid coating	low

A major challenge in this work is understanding and classifying exactly how nanoparticles might be released or emitted from these consumer products, since most manufacturers are reluctant to describe explicitly how nanoparticles, coatings, or other nanostructures are incorporated into products. Some marketing material simply includes the word “nano,” or the expression “silver nano,” alongside the product’s name or description, without a more detailed explanation other than a claim that silver nanotechnology lends anti-bacterial or anti-odor properties.

Nanomaterial Disposal

A review by Bystrzejewska-Piotrowska et al.[21] urged that policymakers define waste management practices for nanotechnology-related consumer products before disposal of the first nanoproducts begins. As shown in Figure 2.3, the disposal step applies to both industrial and consumer waste. The two major opportunities for aerosolization of nanosilver during disposal are incineration and treatment of liquid waste. At this point in the lifecycle, nanosilver has been mixed with other components of the industrial or municipal waste stream. In liquid waste, some silver may remain in a pure form, but some will be present as dissolved ions and complexed with ligands. Hence, aerosolized particles may consist of pure nanosilver or inorganic silver salts such as silver chloride and silver iodide, mixed with other components of the waste.

Consumer products and industrial waste that contain nanosilver may become a source of airborne nanosilver through municipal or industrial waste incineration. Incineration may lead to the vaporization of metals, which subsequently condense and form airborne nanoparticles upon cooling. One study reproduced incineration processes for simulated ash containing metals and detected a high concentration of nanoparticles under 10 nm.[40] Particulate emissions from incinerators are typically estimated using data from the US Environmental Protection Agency's (EPA) AP 42 compilation of emission factors, but the document does not yet address nanoparticles, let alone nanosilver.

nanosilver could become dispersed into the atmosphere in the vicinity of wastewater treatment plants, when aeration takes place promoting droplet suspension. Numerous papers describe the dispersion of bioaerosols around aeration tanks.[41-44] If these microorganisms are subject to aerosolization and transport in the atmosphere, it is likely that nanoparticles could be as well.

Laboratory-scale aerosolization

Nanosilver has been aerosolized in the laboratory using various methods, typically as part of inhalation toxicology studies. Understanding the differences between techniques is important for those wishing to produce the most environmentally relevant types of particles. Of course, characteristics of nanosilver aerosolized during the product life cycle have yet to be determined, so these two efforts—aerosolization for laboratory studies and environmental characterization—are intimately linked. Most of the reviewed studies aimed to provide a nanoparticle-rich aerosol

flow for *in vivo* or *in vitro* toxicity studies or to produce high-purity nanosilver in the gas phase at industrial scales.[45] A smaller portion of studies used a known silver aerosol for other research purposes, such as testing a new filtration technique[46] or measuring diffusional losses to tubing.[47]

Table 2.2 catalogs the six aerosolization methods that appear in the literature. Atomization, also referred to as nebulization or spray, has been used for inhalation toxicity and therapeutic studies.[47-52] This method relies on the forces of an air jet or high voltage (electrospray) to produce a spray from a liquid solution or suspension. The aerosol can subsequently be directed through a diffusion dryer containing desiccant to remove excess humidity and reduce the liquid content of the particles. In these studies, spherical nanosilver particles of aerodynamic diameters as low as 5 nm were synthesized and characterized.

Table 2.2. Aerosolization methods used to synthesize nanosilver.

aerosolization method	references
spray atomization of nanosilver suspension, colloid or silver salt solutions	47, 49-52, 117
liquid flame spray, flash pyrolysis, or corona discharge of super- or ultrasonically atomized silver colloid spray	53-56
evaporation-condensation (ceramic or other furnace heaters, or using heated silver wires)	3, 10, 31-32, 46, 57-63, 78, 118-119
chemical vaporization using arc plasma, or spark discharge generators (SDG)	37, 45, 64-65, 120
dry powder dispersion (e.g., brush dust generator)	50
manual pouring or handling of particles	66

Most size distributions were narrow, with geometric standard deviations ranging between 1.2 and 1.88. There are three different types of nebulizers: jet, ultrasonic, and piezo-electric crystal; and the degree of aggregation in the particles produced depends on the nebulization method and particle hydrophobicity.[48] Ultrasonic nebulizers with hydrophilic particles produced less aggregated aerosols than did jet nebulizers with hydrophobic particles.

The size distribution of nanosilver produced by atomization can be further narrowed by subjecting the aerosol intense heating and cooling in an inert atmosphere (generally N_2) to vaporize and re-condense silver. This method, known as liquid-flame spray,[53] and another similar method described as flash pyrolysis, involves the exposure of a liquid spray to a high-temperature H_2/O_2 (hydrogen-oxygen) flame. The liquid solvent evaporates, and the product species can decompose or volatilize and re-condense to form aerosols with narrow size distributions and small size (10-50 nm).[54-56] Ku et al.[57] affirmed that the shape and crystal structure of the final product depends on the probability of droplet collision and sintering (heating at temperatures lower than the melting point of metals).

Metal evaporation and condensation in ceramic heaters is a widely used method for producing small (in some cases < 10 nm)[10] and relatively monodisperse nanoparticles.[57] A small (dimensions < 0.5 cm) block of silver is placed into a furnace at 1100 ^{o}C. An inert gas runs through the furnace and carries the metal vapors until the aerosol is cooled, and nanoparticles are formed.[3, 10, 31-32, 57-63] Another method for generating airborne nanosilver is chemical vaporization of solid precursors using arc plasma discharge or spark discharge generators.[37, 45, 64-65] Only a few studies have produced aerosols from the physical handling of powders, whether manually or mechanically (e.g., using a brush dust generator); these methods often produce aerosols with large agglomerates and broad size distributions.[32, 50, 66]

In summary, most studies used a relatively pure silver aerosol, carried by a chemically inert gas, such as argon (Ar) or nitrogen (N_2). This approach assures well controlled experiments and minimizes variation. Nevertheless, nanosilver properties such as size, degree of aggregation, shape, and crystal structure can vary depending on the synthesis method, so it is imperative that researchers report all details about the aerosolization method to enable proper interpretation of and comparison between results. Even though other papers describe nanosilver that may be engineered in many different shapes (spheres, cubes, rods, etc),[15] all of the nanosilver aerosols that were produced for the studies cited in this work reported spherical morphology.

A fundamental question arises regarding the representativeness of these aerosols to real-life human exposure scenarios. For instance, the work of McMahon et al.[5] showed that nanosilver tends to tarnish quickly due to sulfur chemisorption to the particles' surfaces. A similar process could affect nanosilver that is released into the atmosphere, where sulfur is present in many

forms. Since nanoparticle toxicity may be influenced by characteristics such as aggregation, morphology, crystal structure, composition, and coating,[22, 67] it is critical to understand the characteristics that airborne nanosilver would exhibit under environmentally relevant, and not just laboratory, conditions.

SAMPLING AND CHARACTERIZATION OF AIRBORNE NANOPARTICLES

Historically, the most common metric of airborne particles has been the mass concentration ($\mu g\ m^{-3}$).[29] Because of the very small mass of individual nanoparticles, their concentrations in the environment are better described in terms of particle surface area (m^2 of surface area m^{-3} of air) or particle counts (number of particles cm^{-3}).[1, 9, 34, 67-69] More recent aerosol studies describe the use of state-of-the-art single-particle analysis techniques for characterizing carbonaceous, metallic, oxide, or organic aerosols.[70]

Existing standard methods for analysis of airborne silver particles will not necessarily be effective for nanosilver. OSHA recommends a filter-based sampling method for silver, using a mixed cellulose ester filter (MCEF) with a pore size of 0.8 μm. Even though the pore size is larger than nanoparticles, they deposit to the filter anyway mainly by Brownian diffusion, which can carry them out of streamlines to the filter surface. The recommended analytical methods are atomic absorption spectroscopy (AAS) or inductively coupled argon plasma mass spectroscopy (ICP-MS).[71] Samples are desorbed from filters using water extractions or mineral acid digestions, often involving hydrochloric and nitric acids.[72-73] Drake et al.[74] also suggest filtration, using polytetrafluoroethylene (PTFE) filters with a pore size of 2 μm for differentiating metallic silver and soluble silver compounds. Unless airborne concentrations are very high, it will be challenging to collect sufficient mass of nanosilver in ambient samples to enable use of the recommended analytical techniques.

A second problem with standard filtration methods is that certain artifacts are magnified with nanoscale particles. The main factor that affects deposition of the smallest nanoparticles (< 10 nm) is thermal rebound, which occurs when the particles have a thermal velocity higher than their critical “sticking” velocity.[75] It is a function of aerosol temperature and causes collection efficiency to decrease.[46] However, Heim et al.[76] attempted to differentiate electrostatic and other

effects from true thermal rebound effects and did not observe thermal effects on particles as small as 2.5 nm.

Many advanced techniques for the characterization of aerosolized nanoparticles are now available. Table 2.3 summarizes the analytical methods used in published experimental research on nanoparticles, including nanosilver. Most of the studies used the techniques described in the preceding section to generate synthetic test aerosols consisting of AgNPs[3, 5, 10, 31-32, 37, 45, 49-63, 77-78] or other types of nanoparticles.[34, 47-48, 64, 79] Electron microscopy has been used to characterize the size and morphology of nanoparticles. Various spectroscopic and diffraction techniques have been used to describe the crystal structure and chemical composition of nanoparticles. Of the techniques that have been applied to nanosilver, only a subset of those measuring the size distribution (SMPS, APS, OPC, EDM) are applied to the aerosol in real time. Nanosilver surface area has been characterized using the Brunauer, Emmet, and Teller (BET) method[80] and estimated through calculations based on TEM imaging.[57, 81] Characterization techniques for surface charge, such as the application of an aerosol electrometer,[82] have been used for other types of aerosol particles but not nanosilver to our knowledge.

Some studies have used these techniques to characterize ambient (indoor or outdoor) nanoparticles,[34, 66, 82-84] but none of them have attempted to detect nanosilver. Measurements of airborne silver have focused on the total silver concentration in the air samples, usually by collecting particulate matter on filters, extracting them, and analyzing for chemical composition and total silver concentration. Methods used include ICP-AES (inductively coupled plasma atomic emission spectrometry), ICP-MS, and neutron activation analysis.[30, 74]

Card et al.[22] warned that a number of published studies on the inhalation toxicity of nanoparticles do not sufficiently describe particles they used. We attribute this deficiency to the lack of a well-defined standard set of nanoparticle characteristics to be described when performing airborne nanoparticle studies. Numerous authors have emphasized the need for a standard set of nanoparticle properties for environmental and human toxicity studies.[22, 85-87] Based on these papers and on the specific scenario of airborne nanoparticles, we recommend that the following nanoparticle characteristics be described in studies involving the aerosolization of nanosilver: size distribution (of aerosol), chemical composition (of core and

surface), shape, crystallinity, surface area, surface charge (whether aerosol is neutralized or charged prior to deposition), and purity of sample.

Table 2.3. Analytical methods used for characterizing nanosilver.

characteristic	technique	used for nanosilver?	references
morphology	transmission electron microscopy (TEM)	yes	3, 31, 37, 50, 53-55, 57, 59-61, 64-66, 77, 79, 84, 98, 105, 108, 117, 119, 121-122
	scanning electron microscopy (SEM)	yes	46, 48, 50, 54-55, 59, 66, 79, 108
crystal structure	X-ray diffraction (XRD)	yes	53, 59-60, 77, 84, 117, 122
	selected-area diffraction (SAED)[a]	yes	77, 83-84, 105
composition	aerosol photoemission spectrometry (APE)	yes	45
	energy dispersive X-ray spectroscopy (EDS)	yes	37, 60, 64, 66, 84, 108
	scanning auger spectroscopy (SAS)	yes	5
	inductively coupled plasma mass spectrometer (ICP-MS)	yes	51
surface composition and functionality	attenuated total reflection-fourier transform infrared (ATR-FTIR) spectroscopy	no	117, 122
	X-ray photoelectron spectroscopy (XPS)	no	117, 122
aerosol size distribution	scanning mobility particle sizers (SMPS) or similar setups[b]	yes	3, 31-32, 34, 46-47, 49, 51-53, 56-59, 62-63, 66, 78-79, 119
	TEM imaging	yes	60, 79
	aerodynamic particle sizer (APS)	yes	49, 52
	optical particle counters (OPC)	no	49-50
	DMA + electrometer (EDM)	yes	37
surface area	Brunauer–Emmett–Teller algorithm (BET) nitrogen adsorption	no	50, 80, 117, 122
surface charge	aerosol electrometer (AE)	no	82

[a] Selected-area diffraction is commonly incorporated into transmission electron microscopes; [b] Fast Mobility Particle Sizers (FMPS) or set-ups with Differential Mobility Analyzers (DMA) + Ultrafine Particle Counters (UCPC).

FATE AND TRANSPORT OF SILVER NANOPARTICLES IN THE ENVIRONMENT

Historically, airborne particulate silver has been found mainly near smelters that process silver-rich ores.[15, 30, 88] Silver is a frequent byproduct of nickel, lead-zinc, copper, platinum and gold ore processing in North America and South Africa.[26] Chow et al.[89] identified silver concentrations up to 0.03 $\mu g\ m^{-3}$ in $PM_{2.5}$ in Mexico City. Lee at al.[90] detected silver in atmospheric aerosols in Atlanta. Although the exact size of the atmospheric silver was not identified in these studies, if it originated during a combustion process, it was likely to fall in the nanoscale size range initially. Atmospheric processing could then lead to growth of the particles into the accumulation mode, 100-2000 nm. Other studies, some dating as far back as 1965,[91] have detected silver in rain water resulting from cloud seeding for which silver iodide was used. With such use, there is the possibility for incorporation of silver into atmospheric particulate matter.

Fate of nanoparticles is determined by the physical and chemical properties of the particles and environmental factors. Once nanoparticles are released into the environment, they may follow any one of countless paths through soil, water, and/or air. In the atmosphere, nanosilver may (1) remain suspended as individual particles, (2) agglomerate, aggregate, or coagulate (agreement upon terminology is lacking) amongst themselves or with other particles, (3) become coated by inorganic or organic compounds that condense on pre-existing particles, (4) dissolve in slightly acidic conditions[4] (into cloud or fog droplets), releasing silver ions, or (5) chemically react with other compounds (such as organic matter or atmospheric oxidants).[4, 24, 50, 68, 92-95] Finally, they will be removed from the atmosphere back to terrestrial ecosystems by dry or wet deposition. A combination of these phenomena is the most likely scenario, and the order in or extent to which they occur is of great importance in determining the health and environmental risks nanosilver may pose.

TOXICITY OF AIRBORNE SILVER NANOPARTICLES

Ecotoxicity

While it is possible that airborne nanosilver could exhibit ecotoxic effects, the main route for their ecotoxicity is expected to be the aqueous phase. Since the focus of this paper is on airborne nanosilver, we direct the reader to other reviews on the ecotoxicity of nanosilver and related materials (aggregates and ions) in water systems.[4, 15, 25, 96] Nanosilver has been shown to be

toxic to bacteria (e.g., *E. coli* and *Staphylococcus aureus*), fungi (e.g., *Aspergillus sp.* and *Penicillium sp.*), and green algae (e.g., *Chlamydomonas reinhardtii*).[93, 96-97] Here, we describe mechanisms of ecotoxicity that might also be applicable to the inhalation exposure route.

Specific nanosilver characteristics have been discovered to cause microbial toxicity. Navarro et al. [97] demonstrated that the environmental toxicity of nanosilver may be related to their shape and size, but more importantly may depend on the Trojan-horse mechanism (described in the following subsection), which facilitates release of silver ions inside cells. A study by Khaydarov et al.[96] showed that smaller nanosilver have a greater antibacterial/antifungal efficacy than do larger ones. Choi and Hu[98] demonstrated that nanosilver was more toxic to nitrifying bacteria than were silver ions or silver chloride colloids. Additionally, neither the ions nor colloids disrupted cell integrity at the tested concentrations. Fabrega et al.[93] and Lok et al.[95] also reported that antibacterial activities could not be explained solely by the presence of ions or the mass concentration of silver. These results support the Trojan-horse hypothesis, although the authors hypothesized that nanosilver can attach to the outside of cell membranes and induce oxidative stress without compromising the membrane.[98]

Silver ion release seems to be an important toxicological mechanism for nanosilver in the environment, as toxicity has been observed mainly in the aqueous phase and is proportional to the concentration of free silver ions.[99] When dispersed in aquatic systems, ionic silver is extremely toxic to certain organisms, especially bacteria, phytoplankton and fish.[4, 97] The ion's toxicity is thought to be due to its attraction to thiols (HS^-), which are present in proteins and enzymes.[4, 23] Correspondingly, studies show that when sulfide and thiosulfate are present in the water to complex with silver ions, their toxicity to microorganisms declines remarkably,[4] since silver is no longer bioavailable. These results suggest that atmospheric processing of airborne nanosilver could alter its toxicity.

Human toxicity

Existing Guidelines for Airborne Silver

There are several occupational guidelines and exposure limits in the US for airborne silver. All are defined on a mass basis.[13, 74] The Occupational Safety and Health Administration (OSHA)[71] has adopted the Threshold Limit Value on a Time-Weighted Average (TLV-TWA), for a 40-hr per week exposure, from the American Conference of Governmental Industrial Hygienists

(ACGIH), of 0.1 mg m^{-3} for metallic silver and 0.01 mg m^{-3} for soluble silver compounds. The National Institute for Occupational Safety and Health (NIOSH) has set a limit for Immediately Dangerous to Life or Health Concentration (IDLH) of 10 mg m^{-3}.[100] Presently, there are no air quality standards for nanoparticles. The closest is the National Ambient Air Quality Standard for fine particulate matter of diameter 2.5 μm or less ($PM_{2.5}$). The 24-hr and annual standards of 35 and 15 μg m^{-3} are the same order of magnitude as the TLV-TWA for soluble silver. Both NIOSH[101] and the Environmental Protection Agency (EPA)[102] are performing research to advance understanding of critical topics in nanotechnology to fill knowledge gaps and guide regulations in the future, but it is likely to be years before sufficient information is available to establish new standards.

General Particle Inhalation Toxicity

Inhaled particles can lead to inflammation in the respiratory and cardiovascular systems, and known health effects include asthma complications, chronic bronchitis, and respiratory tract irritation and infections.[20, 92, 103] Particle size and surface area are important determinants of inhalation toxicity.[8, 22, 24] Numerous studies have demonstrated that airborne nanoparticles, regardless of chemical composition, pose a potential hazard to the lungs.[8-9, 66, 92, 104] Nanoscale particles are capable of penetrating further into the respiratory system than are larger, micrometer-scale particles, and they can also permeate through cell membranes of organisms and interact with subcellular structures.[8, 22, 24]

Nanoparticles' shape, crystal structure, and composition may present additional risk.[1, 9, 20-22, 64, 85] For instance, Bang et al.[83] asserted that crystalline particles seem to be more damaging to lung epithelial cells than are amorphous structures. In the studies performed by Duffin et al.,[9] in vitro and in vivo inflammation was not a function of nanoparticle mass, but of surface area. Also, nanoparticles may serve as carriers of pollutants that would otherwise not become airborne and enter human lungs.[4, 61, 92] Nevertheless, uncertainty and disagreement still exist on whether the main cause for toxicity is related mainly to physical properties, namely size and shape, or to chemical composition, or a combination of both.[103]

Once inhaled, particles may deposit along the airways, from nasal and oral cavities to alveoli of the lungs, by impaction, sedimentation, interception, Brownian motion, or electrostatics. The efficiency of each mechanism depends strongly on size[29] and also on the local geometry and

flow conditions within the respiratory system. A common misperception is that larger particles are deposited exclusively in the upper respiratory system and that all nanoparticles penetrate to the alveolar region. In fact, while Brownian motion is the dominant deposition mechanism for all nanoscale particles, there are subtleties that lead to deposition in different compartments of the respiratory system (Figure 2.4), depending on the particle's exact size. For instance, particles of aerodynamic diameter 1 nm deposit with > 80% efficiency in the nasopharyngeal region and >10% efficiency in the tracheobronchial region, leaving very few to reach the alveolar region. The deposition efficiency of larger 10-20 nm particles in the nasopharyngeal and tracheobronchial regions is less than 20% (per region); these particles have the highest deposition efficiency, 50%, of any size in the alveolar region. The deposition efficiency of particles 20-100 nm is <40% in the alveolar region. Particles in this size range tend not to deposit in significant amounts in the other respiratory regions.[1, 15, 22, 24, 29, 92] Predicting where airborne nanosilver will deposit in the respiratory system will require accurate sizing of the particles.

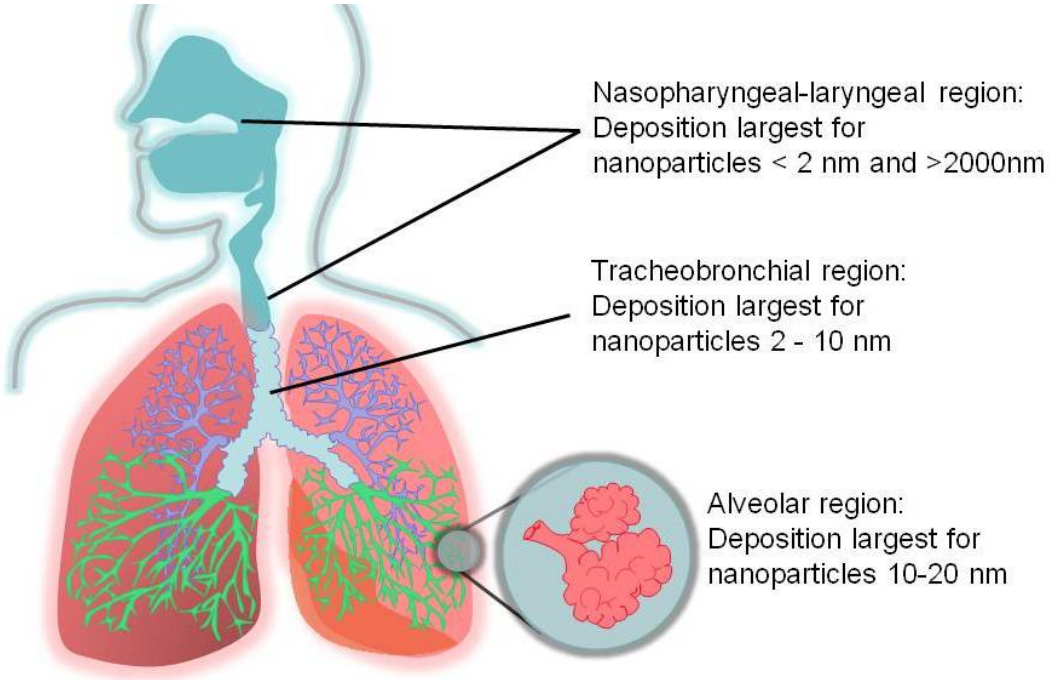

Figure 2.4. Regions of the respiratory system and size (aerodynamic diameter) of particles with the greatest deposition efficiency in each region.

Silver Nanoparticle In Vitro Studies

In vitro studies have demonstrated that the toxicity of silver nanoparticles can be higher than that of other nanomaterials.[105-107] Soto et al.[105] showed that nanosilver was more cytotoxic than all other nanomaterials tested, including TiO_2, Fe_2O_3, Al_2O_3, ZrO_2, Si_3N_4, and carbon nanotubes. Some studies suggested that the mechanism of toxicity of inhaled nanoparticles is increased oxidative stress.[17, 20, 106, 108] It is caused by the formation of intra- or extracellular reactive oxygen species (ROS), such as oxygen ions or radicals, and peroxides at the surface of or within cells. ROS may induce inflammatory processes in the human nose, lung, and cardiovascular system.[9, 20, 67] Numerous in vitro studies[1, 8, 20, 23, 67, 69, 92, 104] on the effects of particles in the respiratory system concentrate on their potential to induce oxidative stress as well as cell lipid peroxidation (oxidative degradation of the lipids present in cell membranes), which leads to cell damage and rupture. Nanoparticles may also interfere with metabolic activities inside the cell.[8-9, 69, 85, 104, 109] In vitro studies with nanosilver reported that it could enter cells and damage DNA.[4, 17, 20, 22, 103] Wijnhoven et al.[15] hypothesized that "toxic effects of silver substances are proportional to the rate of release of free silver ions from them" (p.3).

Silver Nanoparticle In Vivo Studies

The results of multiple inhalation studies with nanosilver in animals justify concern about this route of exposure.[3, 31, 78, 110] Tang et al.[111] showed that nanosilver was capable of translocating through rats' main organs in the form of particles, while silver microparticles could not. Rosenman et al.[112] found an association between decreased night vision and exposure to airborne silver nitrate and oxide. Oral administration of nanosilver to rats was related to a decrease in liver function.[113] Rats exposed to a nanosilver aerosol showed an increase in neutral mucin (substance found in mucous secretions) production in lung tissues and the presence of foamy macrophages in alveolar tissues.[78] Macrophages are large immune system cells that phagocytize foreign materials and degenerated cells, and their presence suggests that nanosilver deposited in the rat's alveoli. Wijnhoven et al.[15] suggested that contact of nanosilver with the olfactory nerve during respiration may constitute an exposure route to the brain. Still, there is a lack of conclusive information on the relative toxicity of nanosilver versus nanoparticles of other compositions, since there are no standardized methods for in vitro studies that would allow for direct comparison between results.[4, 20, 103]

Silver has been found to be a potent enzyme inhibitor.[4] In rats, nanosilver exposure caused decreased liver function due to oxidative stress, and silver was the most toxic of six different metallic nanoparticles.[107] Studies involving the kidneys and cardiovascular system were inconclusive. Park et al.[104] reported that nanosilver showed a lower cytotoxicity than did zinc (Zn) or nickel (Ni) nanoparticles to human alveolar epithelial cells, but the samples consisted of 150-nm nanosilver, which may have been too large for optimum ion release. Two studies found that nanosilver and/or its released ions directly or indirectly resulted in oxidative stress and may have interfered with metabolism inside the cell.[17, 104] The relative impacts of nanosilver versus silver ions remain unclear, and the state in which silver travels through the bloodstream, whether as nanoparticles or complexed silver ions, is also unknown.

DISCUSSION OF SILVER NANOPARTICLE TOXICITY

The Trojan-horse effect (Figure 2.5) has been proposed as the mechanism for inhalation toxicity of nanosilver. Some semiconductor and oxide nanoparticles are known to affect lung epithelial cells via this mechanism.[8] According to this theory, if a nanosilver is able to cross the cell membrane, it may continuously release silver ions once inside. Its toxic effects would be stronger than those of silver ions by themselves because the ions would be largely consumed before breaching the cellular membrane. Inside the cell, the ions can form ROS and cause lipid peroxidation.

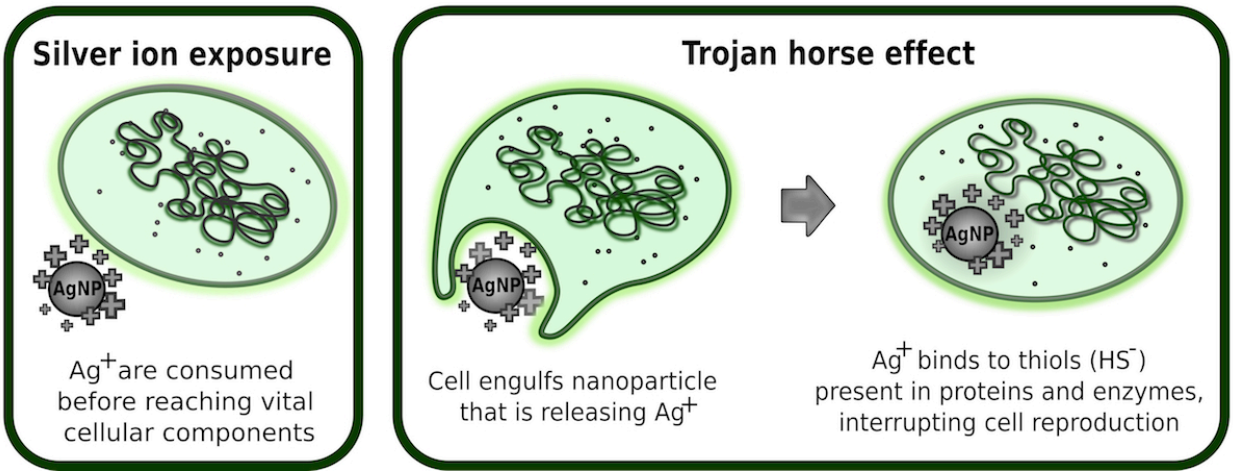

Figure 2.5. Silver ion exposure versus Trojan-horse effect.

Various authors have affirmed the potential for inhalation exposure to a wide variety of nanoparticles.[22, 61, 104] An array of properties, such as size, density, crystal structure, surface charge, and composition may influence their toxicity,[22, 64, 81, 104] and these properties have not yet

been characterized thoroughly for airborne nanoparticles. Many fundamental uncertainties remain about the specific physical and chemical properties of airborne particles that cause known health risks.[31, 67]

CONCLUSIONS

Nanosilver is gaining attention from the academic community, not only because of their antimicrobial effects and product applications, but also because of adverse health effects and environmental exposure scenarios. The fate of nanosilver in the environment as well as its short- and long-term health effects cannot yet be described in detail.[31, 114] There are currently no official government registries or regulations for products containing nanomaterials, as exist for the same materials in bulk form.[4, 7, 115] Thus, understanding the fate of these materials in the environment by studying their physical properties and chemical stability is important for predicting environmental exposure to nanosilver.

The current state of knowledge on the toxicity of nanosilver points toward a potential threat via the inhalation exposure route. Nanoparticle size, chemical composition, crystal structure, and surface area are expected to be important variables in determining toxicity, and the rate of silver ion release is also expected to be a major factor. Still, there are fundamental uncertainties about the specific physical and chemical properties of airborne particles that are responsible for health effects. Thus, to better understand the risks associated with airborne nanosilver, it is vital to carefully describe the conditions under which it could become airborne and available for inhalation.

The lifecycle of silver nanoproducts contains multiple opportunities for nanosilver aerosolization, throughout production, use, and disposal of these products. The domestic use of consumer products that contain nanosilver is a potential source of silver-containing airborne particles in the household. Nanoproducts such as sprays, hair dryers, and misting humidifiers are especially worrisome. Toxicity studies using laboratory-generated particles are important for identifying possible hazards, but exposure characterization including thorough physical and chemical descriptions of the particles is needed to help determine whether or not nanosilver poses a real risk.

Regulatory agencies will need to move rapidly toward new metrics to keep pace with the changing paradigms introduced by nanotechnology. We agree with Bystrzejewska-Piotrowska et

al.,[21] who stated that Amara's law may apply to the effects of nanotechnology. The law is, "We tend to overestimate the effect of a technology in the short run and underestimate the effect in the long run".[116] We might be overestimating the short-term benefits of nanosilver and its antimicrobial properties, while underestimating their long-term effects, including those initiated by airborne exposure to nanosilver.

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Chapter 3: Silver nanoparticles and total aerosols emitted by nanotechnology-related consumer spray products

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ABSTRACT

Products containing silver nanoparticles are entering the market rapidly, but little is known about the potential for inhalation exposure to nanosilver. The objectives of this work were to characterize the emissions of airborne particles from consumer products that claim to contain silver nanoparticles or ions, determine the relationship between emissions and the products' liquid characteristics, and assess the potential for inhalation exposure to silver during product use. Three products were investigated: an anti-odor spray for hunters, a surface disinfectant, and a throat spray. Products emitted 0.24 – 56 ng of silver in aerosols per spray action. The plurality of silver was found in aerosols 1 – 2.5 μm in diameter for two products. Both the products' liquid characteristics and the bottles' spray mechanisms played roles in determining the size distribution of total aerosols, and the size of silver-containing aerosols emitted by the products was largely independent of the silver size distributions in the liquid phase. Silver was associated with chlorine in most samples. Results demonstrate that the normal use of silver-containing spray products carries the potential for inhalation of silver-containing aerosols. Exposure modeling suggests that up to 70 ng of silver may deposit in the respiratory tract during product use.

KEYWORDS: aerosol, emissions, consumer product, nanoparticle, silver, spray.

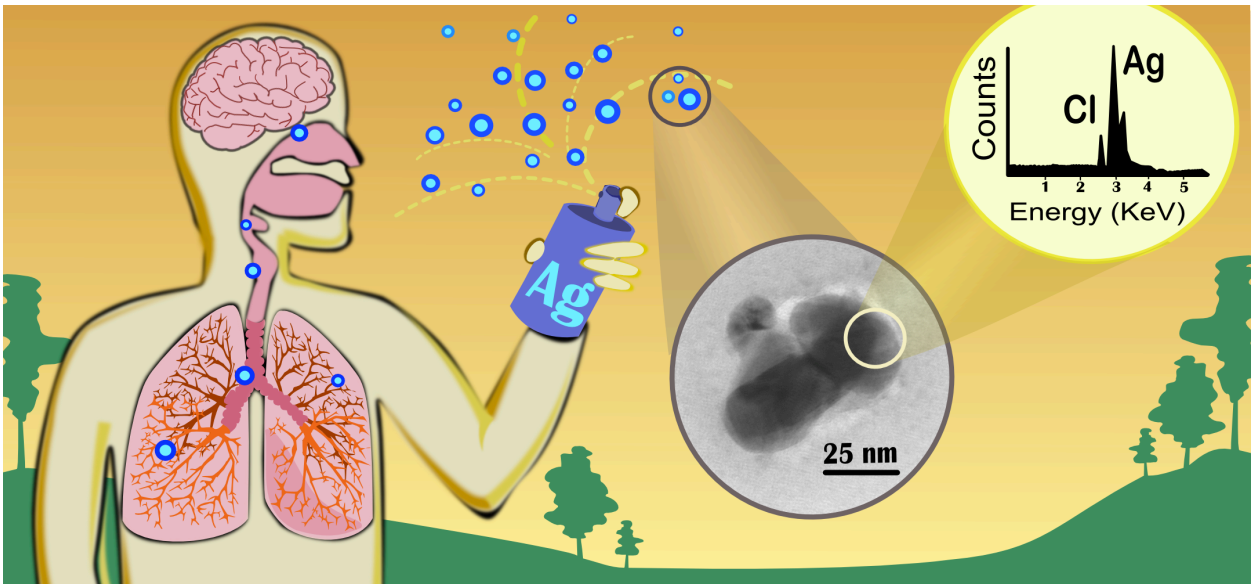

Figure 3.1. Silver nanoparticles and total aerosols emitted by nanotechnology-related consumer spray products

INTRODUCTION

Policies and regulations for responsible incorporation of nanomaterials into consumer products are still under development,[1-4] yet the introduction of new products that have the potential to aerosolize engineered nanoparticles is proceeding swiftly.[5] Silver nanoparticles (nanosilver) are increasingly popular additives to consumer products for antimicrobial purposes.[6,7] Normal use of several types of nanotechnology-based consumer products, such as sprays, humidifiers, and hairdryers, may to lead to inhalation exposure of engineered nanoparticles, but the aerosol emission rates and characteristics — important for assessing risk — are largely unknown.

Inhalation exposure to silver can cause respiratory tract irritation and an irreversible bluish-grayish discoloration of the skin (argyria) or eyes (argyrosis).[15-17] Silver polishers exposed to silver and other metals developed bronchitis, emphysema, and a reduction in pulmonary volume.[18] Occupational exposure to silver dusts and fumes via inhalation is regulated on the basis of mass concentration. The American Conference of Governmental Industrial Hygienists (ACGIH) recommends a threshold limit value (TLV) of 0.1 mg m^{-3} for metallic silver and 0.01 mg m^{-3} for soluble silver compounds, and the Occupational Safety and Health Administration (OSHA) has set a permissible exposure limit (PEL) 0.01 mg m^{-3} for metallic and soluble silver compounds combined. The Environmental Protection Agency (EPA) does not have an inhalation reference concentration for silver, but its oral reference dose for silver is 0.005 mg kg^{-1} day^{-1}.

It is well established that inhalation of nanoparticles is associated with adverse health effects,[5,8] and recent studies justify concern about exposure to nanosilver. Soto et al.[19] found that nanoparticle cytotoxicity was greater for cells exposed to nanosilver than to other materials (e.g., titanium dioxide, iron oxide). Sung et al.[20] reported chronic alveolar inflammation in rats exposed subchronically to airborne silver nanoparticles, with the lungs and liver as the main target organs for silver accumulation. These authors suggested a threshold of 0.1 mg m^{-3} for no adverse effects in rats for inhalation exposure to ~20-nm silver nanoparticles. Even in those studies in which only minimal toxicity was found, authors suggested that chronic effects might arise following long-term exposure to nanosilver.[21]

We are aware of only two studies of aerosols generated from nanotechnology-related consumer products. Hagendorfer et al.[22] reported no measurable release of nanoparticles from a product containing silver nanoparticles when it was dispensed from a pump spray bottle; aerosol emissions were only detected when this product was applied from a pressurized spray bottle. Norgaard et al.[23] assessed the VOC and aerosol emissions from four types of spray products used for surface-coatings (none containing silver), three of which used manual spray pumps and one of which used a pressurized gas can.

More data on the rates and characteristics of nanoparticles that are released during the use of consumer products are needed for studies of the toxicity[24] and environmental fate and transport of engineered nanomaterials.[25] Although toxicity studies have been successful in assessing dose-response relationships for specific types of nanoparticles, the use of laboratory-generated, monodisperse, high purity nanoparticles (for the most part, uncoated) carried by purified air streams may not represent realistic human exposure scenarios. There are concerns about the inhalation toxicity of silver nanoparticles, and there are consumer products that contain nanosilver, but we do not know the amount or characteristics of aerosols that may be released during use of the products. This gap in knowledge is addressed by the objectives of this work: to characterize the emissions of aerosols from consumer products that claim to contain silver nanoparticles or ions, determine the relationship between aerosol emissions and the products' liquid characteristics, and assess the potential for inhalation exposure to silver during product use.

EXPERIMENTAL METHODS

Products tested

Three spray products were chosen for this study based on manufacturers' claims that they contain silver and the products' potential for generating aerosols during normal use:

- An anti-odor spray for hunters (16 ounces, Silver Scent, SilverScent Products, Mechanicsville, VA), which lists "patented nano-silver" and water the only ingredients. The silver concentration was not advertised. Two different bottles were tested.

- A surface disinfectant (4 ounces, SilverClene 24, Agion, Wakefield, MA), whose label advertises "electrolytically generated Ag^{+}" (0.003%), citric acid (4.840%) and "other ingredients" (95.157%). The company's website advertises the use of zeolite to deliver silver ions in its products.

- A throat spray (2 ounces, Wellness Colloidal Silver Throat Spray, Source Naturals, Santa Cruz, CA). "Colloidal silver (30 ppm)" is the only ingredient listed.

Real-time aerosol characterization

To characterize aerosol emissions from products, we sprayed them inside a 0.52-m^3 polyethylene chamber (Atmosbag, Sigma-Aldrich, St. Louis, MO) (Figure 3.2). The chamber was initially filled with air that was conditioned though a hydrocarbon trap, two desiccant dryers, and a high efficiency particulate air (HEPA) to a relative humidity < 10% and particle concentration < 10 cm^{-3}. We used a scanning mobility particle sizer (3936NL SMPS, TSI, Shoreview, MN), consisting of a long differential mobility analyzer and an ultrafine condensation particle counter (CPC 3025A, TSI), and a six-channel optical particle counter (OPC, Aerotrak, TSI) to measure concentrations and size distributions of aerosols with diameters < 0.7 μm and 0.3 – 10 μm, respectively. We also used a diffusion charger (DC 2000 CE, EcoChem) to measure aerosol surface area.

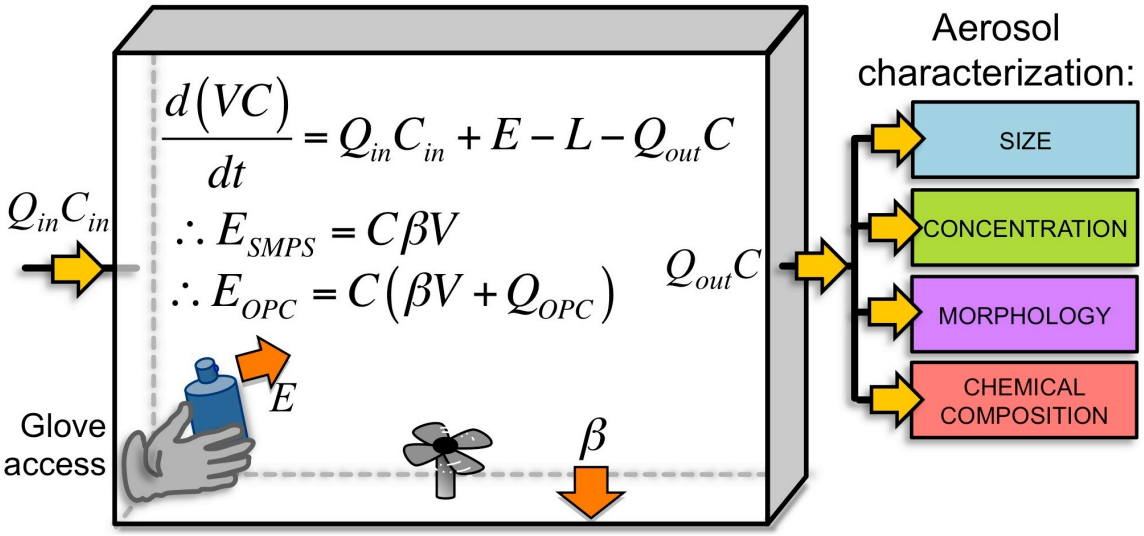

Figure 3.2. Experimental setup and mass balance equations used to determine aerosol emission rates (further described in the supporting information).

We designed a spraying scheme to maintain aerosol concentrations relatively constant inside the chamber in order to facilitate modeling and calculations. Before each experiment, we placed the product in its original bottle inside the chamber, sealed the chamber, and purged it for at least 2 h. Using the chamber's built-in gloves, we manually sprayed products initially at a higher frequency to build aerosol levels and then at a lower frequency to maintain aerosol counts at relatively constant levels (relative standard error < 10%). The hunter spray, which had a larger spray pump compared to other products, was sprayed once every 5 s for the first 2 min and then once every 2 min for the remaining time. The throat spray and the disinfecting spray, which had similarly sized, smaller spray pumps, were sprayed once every 5 s for the first 4 min and then once every 1 min for the remaining time. The total spraying time was 30 min for all products.

Running all aerosolization characterization methods simultaneously would have resulted in an excessively high flow rate through the chamber, so we repeated the spraying scheme three times for each product, once for each of the following measurement scenarios (described in greater detail in Table S1 in the supporting information): (1) concentration and size distribution (0.3 – 10 μm) with the CPC and OPC, (2) concentration and size distribution (< 0.7 μm) with the SMPS, and (3) collection of aerosol samples onto filters and electron microscopy grids. After each experiment, we wiped the chamber's interior walls with a moist paper towel and let the chamber air dry over night. We weighed the product before and after each experiment. As a

control, we repeated the experiments using each product's spray pump with a bottle filled with ultrapure water.

By modeling the chamber as a continuously stirred tank reactor (Figure 3.2) and using size-resolved aerosol concentrations (C, in ml^{-1}), we developed emission factors describing the total amount of particles emitted per spray action (i.e., a single squeeze of the pump) of each product. They can also be converted to emission factors in terms of volume of product sprayed by dividing by the volume per spray action. We set the inlet flow rate (Q_{in}) to zero for SMPS measurements and equal to the OPC flow rate (2.83 l min^{-1}) for measurements using the OPC and CPC together, such that dV/dt was equal to -300 ml min^{-1}, the SMPS/CPC's flow rate, in both cases. We assumed that the particle concentration in the inlet air was zero ($C_{in} = 0$) and that wall losses followed a first-order decay process ($L = \beta VC$), where β (in s^{-1}) is the size-specific aerosol wall loss coefficient, which was determined experimentally for this chamber. We solved the mass balance equation in Figure 3.2 for the size-specific aerosol emission term (E, in s^{-1}).

Different spray pumps may generate different droplet size distributions depending on the geometry of the nozzle and other design parameters, and to control for this variability, we also used a laboratory atomizer (3076, TSI, Shoreview, MN) to produce aerosols from the liquid products. This type of atomizer is used in many in vivo inhalation toxicity studies to produce aerosols from liquid solutions and suspensions. Aerosols were subsequently dried and neutralized and then characterized using the SMPS and the OPC.

Silver characterization in aerosols

We collected aerosol samples on PTFE (Teflon) filters using a 4-stage cascade impactor (Sioutas, SKC, Eighty Four, PA) and extracted them using a method adapted from Benn and Westerhoff,[26] Drake et al.[27], and OSHA ID-206 and ID-125G. We placed the filters in beakers covered with watch glasses and heated them to ~90°C in 20 ml of aqua regia—a 1:3 mixture of reagent-grade nitric acid (HNO_3, 69%, Sigma-Aldrich, St. Louis, MO) and hydrochloric acid (HCl, 38%, Sigma-Aldrich, St. Louis, MO)—until the solution became clear. Then, we removed and rinsed the watch glasses using dilution acid (5% aqua regia), continued heating the solution until it reduced to < 10 ml in volume, and restored it to 10 ml with ultrapure water. We quantified silver by inductively coupled plasma mass spectrometry (ICP-MS, Thermo Electron X-Series

ICP-MS, Waltham, MA). We were unable to perform this characterization step with the first bottle of the hunter spray because we had consumed it entirely in other experiments.

We also collected aerosol samples onto TEM grids using a custom-built thermophoretic precipitator (TP). The flow rate was 12.2 ± 0.2 ml min^{-1}, and top and bottom temperatures were maintained at 61.6 ± 0.4°C and 18.2 ± 0.1°C, respectively. We visualized grids using an environmental scanning electron microscope with electron dispersive X-ray spectrometry capabilities (ESEM, FEI Quanta 600 FEG, Hillsboro, OR), operated under high vacuum, and a transmission electron microscope (TEM, FEI Philips EM420, Hillsboro, OR).

Liquid characterization

To separate silver by size, we diluted products 1:10 in ultrapure water (11 ppb total organic carbon, 18.2 MΩ-cm) and successively filtered them through 1-, 0.45-, and 0.1-μm pore size, 47-mm diameter hydrophilic PTFE filters (Millipore Omnipore, Billerica, MA). We placed 4 ml of 0.1-μm-filtered samples in 3-KDa-cutoff centrifuge filtering units (Millipore Amicon Ultra, Billerica, MA) in attempt to separate ionic and nanoparticulate silver. We diluted bulk and size-fractionated samples of each product 1:100, added 20% aqua regia, and analyzed them for total silver using ICP-MS. We assessed silver ion sorption (i.e., loss) to filter media by repeating the same procedure with an ionic silver solution, obtained by dissolving ~30 mg l^{-1} silver nitrate (Fisher Scientific, Fair Lawn, NJ) in ultrapure water. Loss of ionic silver to PTFE filters was negligible, but loss to the 3-KDa centrifuge filtering membrane was 40 ± 1%. We collected and analyzed all samples in triplicate. We rinsed all glassware in aqua regia, then multiple times in ultrapure water, and let it air dry before use.

Bulk and 1-μm filtered samples were placed in a sonicating bath (85 W, American Scientific Products, McGaw Park, IL) for 60 s and analyzed by dynamic light scattering (cumulant method, DLS, Zetasizer, Malvern, Worcestershire, UK). The disinfecting spray could not be analyzed by DLS because it was a thick detergent liquid with unknown constituents; a refractive index of the material of interest is needed, and the suspension liquid must be known. Bulk and 1-μm filtered samples were also analyzed on a UV-VIS spectrophotometer (Varian Cary 5000, Santa Clara, CA). Aliquots of 10 μl of each product were placed onto holey-carbon coated copper TEM grids (SPI, West Chester, PA) and allowed to air dry inside a desiccator for approximately 2 h. These grids were then visualized by SEM/EDS and TEM.

RESULTS AND DISCUSSION

Characterization of liquid products

Total silver concentrations in the liquid phase were 12.5 ± 1.8 ppm (mean ± standard error) for the hunter spray, 27.5 ± 0.4 ppm for the disinfecting spray, and 23.7 ± 1.2 ppm for the throat spray. These values were 8% and 21% lower than the advertised concentrations of 30 ppm for the disinfecting spray and the throat spray, respectively. The hunter spray did not advertise its silver concentration. The silver mass distribution by particle size in liquid media varied greatly between products (Table 3.1). While most of silver in the hunter spray was associated with relatively large particles (> 450 nm), that in the disinfecting spray was nearly all ionic (< 3 KDa). Silver in the throat spray was bimodally distributed; nearly 70% was nano-sized (between the 3 KDa and 100 nm cutoffs), and ~20% was associated with large particles (> 1000 nm). Results indicate that the percentage of ionic silver lost due to sorption to the 3-KDa centrifuge filtering membrane is not easily transferable to more complex silver-containing solutions, such as the products tested, because correcting the disinfecting spray's 3-KDa filtrate for 40% loss would have produced a concentration much higher than the total measured in the bulk liquid.

Table 3.1. Size-resolved silver concentrations (mean ± standard error) in liquid media (ppm)

size cutoff	hunter spray 2	disinfecting spray	throat spray
> 1000 nm	6.5 ± 2.1	0.2 ± 0.5	4.8 ± 0.9
450 – 1000 nm	2.8 ± 0.7	0.4 ± 0.3	0.7 ± 0.1
100 – 450 nm	0.8 ± 0.2	-1.6 ± 1.3[a]	0.8 ± 0.2
3 KDa – 100 nm	1.7 ± 0.1	1.8 ± 0.7	16.5 ± 0.2
< 3 KDa	0.7 ± 0.1	26.6 ± 0.4	0.8 ± 0.1
Total (bulk)	12.5 ± 1.8	27.5 ± 0.4	23.7 ± 1.2

[a] Negative value resulted from a higher silver concentration on 100-nm filtered samples relative to 450-nm filtered samples. Differences in concentrations amongst all filtration steps for this product were within 2 standard deviations of the average value, so nearly all silver was present in ionic form.

There was no detectable UV-VIS absorbance with the hunter spray and the disinfecting spray. The throat spray's absorbance peaked at 419 nm, which is indicative of silver nanoparticles in

suspension. Size distributions obtained by DLS indicated that a wide range of particle sizes were present in the products. The high polydispersity indexes (0.63 for the throat spray and 0.43 for the hunter spray) render the results highly uncertain, since DLS is a technique more suitable for unimodal, monodisperse suspensions. EDS spectra from liquid samples analyzed by TEM and SEM showed that silver was associated with chlorine in all products; most particles did not contain elements other than silver and chlorine. There was no microscopic or spectroscopic evidence of the presence of zeolite in the disinfecting spray. TEM and SEM images of particles in the liquid products, as well as size distributions obtained by DLS and UV-VIS absorption spectra, are presented in Appendix A.

Total aerosol emissions

Spraying produces aerosols that consist of water, solutes, and possibly solids, and some of the water will evaporate until the aerosols reach equilibrium, which depends on their initial diameter, ambient humidity, and solute concentrations. Figure 3.3 shows size distributions of total aerosols, not just those containing silver. The three products emitted aerosols spanning a wide range of sizes, from nanoscale up to 10 μm, and it is possible that they also produced larger droplets that settled quickly. The disinfecting spray and the first bottle of hunter spray produced bimodal size distributions, peaking around 20 nm and then again around 500 nm. The second bottle of hunter spray and the throat spray produced similar size distributions, with most aerosols larger than 500 nm.

Interestingly, two different bottles of the hunter spray produced different aerosol size distributions. We acquired the bottles approximately 5 months apart and noticed that they had different appearances; the liquid in the first bottle was clear, while that in the second was milky-white, even though the packaging, labeling, and directions were identical. The difference in size distributions between the two bottles of hunter spray, which had the exact same type of nozzle, is evidence that both the product's liquid characteristics and the spray nozzle play roles in determining the aerosol size distribution. The results serve as an example of the great variability that can be expected within consumer products, which makes generalizing results, and thus, regulating products on a case-by-case basis, challenging.

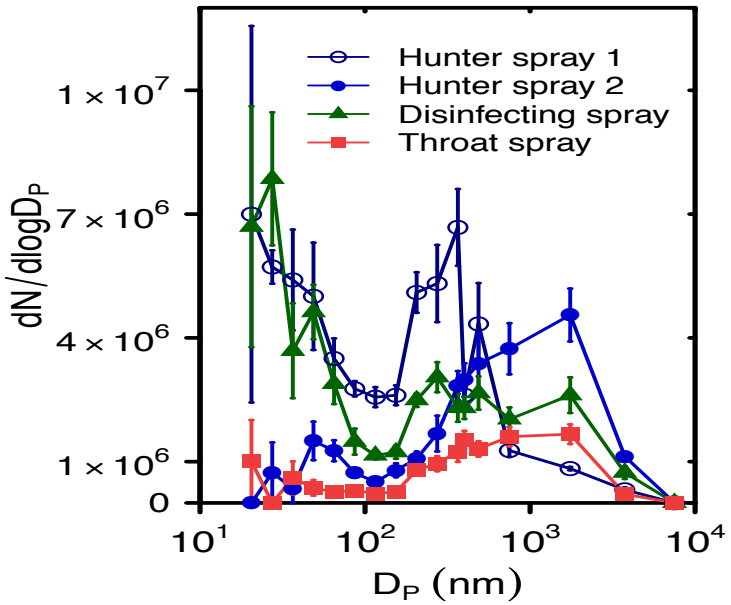

Figure 3.3. Size distributions of total (silver and other) aerosols released per spray action (standard errors shown).

Table 3.2 shows the total aerosol emission factors per spray action and per volume sprayed for each product, as well as the median diameter of the size distributions shown in Figure 3.3 and the amount of product dispensed per spray action. On the basis of spray action, the throat spray had the lowest emission factors. Across all products, emission factors varied by less than a factor of 10 for aerosols < 0.75 μm and by less than a factor of 3 for aerosols 0.3-10 μm. On the basis of the volume sprayed, emission factors varied by a factor of 14 for aerosols < 0.75 μm and by a factor of 7 for aerosols 0.3-10 μm. The disinfecting spray and the first bottle of hunter spray produced aerosols with the smallest median diameters, while the second bottle of hunter spray and the throat spray produced aerosols with similar, larger median diameters. Because aerosol concentrations were very low in the chamber (100 – 400 cm^{-3}), surface area concentrations were below the limit of detection.

Aerosols generated by the atomizer were very different from those generated by the products' original spray bottles. Atomized aerosols had unimodal size distributions, with median diameters between 1.8 and 2.8 times smaller than their spray bottle counterparts (Table A.2 in Appendix A). The median diameters of the atomized aerosols were similar across different products (~78 – 85 nm), probably because the size of aerosols generated is more dependent on the air pressure applied at the atomizer nozzle than on solute concentrations. The aerosol number concentration, however, varied greatly between products. This is probably because the number

of aerosols generated is more dependent on the solute concentrations than on the pressure applied at the atomizer nozzle. The disinfecting spray produced over 10 times more aerosols compared to the hunter spray and the throat spray. These results confirm that the differences observed in aerosol emission rates between products were not exclusively due to the use of different spray pumps.

Table 3.2. Aerosol emission factors and characteristics for each product (mean ± standard error).

characteristic	hunter spray 1	hunter spray 2	disinfecting spray	throat spray
emissions per spray action				
aerosols < 0.75 μm	$(5.6 \pm 0.5)\times10^{7}$	$(1.5 \pm 0.1)\times10^{7}$	$(4.0 \pm 0.6)\times10^{7}$	$(7.3 \pm 0.9)\times10^{6}$
aerosols 0.3 – 10 μm	$(4.4 \pm 0.4)\times10^{6}$	$(8 \pm 3)\times10^{6}$	$(7 \pm 1)\times10^{6}$	$(3 \pm 1)\times10^{6}$
emissions per ml of product				
aerosols < 0.75 μm	$(7.5 \pm 0.6)\times10^{7}$	$(1.9 \pm 0.1)\times10^{7}$	$(2.6 \pm 0.4)\times10^{8}$	$(4.1 \pm 0.5)\times10^{7}$
aerosols 0.3 – 10 μm	$(5.9 \pm 0.6)\times10^{6}$	$(1.4 \pm 0.2)\times10^{7}$	$(4.4 \pm 0.6)\times10^{7}$	$(2.5 \pm 0.4)\times10^{7}$
median diameter (nm)	167 ± 9	217 ± 23	150 ± 12	219 ± 27
product volume per spray action (ml)	0.747 ± 0.008	0.778 ± 0.013	0.157 ± 0.005	0.181 ± 0.001

During the experiment, particle concentrations in the 14 – 750 nm size range were elevated by 99 – 405 cm^{-3} above background levels inside the chamber. In the 300 – 500 nm size range, concentrations were 29 – 85 cm^{-3} above background. These concentrations were similar to those measured by Norgaard at al.[23] but their aerosols were more evenly distributed over a wide range of sizes. Comparing size distributions, and, whenever possible, normalized emission rates (i.e., emission factors) between products and between different studies is more appropriate than comparing concentrations, which are subject to experimental circumstances. Emission factors can also be affected by variables such as temperature and humidity, so adoption of a set of standard environmental conditions for the investigation of nanoparticle emissions would be useful.

Silver in aerosolized products

Silver aerosol emission factors in mass of silver emitted per spray action, segregated by particle size, are shown in Table 3.3. For the hunter spray and the throat spray, silver was present over a wide range of aerosol sizes, with the mode at 1 – 2.5 μm. Silver aerosol emissions were very low for the disinfecting spray, and there was no dominant size. Even though total silver concentrations in the liquid media varied by a factor of only 2.2 between products, the amount aerosolized by the throat spray was 4.6 and 230 times higher than by the hunter spray and disinfecting spray, respectively. These emission factors can be converted to mass per volume of product used by dividing by the volume of product dispersed per spray action in Table 3.2.

Table 3.3. Size-resolved silver emissions in aerosol, per spray action (ng).

size cutoff	hunter spray 2	disinfecting spray	throat spray
> 2.5 μm	2.4 ± 0.3	0.04 ± 0.02	16.6 ± 2.8
1 – 2.5 μm	6.1 ± 1.8	0.04 ± 0.02	24.9 ± 7.3
0.5 – 1 μm	2.6 ± 0.8	0.10 ± 0.08	10.2 ± 3.1
0.25 – 0.5 μm	0.7 ± 0.3	0.04 ± 0.01	2.8 ± 0.9
After filter [a]	0.1 ± 0.1	0.03 ± 0.02	1.2 ± 0.2
Total	12.0 ± 2.7	0.24 ± 0.12	55.6 ± 8.2

[a] A Teflon filter that is expected to collect all particles that pass the 0.25-μm cutoff with near 100% efficiency.

In Tables 3.1 and 3.2, the size cutoffs refer to particles that may be only partially composed of silver; other elements and water may also be associated with the particles. The size of silver-containing aerosols emitted by the products was largely independent of the silver size distributions in the liquid phase. Rather, the aerosol size distribution produced from the spray pump itself governed the release of silver. For liquid products that contained mainly particulate silver (hunter spray and throat spray), over 90% of aerosolized silver was found in aerosols larger than 500 nm in size. The disinfecting spray contained mostly ionic silver and produced very low amounts of silver-containing aerosols that could be sampled using this impactor.

The silver content of aerosols in terms of mass of silver per volume of particles could be estimated based on total aerosol size distributions (Figure 3.3 and Table 3.3), but the different sampling methods (impactor for silver mass v. SMPS and OPC for number and size) and coarse

resolution of the impactor, which necessitated assumptions about a representative aerosol size collected on each stage, introduced considerable uncertainty to the calculation. The resulting silver contents as a function of aerosol size spanned several orders of magnitude but were centered around 0.5 – 50 ng mm^{-3}, compared to the expected values of 12.5 – 27.5 ng mm^{-3} found in the products' liquid phases (Table 3.1).

If aerosol emissions were related to liquid characteristics, evaluation of new products would be greatly simplified. However, products' liquid characteristics were sufficient for predicting some only some aerosol characteristics. The product that contained mostly ionic silver in liquid form, the disinfecting spray, yielded aerosols with very low silver concentrations, even though the total aerosol emissions (not just silver) were of the same order of magnitude as those of the other products. It is likely that this product's spray pump produced mostly large droplets, which settled within seconds, too quickly and to be sampled and detected, and too quickly to present much of an inhalation risk. Products that contained particulate silver, either nano-sized (disinfecting spray) or micron-sized (hunter spray), yielded silver-containing aerosols mostly in the 1 – 2.5 μm range, small enough to be inhaled.

Figure 3.4 shows TEM and SEM images of aerosols, or more precisely the particles that remain when aerosols are exposed to a vacuum, generated by the hunter spray. They were spherical and multi-faceted and ranged in size from 13 nm to 400 nm; most were smaller than 100 nm. All of the particles > 100 nm that were observed seemed to be agglomerates of sub-100-nm unit particles. It is likely that the products' manual spray pumps produced large droplets containing multiple nanoparticles, which agglomerated as the droplets dried and shrank. As can be seen in the EDS spectra, some of the particles contained silver and chlorine. Due to the low collection efficiency of the thermophoretic precipitator and low aerosol concentrations in the chamber throughout the experiments (~100 cm^{-3}), samples contained insufficient numbers of particles to permit a size-distribution count based on TEM or SEM images for the disinfecting spray and the throat spray.

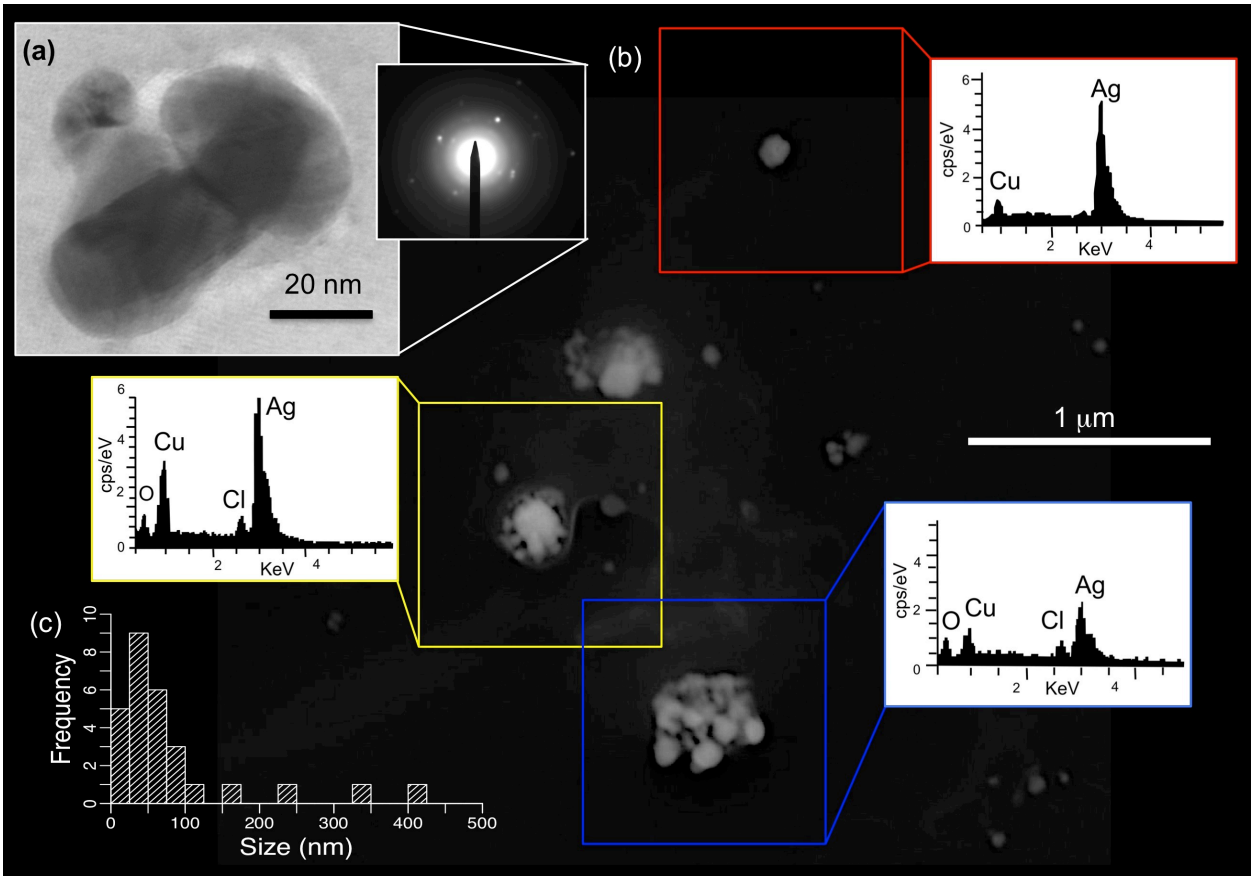

Figure 3.4. Hunter spray aerosols: (a) TEM image and selected area diffraction pattern, with d-spacings roughly matching those of silver crystals, (b) SEM images with EDS spectra of three selected aggregates (EDS spot size of 1 μm outlined), and (c) size distribution of the particles observed (n=28).

INHALATION EXPOSURE TO SILVER

The aerosol emission factors can be used to estimate inhalation exposure to silver. Applying a mass balance in a well-mixed indoor environment, we can calculate the total mass of silver inhaled associated with episodic emissions during product use.[28] For example, if a person pumps the hunter spray 20 times at a rate of 1 spray action per second in a 30 m^3 (4 m × 3 m × 2.5 m) room with an air exchange rate of 0.5 h^{-1},[29] and remains in the room for a total of 10 min, she would inhale 0.62 ng of silver. Based on the size distribution of the silver aerosols (Table 3.3) and size-dependent deposition efficiency in various regions of the respiratory system,[30] we estimate that 0.38 ng of silver would deposit in the respiratory system, with 77% of this in the nasopharyngeal region, 6% in the tracheobronchial region, and 17% in the alveolar region. Of course, actual exposure will differ because emissions will not mix instantaneously and homogeneously throughout the room. In fact, because the hunter spray is supposed to be

sprayed onto the body, actual concentrations in the breathing zone are likely to be higher than modeled, so the resulting inhalation dose of silver is likely to be at least of the order of magnitude of 1 ng for this scenario. Less than 1% of the silver deposited by mass is expected to be nanoscale. If the particles are 100 nm in diameter, this mass corresponds to a number dose of ~300 silver nanoparticles, or more if the actual diameter is smaller. Models of near-source concentrations of aerosol emissions from personal care products, currently not available to our knowledge, would greatly aid evaluation of the inhalation risk posed by spray products containing engineered nanoparticles. We omit the disinfecting spray from modeling because it did not contain silver nanoparticles.

The throat spray's recommended dose is 1 to 2 sprays per day into the mouth, and if under the worst-case scenario all of the aerosols produced were inhaled, 70 ng of silver would deposit in the respiratory system, with 82% of this falling in the nasopharyngeal region, 2% in the tracheobronchial region, and 16% in the alveolar region. Using the suggested maximum subchronic exposure level for Sprague-Dawley rats of 100 μg m^{-3} of ~20-nm silver nanoparticles, suggested by Sung et al.[20], rat body masses from the same reference, and minute ventilation rates from Sung et al.[12], we calculate a maximum inhalation exposure dose of 0.002 mg kg^{-1} day^{-1}, above which alveolar inflammatory responses were observed. The silver dose from the use of the throat spray would be ~16 times below the maximum recommended dose for a 70-kg adult and ~3 times below it for a 15-kg child. This comparison does not account for interspecies variation. Measurements of personal exposure associated with the use of nanotechnology-based products under real-world conditions are needed to complement model-based predictions.

Argyria is also of concern with exposure to silver. Since the throat spray is supposed to be sprayed directly into the mouth, we can assume that nearly all of it will enter the body, either by inhalation or ingestion. Considering the silver concentration in the product and the volume emitted per spray, we calculate the total silver exposure to be 0.009 mg day^{-1}, which is ~40 times below the EPA's reference dose of 0.005 mg kg^{-1} day^{-1} for the development of argyria for a 70-kg adult, but only ~9 times below the reference dose for a 15-kg child. As silver-nanotechnology products grow in popularity, exposure from multiple types of products can be expected and might lead to cumulative exposure levels above the reference dose for argyria.

Evaluation of the health and environmental impacts of nanotechnology requires an accurate description of particles that are released at all stages of a product's life cycle. During product use, the nanoparticles emitted are not necessarily in the same form in which they are added to the product. In addition to engineered nanoparticles, nanotechnology-based products may contain other ingredients such as surfactants, shelf stabilizers, or odor enhancers that may interact with the nanoparticles. When liquid products are sprayed, droplets are emitted, some of the solvent may evaporate, and except with pure suspensions, complex aerosols remain. The engineered nanomaterials may agglomerate with each other, become coated, and/or aggregate with other components of the product that solidify as the droplets dry. Therefore aerosolized nanoparticles' physical and chemical characteristics may not be the same as those of the virgin nanomaterial.

For future work, we recommend a more intense electron microscopy approach, including the use of single-particle chemical characterization for the aerosols using high-resolution TEM coupled with EDS. Additionally, single-particle aerosol mass spectrometry would enable in situ quantification of the size and silver content of airborne particles. Because aerosol characteristics differ greatly with use of a laboratory atomizer versus the consumer products' own bottles, we advise future inhalation toxicity studies to consider carefully the aerosolization method. We also recommend that future studies assess the water content of the aerosols; the low aerosol concentrations in the chamber (100 – 400 cm^{-3}) in this study were not conducive to the use of a diffusion dryer for such an investigation.

The aerosol emission rates and size distributions presented in this work can serve as input to risk assessment models, such as the one developed by Lorenz et al.[31] Results can be used to guide the selection of relevant particle doses in nanotoxicity testing, to predict exposure to emissions from nanotechnology-based product in indoor air quality models, and to develop regulations to ensure consumer safety.

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Chapter 4: Exposure assessment of silver nanoparticles in select children's consumer products

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Status: This chapter has been extracted from a report submitted to the US EPA (Environmental Protection Agency) and the CPSC (Consumer Product Safety Commission) in October 2011. Most of the findings described in this chapter are included in a manuscript being prepared in collaboration with the EPA, to be submitted to Environmental Science & Technology.

ABSTRACT

The goal of this work was to determine if children could potentially be exposed to nanosilver during normal use of consumer products. To achieve this goal, we developed scientific methods for evaluating the presence and release of nanosilver from consumer products and then applied them to quantify the amount of silver contained in and released by the products. We first compiled an inventory of 82 consumer products that claim to contain nanosilver and that may be used by or near children. From that inventory, 13 products were selected for testing. These products included one plush toy (a teddy bear), three fabric products, one set of breast milk storage bags, two sippy cups, three cleaning products (a spray, a surface wipe, and a kitchen scrubber), two humidifiers, and one humidifier accessory. All products had at least one component containing silver. Silver in particulate form was visible in the spray cleaning product, the interior foam and exterior fur of the teddy bear, the baby blanket, and in components of a sippy cup. Silver particles ranged from nanoscale up to 10 μm in size and appeared to be located on the surface and interior of fabric fibers and embedded in the components of the cup. We measured the potential for aerosol exposure associated with running a humidifier, spraying a cleaning product, and handling a fabric product and a stuffed toy. Aerosol concentrations were not significantly elevated above background levels during product use. Among the products tested, the plush toy and the fabric products are most likely to be a source of low levels of bioavailable silver. Given the current state of knowledge, the implications of this finding cannot be predicted at this time because the health effects of nanosilver and mechanisms of toxicity are not yet fully understood.

KEYWORDS: nanotechnology, nanoparticle, consumer product, silver, children.

INTRODUCTION

Silver nanoparticles (nanosilver) are gaining significant attention from the academic and regulatory communities, not only because of their antimicrobial effects and subsequent product applications, but also because of their potential environmental and human health impacts. Numerous in vitro studies have shown that silver nanoparticles are toxic to certain organisms, such as phytoplankton,[1] bacteria,[2,3], algae,[4] and fish[5-7] and also to human cells.[8-10] In vivo studies have also been performed with silver nanoparticles, and they were proven to be toxic to rats and to translocate between organs.[11-15] Luoma[5] reports that silver can be absorbed by the lungs, skin, and gastrointestinal and urogenital tracts, but it is not thought to be toxic to the nervous, cardiovascular, or reproductive systems in humans.

The emerging application of silver nanotechnology (or nanosilver) as an antimicrobial and anti-odor agent in consumer products has created the need to evaluate the potential exposure to silver nanoparticles through multiple routes (dermal, inhalation, and digestive). Children especially may be affected by the normal use of consumer products designed specifically for them, such as milk bottles, pacifiers, and textiles. Children are at a higher exposure risk than adults because (1) they have a higher metabolism and surface-area-to-body-mass ratio than adults, (2) their organs and tissues are still under development, (3) they have more years ahead of them to develop health conditions from chronic exposure to emerging materials,[16] such as nanosilver, and (4) they have a higher tendency to place hands and objects in the mouth.[17] Guney and Zagury[18] call for the development of regulations concerning toxic substances in toys and children's products based on risk assessment rather than on total contaminant content.

Although there have been studies on the toxic effects of silver nanoparticles to human cells and the environment, there still exists a gap in knowledge about realistic human exposure scenarios during the use of nanosilver consumer products, with few published works that only partially cover this subject.[19-22] The mechanisms, forms, and amount of silver released from consumer products containing nanosilver are poorly understood and at present, cannot be predicted on the basis of product type and physical and chemical characteristics. Therefore, new data are needed to describe the size, morphology, chemical composition, and other physicochemical properties of silver contained in and released from consumer products. Whether silver is in the ionic or nanoparticle form is of special interest because the distinction likely has implications for toxicity.[5]

The purpose of this project was to evaluate the hypothesis that nanosilver is released from children's consumer products under conditions of normal, real-world use, making it available for potential exposure of children. Both methods and data were needed to evaluate this hypothesis. Thus, the main objectives of this work were to develop scientific methods for evaluating the presence and release of nanosilver from consumer products and to obtain the experimental data needed to determine if children could potentially be exposed to nanosilver during normal use of consumer products (e.g., sippy cups, plush toys, clothing, etc.). Specific objectives were to (1) prepare an inventory of consumer products that claim to contain nanosilver and that may be used by or near children; (2) chemically characterize the silver content of selected products; and (3) characterize the size and morphology of silver in the products through electron microscopy.

EXPERIMENTAL METHODS

Children's consumer product inventory

The first step of this project was to build an inventory of consumer products that claim to contain nanosilver and that may be used by or near children. Products listed in The Project on Emerging Nanotechnologies (PEN) consumer products inventory were added to our inventory if described as being safe for consumption by children and currently available for purchase by end-consumers in the US market.[23,24] We expanded from this database by searching for products that may have become available more recently.

We searched for products sold over the Internet on commonly used shopping websites and also visited large department stores and international stores in the local area. Our search comprised the stores listed in Table 4.1. Key words used in the online search included, but were not limited to *nanosilver, silver, agion, ag, nanoparticle, antimicrobial, antibacterial, children, kids, baby, toy*, etc. These key words were used in different combinations to generate the maximum number of relevant results. We also searched for products with descriptions that are suggestive of the use of nanosilver, even if it is not explicitly advertised (e.g., "nano toothbrush"). Products previously purchased by EPA researchers were also added to this inventory.

Table 4.1. Listing of stores and websites visited in the search for nanosilver children's consumer products.

name of store	location (link if online store, city if physical)
Amazon	www.amazon.com
Babies "R" Us	www.babiesrus.com
Baby Gap	www.gap.com/browse/division.do?cid=6344
eBay	www.ebay.com
Google Product Search[1]	http://www.google.com/prdhp
Overstock	www.overstock.com
The Children's Place	www.childrensplace.com
CVS	Blacksburg, VA
Eats Natural Foods	Blacksburg, VA
Oasis World Market	Blacksburg, VA
Rite Aid	Blacksburg, VA
K-Mart	Christiansburg, VA
Ross	Christiansburg, VA
Target	Christiansburg, VA
TJ Maxx	Christiansburg, VA
Walmart	Christiansburg, VA

[1] Not an online store, but a shopping-specific search engine that connects the user to a large number of online stores.

The search included, but was not limited to, the following product types:

- Textiles (clothes, blankets, cloth diapers, towels, bags, etc.)
- Disposable diapers
- Stuffed toys (cloth, plush, or other textiles)
- Plastic toys and teething toys
- Milk bottles and sippy cups
- Plates, bowls, food containers, and utensils
- Pacifiers
- Toothbrushes
- Throat or nasal sprays
- Dietary supplements / homeopathic supplements

We compiled an itemized list of 82 available products with descriptions of how nanosilver is present in the product and which route(s) of exposure is(are) most likely (e.g., inhalation, dermal, ingestion).

Table 4.2 presents a summary of products listed in the inventory. The inventory itself is listed in Appendix B. Products listed on websites that did not contain purchasing information (i.e., the means for someone in the US to purchase them) were not added to this inventory. Business-to-business (B2B) websites were generally avoided, yet a few products sold in bulk were included to illustrate examples of items that might enter the US market through distributors (e.g., Appendix B, item 3: pacifiers and item 12: cloth diapers).

Table 4.2. Summary of the inventory of nanosilver children's products.

product type	number of products	item numbers in appendix b
toys	2	1 - 2
pacifiers	1	3
toothbrushes and toothpastes	9	4 - 11
textiles (cloth diapers, clothes, blankets, etc.)	9	12 - 20
food storage (including sippy cups)	6	21 - 26
skin and nail care (gels, lotions, nail polish, etc.)	11	27 - 37
shampoos and hair conditioners	4	38 - 41
throat and nose sprays	5	42 - 46
dietary supplements (liquid and pills)	19	47 - 65
surface disinfectants and cleaners	5	66 - 70
humidifiers	12	71 - 82

Consumer product selection

In collaboration with EPA and CPSC scientists, we selected 14 products for testing. The selected products were limited to types for which CPSC has jurisdiction. Products were either provided by EPA or purchased by Virginia Tech.

Table 4.3 presents a summary of products tested, and Table 4.4 contains detailed descriptions of the products. The final product list includes only 13 items, because item 2 (pacifier) was only available for purchase in bulk and was not acquired for this project.

Table 4.3. Summary of nanosilver products selected for testing.

	product	category	unit price	item number in product inventory
1	Benny the Bear	toys	*	1
2	pacifier (not purchased)	pacifiers	*	N/A
3	Precious Protechtor sleepsuit or babygro	textiles	£12.00	14
4	Precious Protechtor baby blanket	textiles	£15.00	15
5	Precious Protechtor baby scratch mitts	textiles	£5.00	16
6	BreastMilk storage bags	food storage	$11.00	21
7	Sippy Cup #1, light blue	food storage	*	23
8	Sippy Cup #2, dark blue (Anywayup)	food storage	*	23
9	MesoSilver antifungal/antibacterial disinfecting spray (four 125-ml bottles)	surface cleaners and disinfectants	$27.99	66
10	Nature's Fresh Silver Shield surface wipes (one pack of 30)	surface cleaners and disinfectants	$18.99	68
11	Easy-Well Perfect Nano Silver scrubber	surface cleaners and disinfectants	*	69
12	Germ Guardian H1000 tabletop humidifier	humidifiers	$49.99	72
13	Germ Guardian H2000 manual ultrasonic humidifier	humidifiers	$124.78	74
14	Stadler Form Ionic Silver Cube	humidifiers	$25.00	82

* Products purchased by EPA or CPSC.

Table 4.4. Nanosilver products selected for testing.

(1) **Product Name:** Benny the Bear Antimicrobial Toy	**Product Type (likely exposure route):** Toy (ingestion, dermal)
Manufacturer (Country): Pure Plushy (country unknown)	**Retailer:** Overstock.com
Retailer website: http://www.overstock.com/Baby/Benny-the-Bear-Antimicrobial-Stuffed-Toy/2876408/product.html	
Advertised form of silver: "Patented animal toy blends memory foam with silver nanotechnology"	
Comments: Cost: $27.99. Product sold out, according to Overstock.com. We have one bear, purchased by EPA. "Donny the Dog" was also advertised to contain silver nanotechnology.	
(2) **Product Name:** Baby Pink or Blue Precious Protechtor Sleepsuit	**Product Type (likely exposure route):** Clothing (dermal)
Manufacturer (Country): Precious Protechtor (UK)	**Retailer:** Olivers Babycare
Retailer website: http://www.olivers-baby-care.co.uk/baby-pink-or-blue-precious-protechtor-sleepsuit-or-babygro.html	
Advertised form of silver: "designed using so-called 'nano technology' which incorporates the mineral [sic] silver" "Silver has natural antibacterial properties which kill viruses, and it is one of the safest substances for babies and has no side effects"	
Comments: Cost: £12.00. UK vendor website, shipping to the US is available. Product listed on PEN online inventory.	
(3) **Product Name:** Baby Pink or Blue Precious Protechtor Baby Blanket	**Product Type (likely exposure route):** Blanket (dermal)
Manufacturer (Country): Precious Protechtor (UK)	**Retailer:** Olivers Babycare
Retailer website: http://www.olivers-baby-care.co.uk/baby-pink-or-blue-precious-protechtor-baby-blanket.html	
Advertised form of silver: "100% nano-impregnated tricot polyester"	
Comments: Cost: £15.00. UK vendor website, shipping to the US is available. Product listed on PEN online inventory.	
(4) **Product Name:** Precious Protechtor Baby Scratch Mitts by Baby Pink and Blue	**Product Type (likely exposure route):** Clothing (dermal)
Manufacturer (Country): Precious Protechtor (UK)	**Retailer:** Olivers Babycare
Retailer website: http://www.olivers-baby-care.co.uk/precious-protechtor-baby-scratch-mitts-by-baby-pink-and-blue.html	
Advertised form of silver: "designed using so-called 'nano technology' which incorporates the mineral [sic] silver" "Silver has natural antibacterial properties which kill viruses, and it is one of the safest substances for babies and has no side effects"	
Comments: Cost: £5.00. UK vendor website, shipping to the US is available. Product listed on PEN online inventory.	

Table 4.4 (continued). Nanosilver products selected for testing.

(5) **Product Name:** BreastMilk Storage Bags	**Product Type (likely exposure route):** Food storage (ingestion)
Manufacturer (Country): Perfection (South Korea)	**Retailer:** eBay.com
Retailer website: http://cgi.ebay.com/BreastMilk-Storage-Bags-BPA-Free-30-90-Packs-NanoSilver-/320652895280	
Advertised form of silver: "Nano-Silver Process"	
Comments: Cost: $11.00 for 30 200-ml bags, $17.00 for 60 200-ml bags.	
(6) **Product Name:** Sippy cups, 2 ct, blue	**Product Type (likely exposure route):** Sippy cups (ingestion)
Manufacturer (Country): Unknown (UK)	**Retailer:** Unknown
Retailer website: Not Applicable	
Advertised form of silver: Unknown	
Comments: These two sippy cups were purchased in the UK and provided by EPA.	
(7) **Product Name:** MesoSilver® Antifungal/Antibacterial Disinfecting Spray	**Product Type (likely exposure route):** Surface disinfectant (dermal, ingestion)
Manufacturer (Country): Purest Colloids (USA)	**Retailer:** Amazon.com Also at PurestColloids.com
Retailer website: http://www.amazon.com/MesoSilver-Antifungal-Spray-125-Colloidal/dp/B00106J28O/ref=pd_sim_hpc_5	
Advertised form of silver / Directions: "Colloidal Silver"; "World's Smallest Particle Size, less than 1 nm on average" "Use in the kitchen, bathroom and children's room (…) [sic]"	
Comments: Cost: $27.99 for a 125-ml bottle.	
(8) **Product Name:** Nature's Fresh Silver Shield Surface Wipes	**Product Type (likely exposure route):** Surface disinfectant (dermal, ingestion)
Manufacturer (Country): Nature's Sunshine (USA)	**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Natures-Fresh-Silver-Shield-Surface/dp/B004ST0AQ8/ref=sr_1_4?ie=UTF8&s=hpc&qid=1301519636&sr=1-4	
Advertised form of silver: "Contains: (…) , Silver Shield® (silver: colloidal and ionic)"	
Comments: Cost: $18.99 for 30 wipes. Might be used as a toy/surface cleaner near infants.	

Table 4.4 (continued). Nanosilver products selected for testing.

(9) **Product Name:** Easy-Well Perfect Nano Silver Scrubber	**Product Type (likely exposure route):** Surface cleaner (dermal, ingestion)
Manufacturer (Country): Hankook Tamina Co., Ltd (Korea)	**Retailer:** Unknown
Manufacturer website: http://www.hktamina.co.kr/	
Advertised form of silver: "Nano Silver"	
Comments: Product purchased in Seoul by EPA staff (set of 2).	
(10) **Product Name:** Germ Guardian H1000 TableTop Humidifier	**Product Type (likely exposure route):** Humidifier (inhalation)
Manufacturer (Country): Germ Guardian (USA)	**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Germ-Guardian-H1000-TableTop-Humidifier/dp/B000ZPKVMM/ref=sr_1_3?s=hpc&ie=UTF8&qid=1301872292&sr=1-3	
Advertised form of silver: "Uses Nano-Silver technology to stop mold and bacteria from growing in the tank"	
Comments: Cost: $49.99	
(11) **Product Name:** Germ Guardian H2000 Manual Ultrasonic Humidifier	**Product Type (likely exposure route):** Humidifier (inhalation)
Manufacturer (Country): Germ Guardian (USA)	**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Germ-Guardian-H2000-Ultrasonic-Humidifier/dp/B001A075IE	
Advertised form of silver: "Uses nano-silver technology to fight the growth of mold and bacteria in the water so germ-free water is vaporized into the room"	
Comments: Cost: $124.78	
(12) **Product Name:** Stadler Form Ionic Silver Cube	**Product Type (likely exposure route):** Humidifier (inhalation)
Manufacturer (Country): Stadler Form (USA)	**Retailer:** Stadler Form
Retailer website: http://swizz-style.myshopify.com/collections/accessories/products/ionic-silver-cube	
Advertised form of silver / directions: "Releases silver ions into the water" "For use with most humidifiers on the market"	
Comments: Cost: $25.00	

Chemical analysis of select children's consumer products

After the selected children's products were acquired, the next tasks were to confirm that these products indeed contained silver and to quantify their total silver content. The sample preparation method varied according to product type and the most relevant exposure route. Results of the chemical analysis for total silver content determined whether each consumer product or component was subjected to subsequent microscopy and leaching analyses.

For fabrics and plastics, for example, if the product contained a detectable amount of silver, we proceeded to leaching experiments (Step 2) and then, if silver leached from the product, we proceeded to the microscopy analysis (Step 3). If silver did not leach from the product, no further analyses were conducted. Leaching protocols are described in Appendix B and the results from the leaching study are presented in the Chapter 5 of this dissertation.

Liquid product

One liquid nanosilver product was analyzed, the MesoSilver Antifungal/Antibacterial Disinfecting Spray. To determine whether the product contained silver in particulate and/or ionic form, we passed it through a series of filters with the following cutoff sizes: 1000 nm, 450 nm, 100 nm, and 3KDa. For the 1000-, 450-, and 100-nm cutoffs, we used hydrophilic Teflon filters (Millipore Omnipore). For the 3-KDa cutoff, we used a centrifugal filtering unit (Millipore). This type of centrifugal filtering unit is recommended for concentrating proteins ~1 nm or larger;[25] thus it is fair to assume that the silver present in its filtrate is likely to be in ionic form.

Bulk (unfiltered) samples and those from each filtration step were acidified with 10% nitric acid (HNO3, 70% ACS certified, Fisher Scientific), diluted 1:100, and analyzed for total silver concentrations using an inductively coupled plasma mass spectrometer (ICP-MS, X-Series, Thermo Electron).

Figure 4.1 is a flowchart showing the relationship between the three analytical tasks (chemical characterization, leaching, and microscopy) and how analysis proceeded depending on results of each step. For fabrics and plastics, for example, if the product contained a detectable amount of silver, we proceeded to leaching experiments (Step 2) and then, if silver leached from the product, we proceeded to the microscopy analysis (Step 3). If silver did not leach from the

product, no further analyses were conducted. Leaching protocols are described in Appendix B and the results from the leaching study are presented in the Chapter 5 of this dissertation.

Table 4.5 shows the specific leaching media and exposure scenarios applied to each consumer product or product component; the specific tasks conducted depended on the intended consumer use for each product.

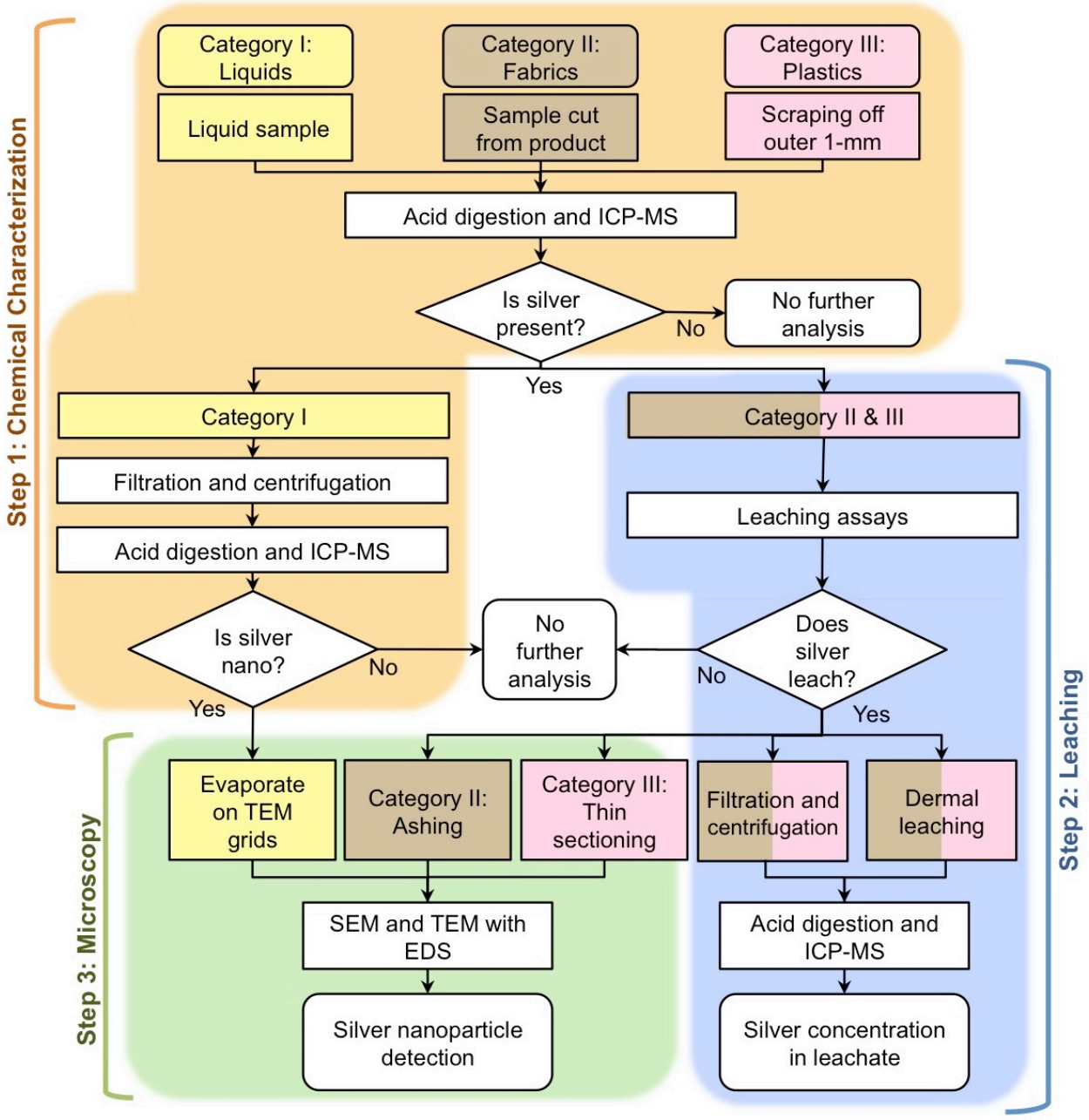

Figure 4.1. Flowchart summarizing the experimental approach for this project.

Table 4.5. Sampling matrix for nanosilver consumer products.

		Task 3: Total Silver	Task 4: Microscopy				Task 5: Leaching								
#	Product (component)		SEM product	SEM (ash)	TEM product	TEM (ash)	Tap Water	Orange Juice	Milk Formula	Artificial Saliva	Synthetic Urine	Synthetic Sweat	Task 5: Dermal	Task 5: Aerosol
1	Benny the Bear (exterior, new)	X	X	X			X			X	X	X	X	X
2	Benny the Bear (exterior, aged)						X			X	X	X		
3	Benny the Bear (interior)	X	X	X		X	X			X	X	X		
4	Precious Protechtor Antibacterial Baby Blanket (new)	X	X	X		X	X			X	X	X	X	
5	Precious Protechtor Antibacterial Baby Blanket (aged)						X			X	X	X		
6	Precious Protechtor Antibacterial Sleepsuit	X												X
7	Antibacterial Baby Scratch Mitts	X												
8	Breast Milk Storage Bags	X							X					
9	Sippy Cup #1 (outside of cup)	X												
10	Sippy Cup #1 (inside of cup)	X												
11	Sippy Cup #1 (rubber ring)	X	X					X	X					
12	Sippy Cup #1 (white plastic rim)	X												
13	Sippy Cup #1 (transparent blue plastic)	X	X					X	X					
14	Sippy Cup #2 (outside of cup)	X												
15	Sippy Cup #2 (inside of cup)	X												
16	Sippy Cup #2 (clear cap)	X	X					X	X					
17	Sippy Cup #2 (spout)	X												
18	MesoSilver Antifungal Spray	X			X								X	X
19	Silver Shield Surface Wipes	X	X		X								X	
20	Easy Well Scrubber	X											X	
21	Germguardian Table Top Ultrasonic Humidifier						X							
22	Germguardian Ultrasonic Humidifier H2000						X							
23	Ionic Silver Cube						X							X

Fabrics and plastics

For fabrics and plastics, we digested samples using HNO_3 and hydrogen peroxide (H_2O_2). Benn and Westerhoff [20,26] applied this digestion method in determining the concentration of silver in socks and other textile consumer products.

To quantify the total silver concentration in the products, we analyzed at least three samples from each product and subjected these to acid digestions according to SOP # AirVT-Nanosilver-001: Preparation of Consumer Product Samples for Silver Analysis by ICP-MS (Appendix B). When appropriate, we considered subsets of samples from different parts of the same product. For example, Sippy Cup #1 had five different components (outside of cup, inside of cup, spout, ring, cap), and Sippy Cup #2 had four different components (outside of cup, inside of cup, lid with spout, and clear spout cover), as shown in Figure 4.2.

Figure 4.2. Different components in Sippy Cup #1 (left) and #2 (right).

Product sample masses were ~500 mg in most cases, but when there was not enough material or the product was a hard plastic, which required shaving of material from the surface, samples weighed less than 100 mg (Table 4.6). Since the surface wipes were packed in a solution containing alcohol, which evaporates quickly, their sample masses had a larger standard deviation. This product was re-sampled with a much larger sample mass (one whole wipe per sample) in attempt to reduce the effect of mass loss by evaporation.

Table 4.6. Number of samples and sample mass for products that were subjected to acid digestion.

	product	sample size (n)	mass extracted for acid digestion (mg)
1	Benny the Bear: exterior	3	502 ± 3
2	Benny the Bear: interior	6	495 ± 3
3	Pacifier[1]	-	-
4	Precious Protechtor sleepsuit or babygro	3	501 ± 1
5	Precious Protechtor baby blanket	3	501 ± 1
6	Precious Protechtor baby scratch mitts	3	501 ± 1
7	breast milk storage bags	6	506 ± 1
8	sippy cup #1, light blue: outside of cup	3	99 ± 1
9	sippy cup #1, light blue: inside of cup	3	99 ± 4
10	sippy cup #1, light blue: rubber ring	3	255 ± 2
11	sippy cup #1, light blue: white rim	3	201 ± 1
12	sippy cup #1, light blue: transparent blue cap	3	101 ± 4
13	sippy cup #2, dark blue: outside of cup	3	57 ± 10
14	sippy cup #2, dark blue: inside of cup	3	103 ± 5
15	sippy cup #2, dark blue: spout	3	100 ± 1
16	sippy cup #2, dark blue: clear spout cover	6	37 ± 13
17	Nature's Fresh Silver Shield surface wipes	6	554 ± 38
-	surface wipes (2nd attempt)	3	7116 ± 912
18	Easy-Well Perfect Nano Silver scrubber	3	505 ± 2
19	non-nano fabric	3	502 ± 2

[1]Not purchased in time for this project.

We placed each sample in a 140-ml beaker and soaked it in 10 – 20 ml of nitric acid (70% ACS certified, Fisher Scientific) for at least 30 min. We then heated samples to ~90°C and added nitric acid in 1-ml aliquots until the material was completely digested. In the case of hard plastics, which do not digest in heated nitric acid, we heated samples for a period of at least 2 hr to dissolve silver into the liquid media. After this period, we removed samples from heat, let them cool, and added 2 ml of hydrogen peroxide (50% ACS certified, Fisher Scientific). We heated samples until effervescence was minimal and then removed them from the hot plate.

After samples reached room temperature, we diluted them to a volume of 100 ml with ultrapure water and passed them through a 450-nm hydrophilic Teflon filter (Millipore Omnipore). Samples were analyzed for total silver concentrations by ICP-MS.

Microscopy analysis of children's consumer products containing silver

After ascertaining whether silver was present in the selected product and quantifying its mass ratio (micrograms of silver per gram of product), we determined whether or not silver was in the form of nanoparticles (smaller than 100 nm in at least one dimension). Analytical methods were tailored for specific product materials. Sample preparation and microscopy analysis of consumer products followed SOP # AirVT-Nanosilver-002: Analytical Methods for Silver Nanoparticle Characterization via Electron Microscopy in Complex Media (Appendix B). Microscopes used for this project are presented in Table 4.7.

Table 4.7. Microscopes used in this project.

name / description of method	instrument	description / rationale for selection	resolution or limit of detection
environmental scanning electron microscope (SEM) with energy-dispersive spectrometry (EDS)	FEI Quanta 600 FEG	imaging and chemical characterization for particles <100 nm, environmental pressure and humidity conditions	~5 nm for imaging and 0.1-1% in mass for EDS (EDS resolution ~1×1×1 μm^3)
transmission electron microscope (TEM)	Philips EM 420	imaging for particles <100 nm	4 nm
high-resolution scanning TEM with EDS, HAADF[1], EELS[2]	FEI Titan 300	world-class HRTEM with multiple chemical characterization capabilities	sub-angstrom resolution for imaging and sub-nm resolution for EDS/EELS.

[1] High angle annular dark field
[2] Electron energy loss spectroscopy

Liquid products that were shown to contain silver were evaporated onto carbon-coated TEM grids and analyzed using the Philips EM 420 TEM at 120 KeV. When possible, images were analyzed using ImageJ software (National Institute of Health) for estimating the primary particle size distributions.

For fabrics and plastics that were shown to contain and leach silver, we cut or sliced pieces of each product, adhered them to SEM stubs using carbon tape, then sputter-coated each sample with gold to make them conductive, and analyzed them by SEM/EDS.

Ashing and TEM

For the baby blanket and Benny the Bear, we adapted a method applied by Benn and Westerhoff,[26] which consists of ashing the material and dusting the residue onto carbon tape for SEM. We partially ashed samples in a muffle furnace at 450 – 500°C. We then suspended samples in water, sonicated them, evaporated them onto carbon-coated TEM grids, and analyzed them using high-resolution TEM/EDS at 300 KeV. Since high-resolution TEM is a costly and time-consuming technique, it was the final step after all other microscopy techniques were employed.

To assess whether or not the ashing method altered the silver particles in the samples, we produced a control sample using a silver nanoparticle suspension applied to a non-nanosilver fabric (100% cotton t-shirt, Hanes). We dispersed this nanosilver suspension over the fabric (100% cotton) at the same silver concentration as observed in the nanosilver fabric samples and then subjected it to the same ashing and microscopy methods that were applied to the nanosilver fabric and Benny the Bear samples.

We used a method described by Heard et al.[27] to synthesize EDTA-stabilized silver nanoparticles. The resulting particles were characterized by dynamic light scattering (DLS, cumulant method) and UV-Vis absorbance spectroscopy. A sample from this nanoparticle suspension was concentrated using a 3-KDa cutoff centrifugal filtering unit (Millipore Amicon-Ultra) for approximately 60 min at 3400 G, and then 10-μl droplets were placed onto copper TEM grids coated with a holey carbon film (SPI). Samples from the nanoparticle suspension were viewed using the Philips EM 420 TEM at 120 KeV. We also diluted samples 1:100, acidified them with 5% nitric acid, and analyzed them by ICP-MS to determine the total silver concentration.

Aerosol experiments

For products with the potential to produce aerosolized nanosilver (sprays, powders, and plush toys) and that were proven to contain silver, we characterized aerosol emissions using methods

our research group has developed at Virginia Tech,[28,29] as described in SOP # AirVT-Nanosilver-004: Characterization of Aerosols Generated from Nanosilver Consumer Products (Appendix B).

We performed the aerosol emissions testing following a protocol designed to mimic real-world scenarios in which these products might be used. We simulated product use in a room that had a floor area of 13.6 m^2 and a volume of 36 m^3. It had carpeted floors, one door, and one window. The room was furnished with two desks, two cushioned chairs, a bookcase, and a wooden wardrobe. Prior to each experiment, an air conditioning unit (AC) was switched on until the room temperature dropped below 25^oC and the relative humidity dropped below 40%. The AC was then turned off, and background measurements were performed for at least 10 min before products were used.

The following products were tested for aerosol emissions:

- An ultrasonic humidifier (Safety 1st), which contained the Ionic Silver Cube in the water tank. For comparison, we repeated experiments using an identical humidifier without the silver cube that served as a blank control ("non-nano").
- The spray antifungal/antibacterial product (MesoSilver). Again, we repeated the experiment using a bottle filled with ultrapure water and with MesoSilver's spray pump as a blank control.
- A nanosilver fabric (Precious Protechtor sleepsuit). We also repeated this experiment with a 100% cotton shirt as a blank control.
- The stuffed toy (Benny the Bear).

Experimental methods were tailored to mimic each product's normal use. We placed the humidifiers in a corner of the testing room and ran them, individually, for 90 min, during which approximately 300 mL of water from the reservoirs were vaporized. We sprayed the disinfectant onto a surface located 1 m above the floor and at a horizontal distance of 0.3 m from the instrument inlets, once per minute for 30 min. We handled the fabric in a repetitive fashion for 30 min: picking it up, shaking it, folding it, and setting it back down on a surface located 0.3 m horizontally from the sampling inlets. We performed a similar procedure with the stuffed toy, by repeatedly picking it up, shaking it, and lightly beating it on the surface.

We monitored the following parameters during the aerosol experiments:

- Temperature and relative humidity
- Concentration and size distribution of aerosols between 14 and 750 nm in diameter, obtained by a Scanning Mobility Particle Sizer (SMPS 3936, TSI)
- Concentration of aerosols ranging from 300 nm to 10 μm in diameter, measured by an optical particle counter (Aerotrak, TSI), in six size channels

RESULTS AND DISCUSSION

Liquid product

Table 4.8 shows the concentration of silver obtained by ICP-MS for the liquid product (MesoSilver Antifungal/Antibacterial Disinfecting Spray) for different particle size cutoffs. This product had an advertised silver concentration of 20 ppm; we measured a concentration of ~27 ppm. About 50% of the silver mass present in the product was in the form of nanoparticles (1 – 100 nm), 24% of it seemed to be in the form of aggregates larger than 1000 nm, and 14% was in ionic form.

We assessed silver ion sorption (i.e., loss) to filter media using an ionic silver solution, obtained by dissolving ~30 mg l^{-1} silver nitrate (Fisher Scientific) in ultrapure water. Loss of ionic silver to Teflon filters was negligible, but loss to the 3-KDa centrifuge filtering membrane was 40% ± 1%. This indicates that the actual proportion of silver in ionic form in the spray product is likely higher than measured.

Table 4.8. Size-resolved silver concentrations in MesoSilver Antifungal/Antibacterial Disinfecting Spray. Values shown with standard errors (n = 3).

size cut-off	silver concentration (ppm)
> 1000 nm	6.5 ± 1.0
450 - 1000 nm	2.3± 0.6
100 – 450 nm	0.7 ± 0.3
3 KDa – 100 nm	13.7 ± 0.6
< 3 KDa	3.9 ± 0.03
total	27.1 ± 0.6

Figure 4.3 shows a TEM micrograph of MesoSilver. The product contains aggregates of widely distributed sizes and primary particles of narrowly distributed sizes. Figure 4.4 shows this

product's primary particle size distribution, estimated using Image J (with a sample size of 253 particles).

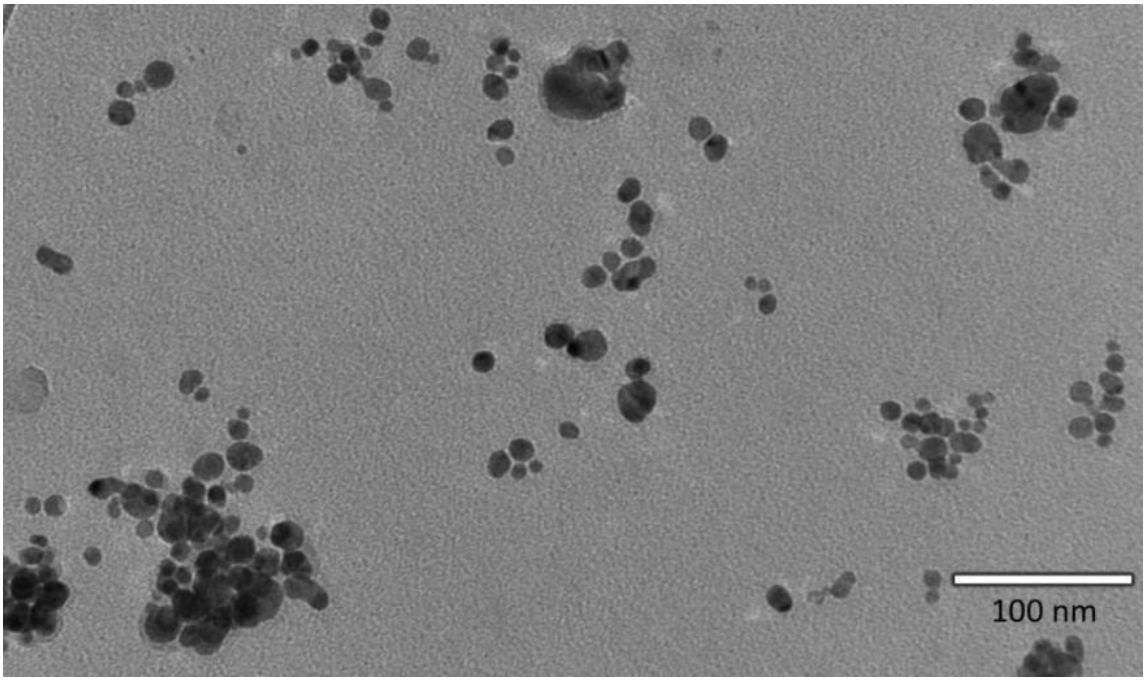

Figure 4.3. TEM micrograph of MesoSilver (disinfecting spray).

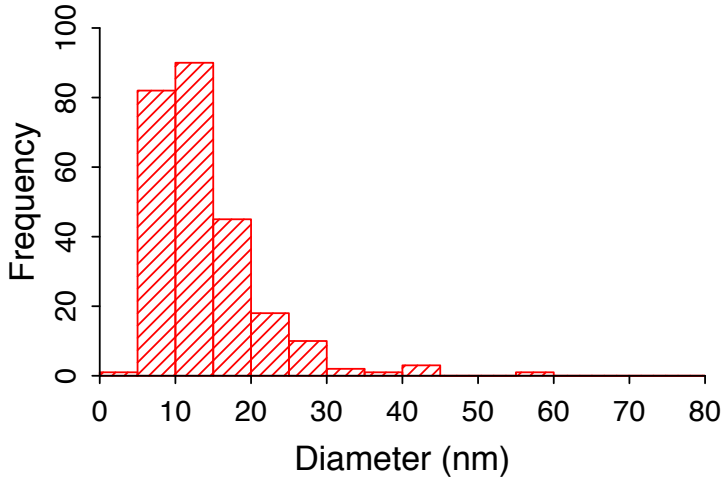

Figure 4.4. Primary particle size distribution of MesoSilver (not counting aggregates).

Even though this product contains very small primary particles (average diameter of 13.8 ± 0.4 nm), they are mostly aggregated, as can be seen in the TEM micrograph (**Figure 4.3**) and the size distribution obtained by DLS (**Figure 4.5**). The DLS size distribution has three peaks: at 0.8 nm, at 3.2 nm, and at 255 nm. DLS results are more accurate when size distributions are unimodal, so the intensities shown in **Figure 4.5** do not directly represent the amount of

particles present in each size range. The UV-Vis absorbance spectrum peaks at 396 nm (**Figure 4.5**), close to the peak for colloidal silver, which occurs in the 400-nm region. The UV-Vis peak location usually increases with particle size,[27,30] but it also depends on factors such as purity,[31] the presence of coatings,[30], oxidation state,[32] and aggregation state.

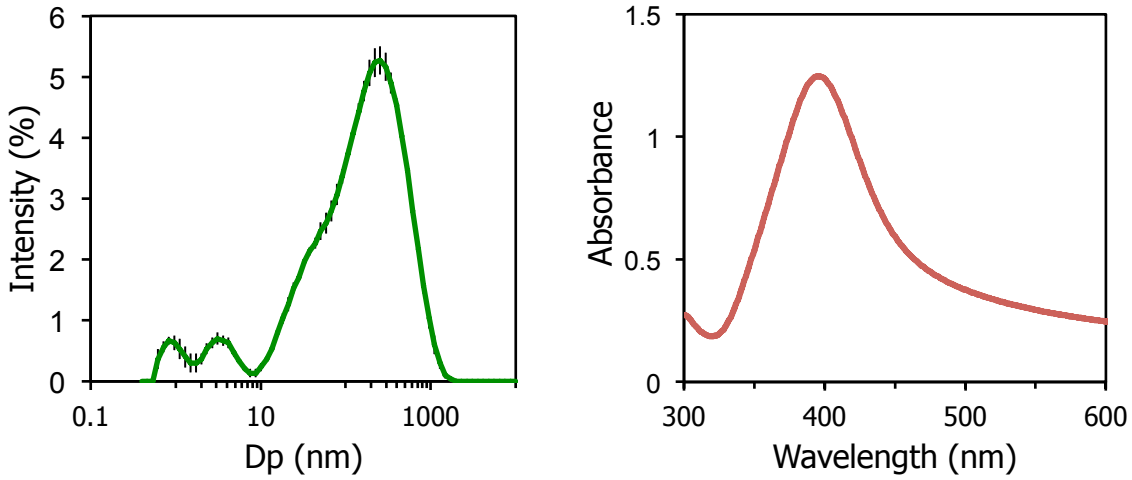

Figure 4.5. DLS size distribution (left) and UV-Vis absorbance spectrum (right) for MesoSilver.

We performed an aerosol experiment using MesoSilver. It was sprayed onto a surface once per minute for 30 min. This period is represented by the yellow shaded area in Figure 4.6, which shows the concentration of aerosols in the room during the experiment. We repeated the same experiment using a bottle filled with ultrapure water with MesoSilver's spray pump as a control.

Figure 4.6 and Figure 4.7 show that aerosol concentrations in the 14 – 750 nm range in the room were not significantly different during use of MesoSilver. Figure 4.8 shows the total aerosol concentrations, ranging from 300 nm to 10 μm in diameter, measured by an optical particle counter (Aerotrak, TSI). The total counts of larger aerosols seemed to increase over time during the use of MesoSilver, but these counts continued to increase even after spraying stopped, which suggests that the increase was caused by fluctuations in the background aerosol levels in the room.

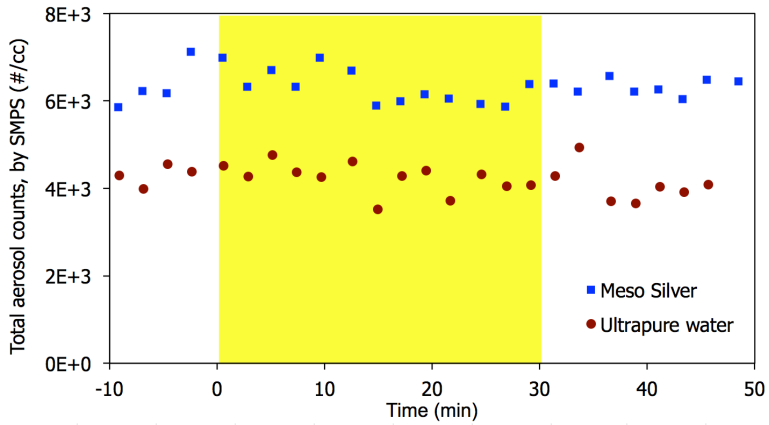

Figure 4.6. Time series of concentrations of aerosols between 14 nm and 750 nm, measured by SMPS, before, during, and after spraying of MesoSilver disinfectant and ultrapure water control. The yellow shaded area indicates the period when the product and control were sprayed.

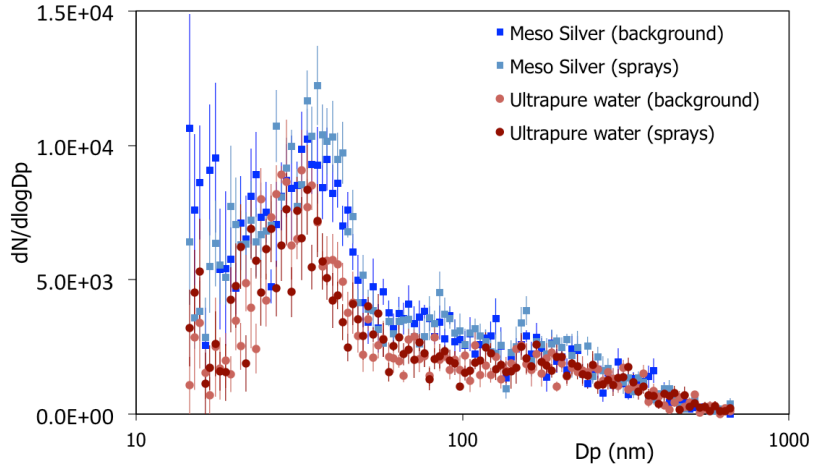

Figure 4.7. Size distributions of aerosols before and during spraying, measured by SMPS.

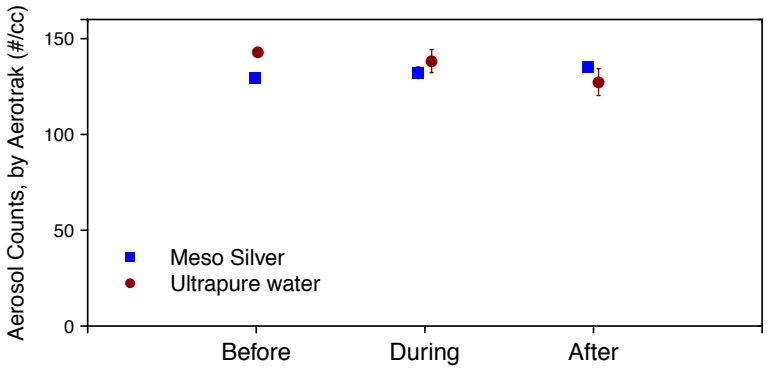

Figure 4.8. Total concentrations of aerosols between 300 nm and 10 μm in diameter, before, during, and after 30 min of spraying, measured by the optical particle counter.

Fabrics and plastics

Table 4.9 shows the total silver content of each product or product component in terms of mass of silver per mass of product. Samples that had a silver concentration lower than 0.5 ppb in the digested liquid were considered to be below the detection limit (ND). The processes used to incorporate nanosilver into the products are unknown, and it is possible that silver may not be homogeneously distributed throughout the product. Such heterogeneity may result in high standard deviations between triplicate samples. When the average result of triplicate samples had a high relative standard error (> 20%), we collected and analyzed three more samples to bring the total sample size to six.

The interior foam of Benny the Bear had a silver concentration 8 times higher than that in the exterior fur. Silver did not seem to be homogeneously distributed throughout the foam, as measurements had a relatively high standard error (10%). All Precious Protechtor fabric samples had similar silver concentrations, averaging 107 ± 2 mg Ag/kg product, which suggests that the products were manufactured from the same type of fabric and that the silver application onto the fabric was spatially homogeneous. Most plastic samples had low or no silver present, with the exception of a rubber ring present in Sippy Cup #1 (24 mg Ag/kg product) and the transparent blue cap in the same product (9.5 mg Ag/kg product). The surface wipe and the kitchen scrubber presented similar silver concentrations (~4 mg Ag/kg product), but the silver was more homogeneously distributed in the scrubber than in the surface wipes.

Table 4.9. Total silver concentration in consumer products (± standard errors).

	product	sample size (n)	total silver concentration (mg ag / kg product)
1	Benny the Bear: exterior	3	0.6 ± 0.1
2	Benny the Bear: interior	6	48.2 ± 5.0
3	Pacifier[1]	-	-
4	Precious Protechtor sleepsuit or babygro	3	109.8 ± 4.1
5	Precious Protechtor baby blanket	3	108.3 ± 1.7
6	Precious Protechtor baby scratch mitts	3	104.2 ± 4.0
7	breast milk storage bags	6	0.9 ± 0.6
8	sippy cup #1, light blue: outside of cup	3	ND[2] (0.24 ± 0.04)

Table 4.9. (continued) Total silver concentration in consumer products (± standard errors).

product	sample size (n)	total silver concentration (mg ag / kg product)
10 sippy cup #1, light blue: rubber ring	3	24.3 ± 2.9
11 sippy cup #1, light blue: white rim	3	4.9 ± 0.2
12 sippy cup #1, light blue: transparent blue cap	3	9.5 ± 1.0
13 sippy cup #2, dark blue: outside of cup	3	ND (0.39 ± 0.01)
14 sippy cup #2, dark blue: inside of cup	3	ND (0.32 ± 0.19)
15 sippy cup #2, dark blue: spout	3	ND (0.45 ± 0.29)
16 sippy cup #2, dark blue: clear spout cover	6	ND[3] (2.12 ± 1.47)
18 Nature's Fresh Silver Shield surface wipes	9	4.5 ± 3.0
19 Easy-Well Perfect Nano Silver scrubber	3	4.6 ± 0.3
20 non-nano fabric	3	ND (0.07 ± 0.02)

[1]Not purchased in time for this project.
[2]Not detected. Silver concentrations in the digestion liquid were below 0.5 ppb.
[3]Since this product's silver concentrations were highly variable between samples (0.2 – 9.3 mg Ag / kg product), we elected to subject this product to leaching.

Standard nanosilver suspension for ashing control

We synthesized silver nanoparticles in suspension for use in experimental controls. The suspension had a silver concentration of 23.8 ppm. According to DLS results, the silver nanoparticles had an average diameter of 36.2 ± 0.2 nm and were monodisperse (polydispersity index of 0.1). The solution was bright yellow and had an absorption peak at 405 nm, which is indicative of non-aggregated silver nanoparticles.

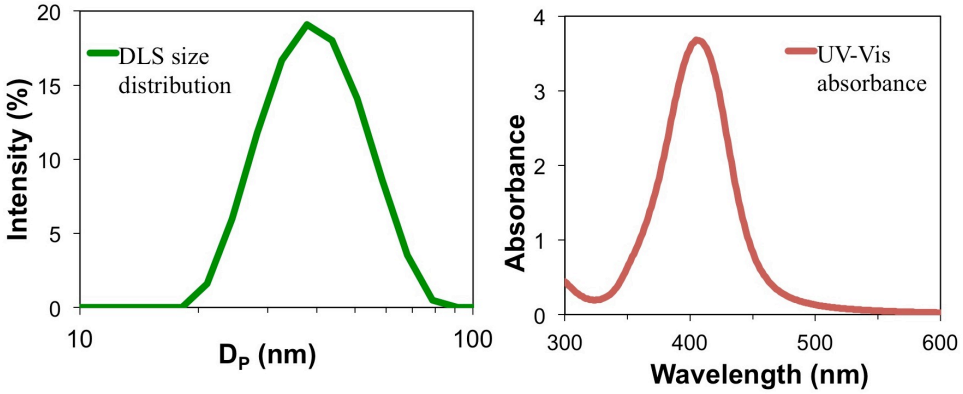

Figure 4.9. DLS size distribution (left) and UV-Vis absorbance spectrum (right).

Figure 4.10 shows a TEM micrograph of the silver nanoparticles synthesized in our laboratory. The average particle diameter, measured by DLS, was 36.2 ± 0.2 nm. The average particle diameter estimated by Image J (image processing and analysis software) was 25.1 ± 0.6 nm (Figure 4.11). Particle diameters measured by DLS are usually larger than those measured from TEM micrographs, probably because a few layers of water molecules that surround each particle affect the particles' scattering properties.

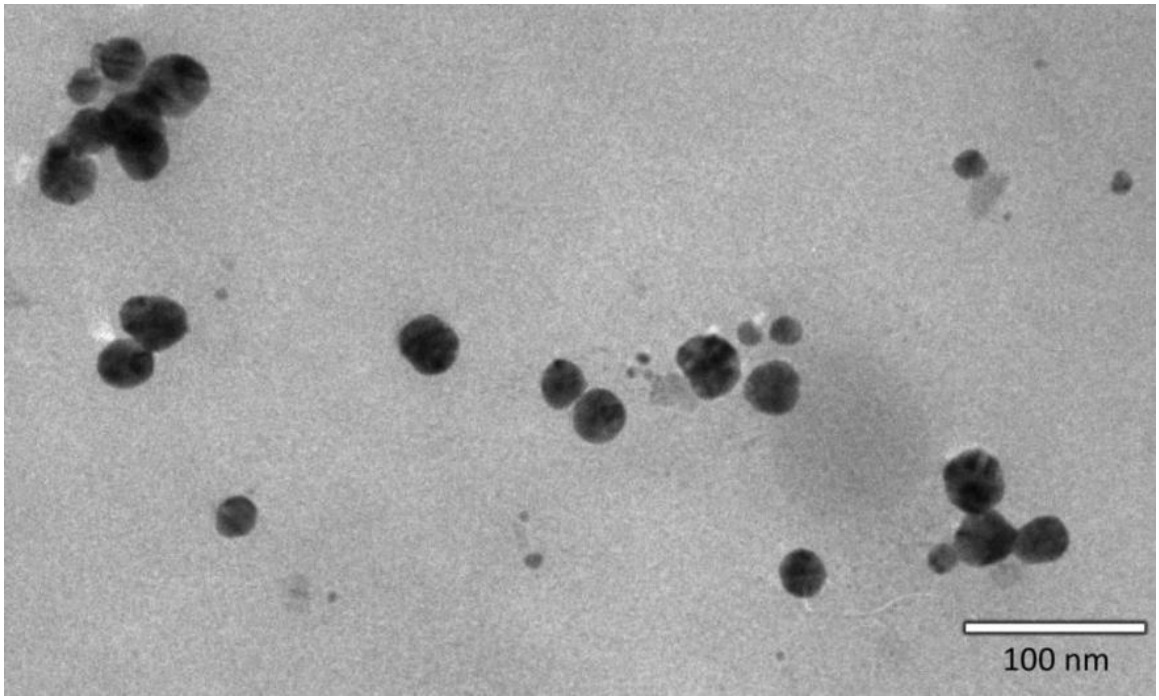

Figure 4.10. TEM micrograph of nanosilver particles synthesized in our laboratory.

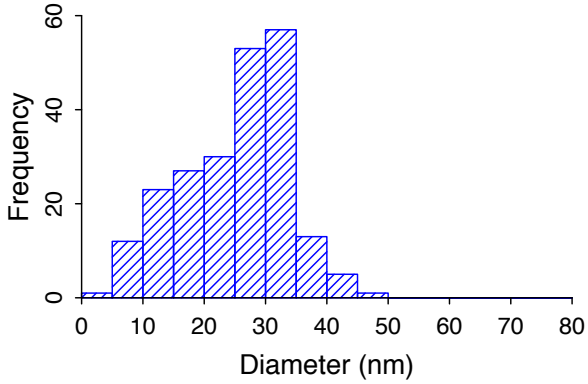

Figure 4.11. Particle size distribution of nanosilver particles in liquid media, estimated using Image J (sample size = 222 particles).

Figure 4.12 shows an SEM image of the control fabric (100% cotton t-shirt) treated with the standard nanosilver suspension. This image was obtained using the backscattered electron

detector, through which heavy elements shine brighter. The bright spots observed on the fiber are silver nanoparticles and nanoparticle aggregates. EDS spectra for this sample can be found in Appendix B.

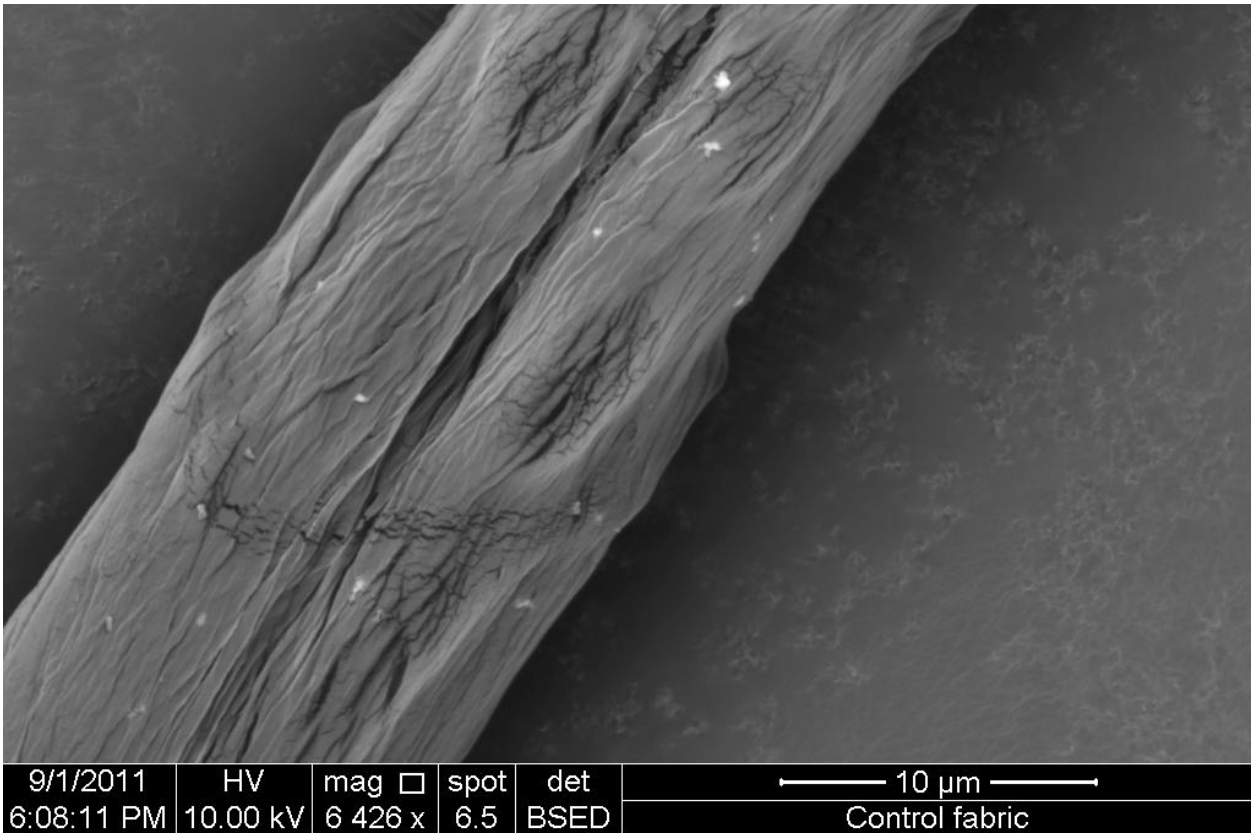

Figure 4.12. SEM micrograph of the control fabric treated with a standard nanosilver suspension (obtained in backscattered mode).

Figure 4.13 shows an HR-TEM micrograph of an ash sample from the control fabric treated with the standard nanosilver suspension. We collected EDS spectra from 10 particles in this image, and they were confirmed to contain silver (Appendix B). Comparing this image with the micrograph of the nanosilver suspension (Figure 4.10), we can infer that the single primary particles remained unaltered, but the aggregated particles were sintered into larger particles that exhibit dark features and have irregular shapes. However, since the nature of surface coatings applied to the consumer products' particles is unknown, we cannot affirm with full certainty that those particles behave exactly as the EDTA-stabilized particles under heating due to possible differences in surface functionality.

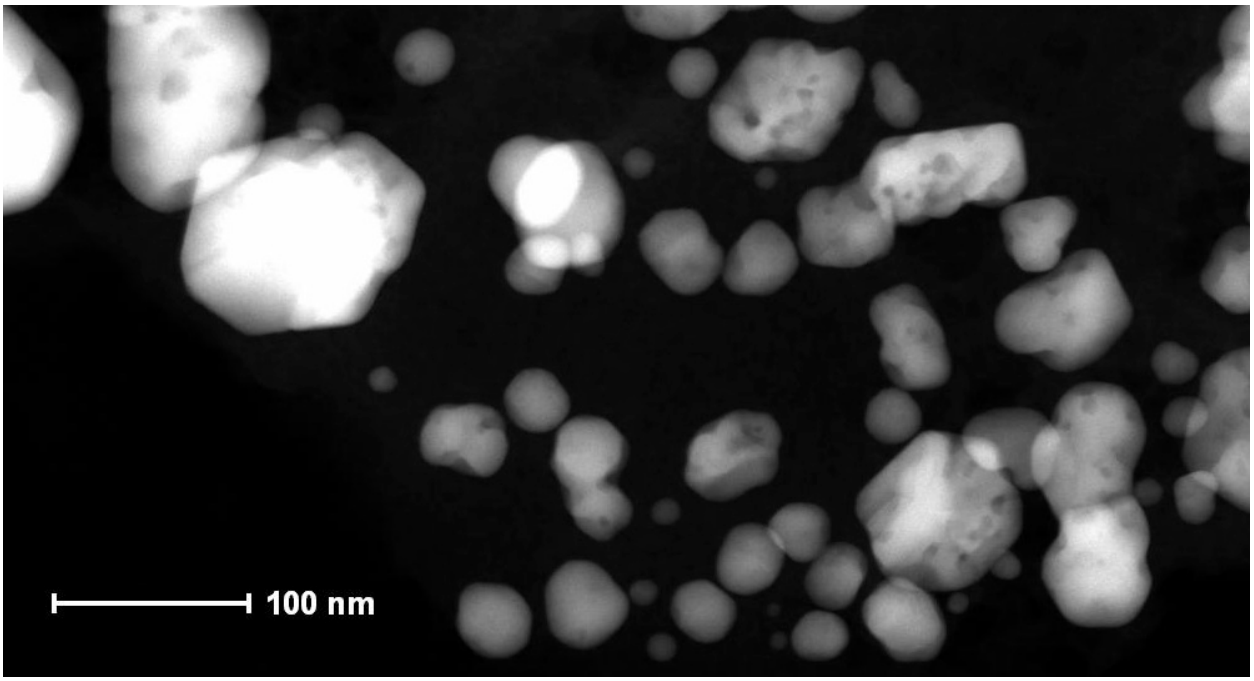

Figure 4.13. High angle annular dark field (HAADF) micrograph of ashed sample from the cotton t-shirt treated with a standard nanosilver suspension.

Microscopy analysis of fabrics and plastics

Figure 4.14 shows an SEM micrograph of a fiber from the baby blanket. The circle indicates a location where a silver peak was observed using EDS (spectrum shown in Appendix B). Although we were not able to observe silver in other locations in this image, it is likely that the small bright spots are silver nanoparticles that did not emit a strong enough signal to be detected by this EDS, which has a spot size of 1 μm × 1 μm.

Figure 4.15 shows an HR-TEM micrograph of an ashed sample from the baby blanket. The sample is composed of many small (6 – 15 nm) silicone dioxide particles, probably a byproduct from the ashing of the fabric, and larger (~40 nm), multifaceted silver nanoparticles. Figure 4.16 shows a micrograph from the same sample in detail. The silver nanoparticles do not exhibit the same dark features that would indicate sintering of smaller units. The EDS spectra from this sample are shown in Appendix B. We estimate the average particle diameter to be 23.8 ± 0.7 nm (Figure 4.17).

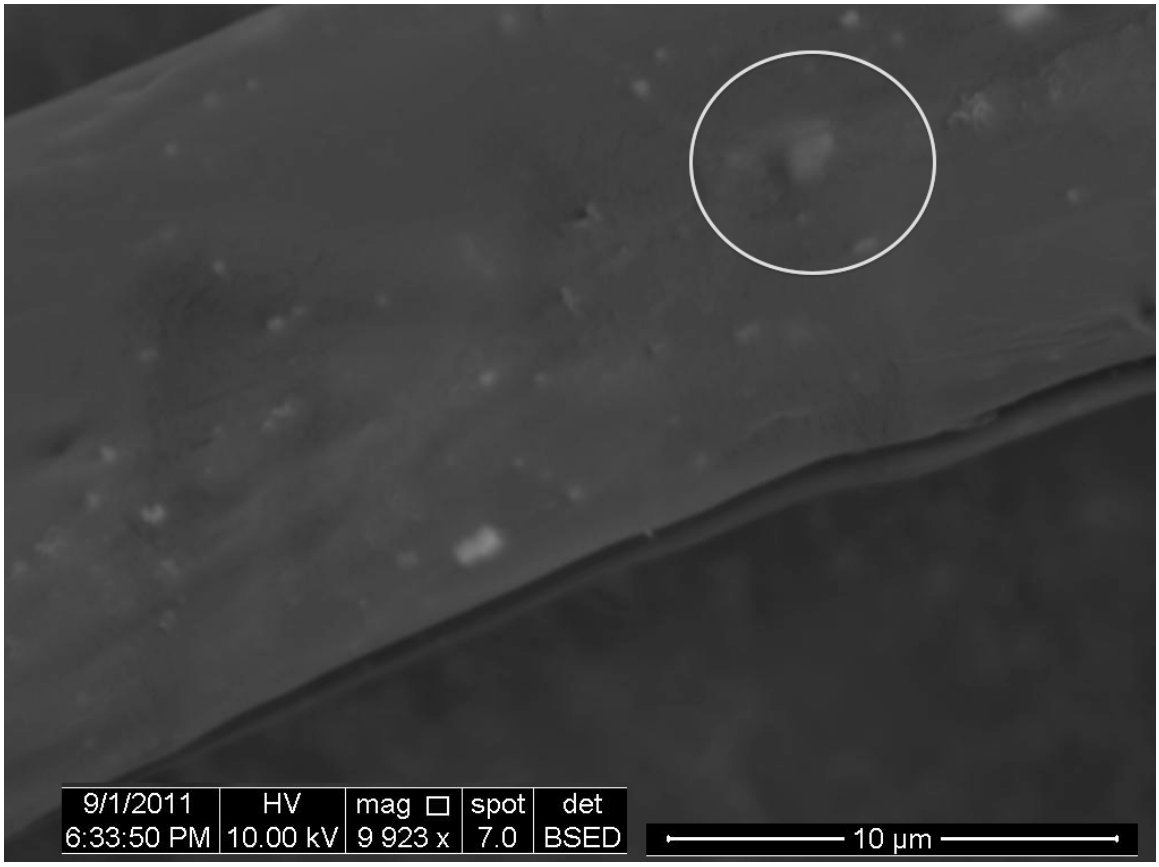

Figure 4.14. SEM micrograph of the baby blanket (obtained in backscattered mode).

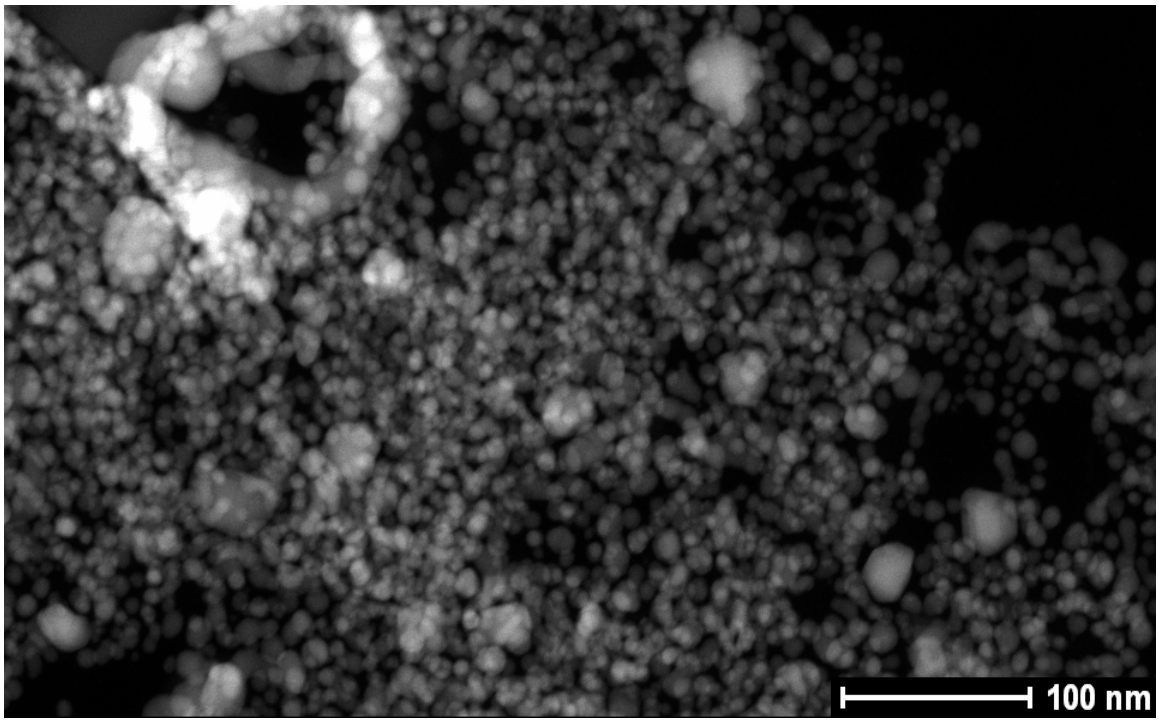

Figure 4.15. HAADF micrograph of ashed sample from the baby blanket.

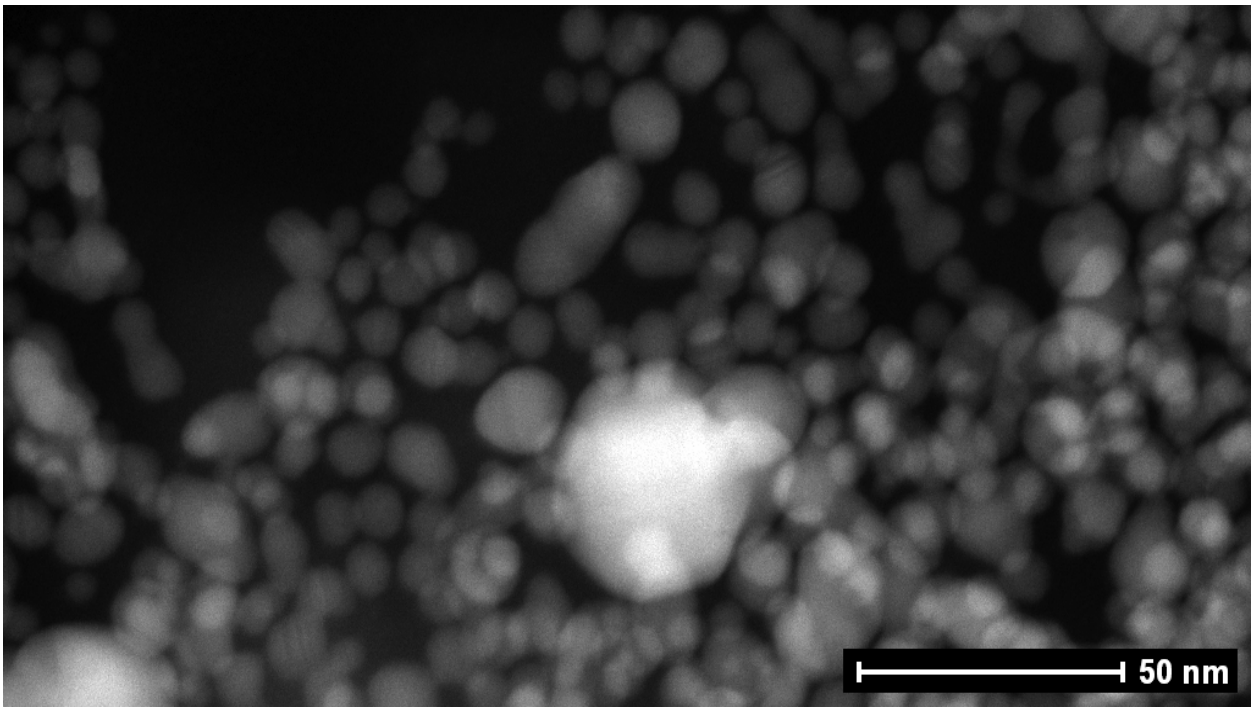

Figure 4.16. HAADF micrograph of ashed sample from the baby blanket (detail showing one silver nanoparticle).

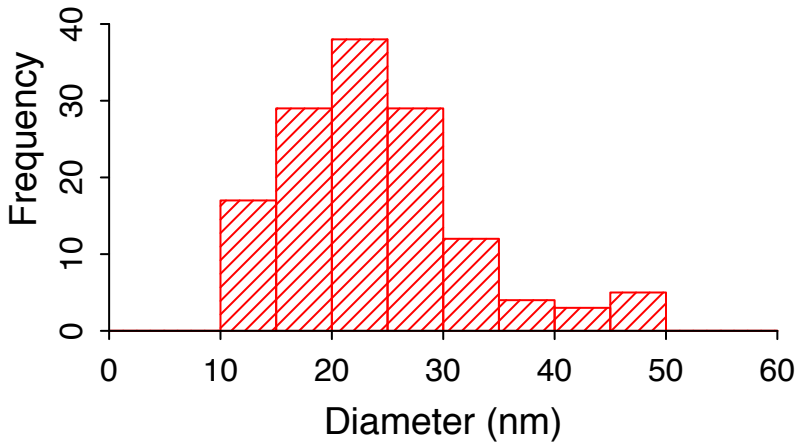

Figure 4.17. Particle size distribution of nanosilver particles visualized by HR-TEM, estimated using Image J (sample size = 137 particles).

We were able to locate a few silver-containing particles in the fibers of Benny the Bear's fur using SEM, indicated by the circles in Figure 4.18.

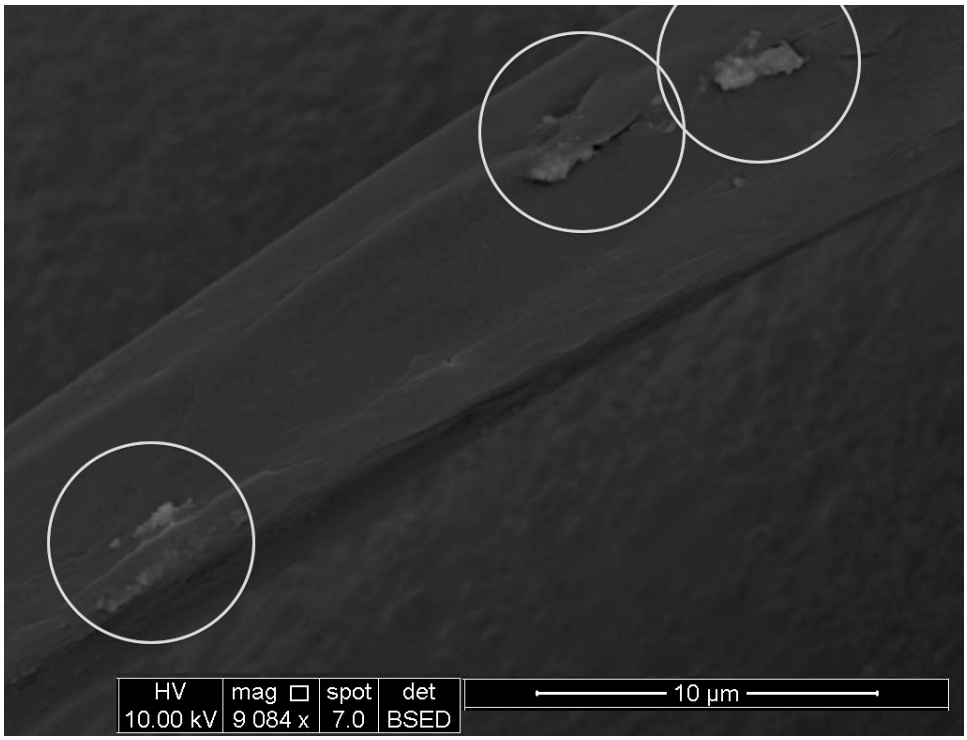

Figure 4.18. SEM micrograph of a fiber from Benny the Bear's exterior (backscattered mode).

Using SEM, we were able to find a wide range of silver-containing particles, from ~100-nm particles/aggregates to >2000-nm aggregates. These appear as bright spots in Figure 4.19.

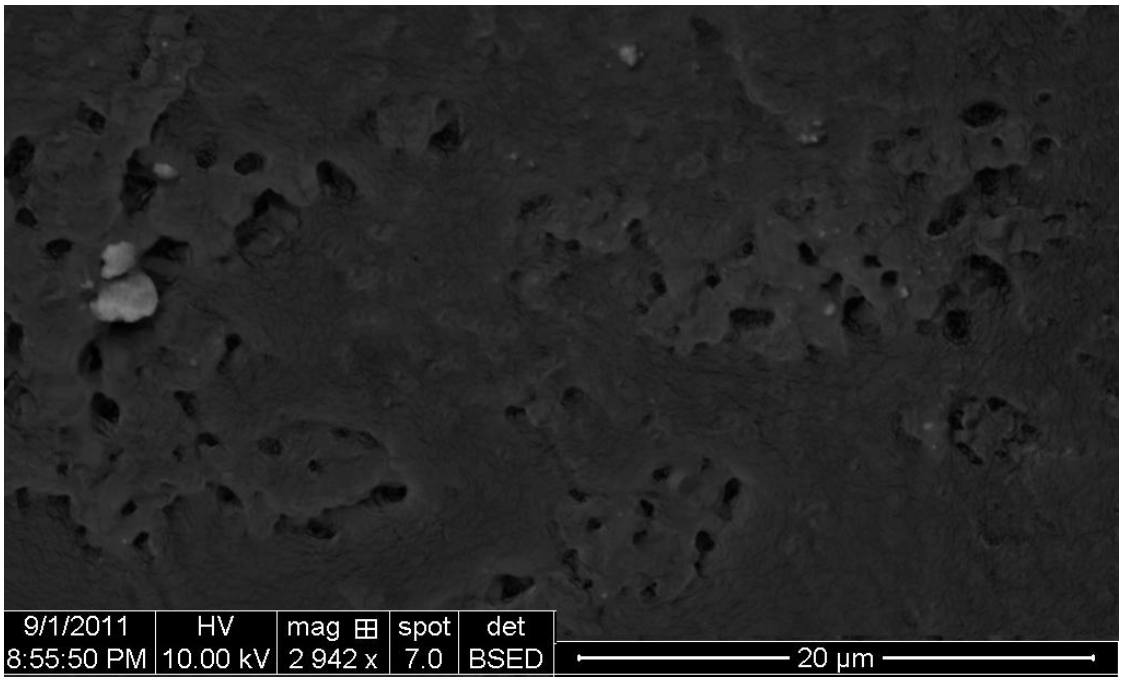

Figure 4.19. SEM micrograph of Benny the Bear's interior (obtained in backscattered mode).

In the ashed samples of Benny the Bear's interior, silver nanoparticles were not as abundant as in the other ashed samples. Figure 4.20 shows a silver nanoparticle aggregate consisting of primary particles that are 15 - 21 nm in diameter.

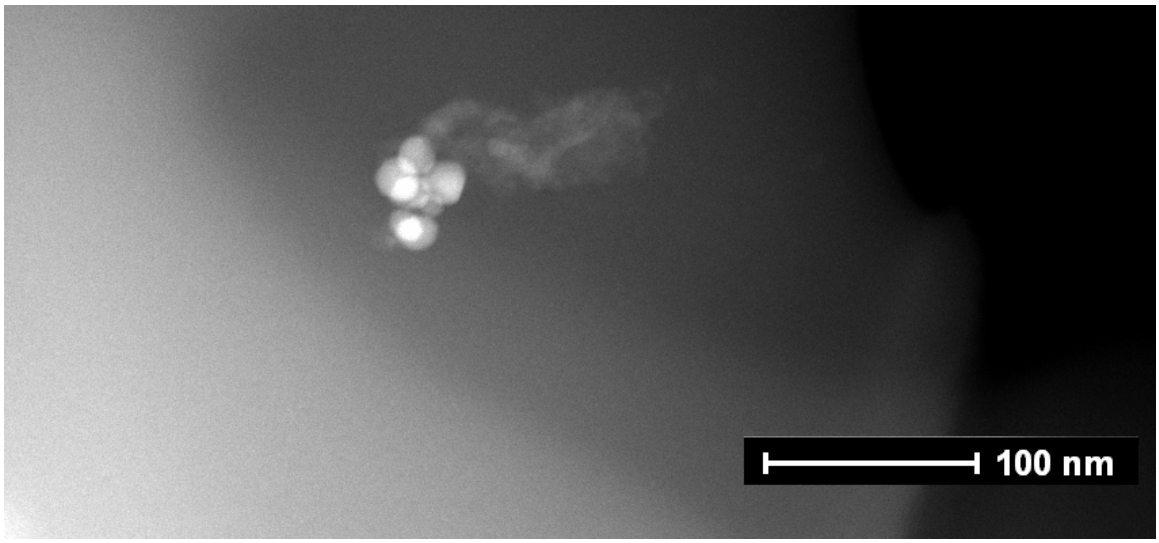

Figure 4.20. HAADF micrograph of ashed sample from the interior foam of Benny the Bear.

For the plastic samples (sippy cups), we were able to identify silver-containing particles using SEM/EDS on Sippy Cup #1.

Figure 4.21 shows one silver-containing particle (~2 μm in size) from the rubber ring from Sippy Cup #1. **Figure 4.22** is an SEM micrograph of the transparent blue cap from Sippy Cup #1, showing relatively large silver particles ranging from ~600 nm – 10,000 nm in diameter.

We were not able to observe silver-containing particles in the surface wipe or the clear spout cover from Sippy Cup #2 under SEM or TEM.

We measured the potential for aerosol exposure associated with handling of the nanosilver fabric and the stuffed toy. The same procedure was also performed with a cotton t-shirt as a non-nanosilver control and with Benny the Bear. **Figure 4.23** shows a time series of total concentrations of aerosols between 14 and 750 nm in diameter, where the yellow shaded area represents the 30-min period when materials were shaken.

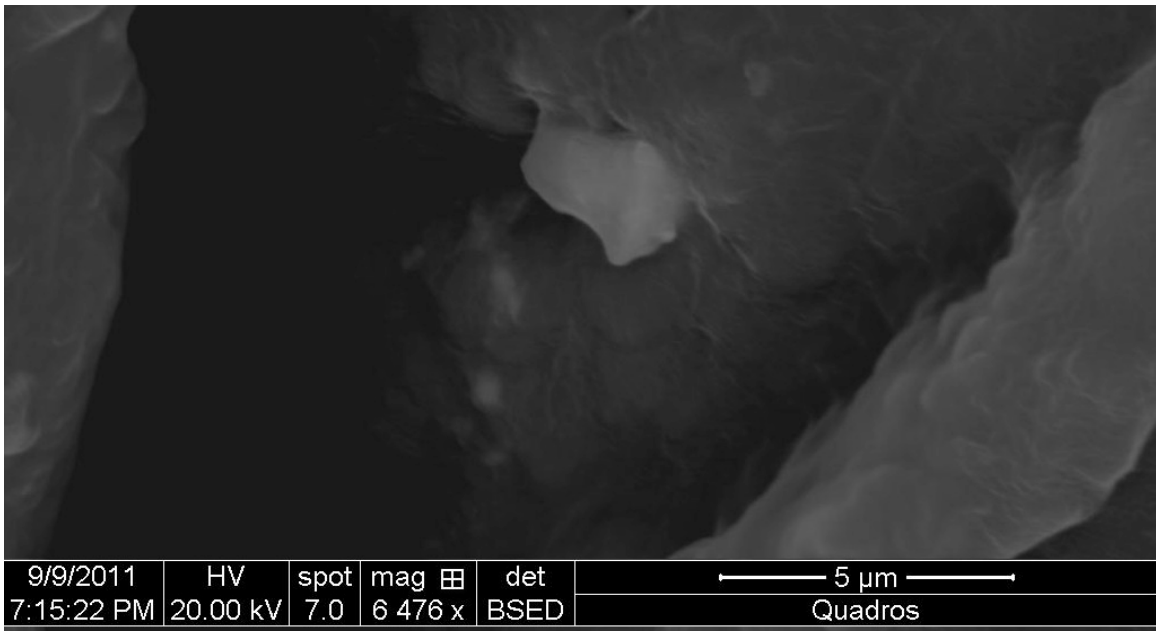

Figure 4.21. SEM micrograph of the rubber ring from Sippy Cup #1 (backscattered mode).

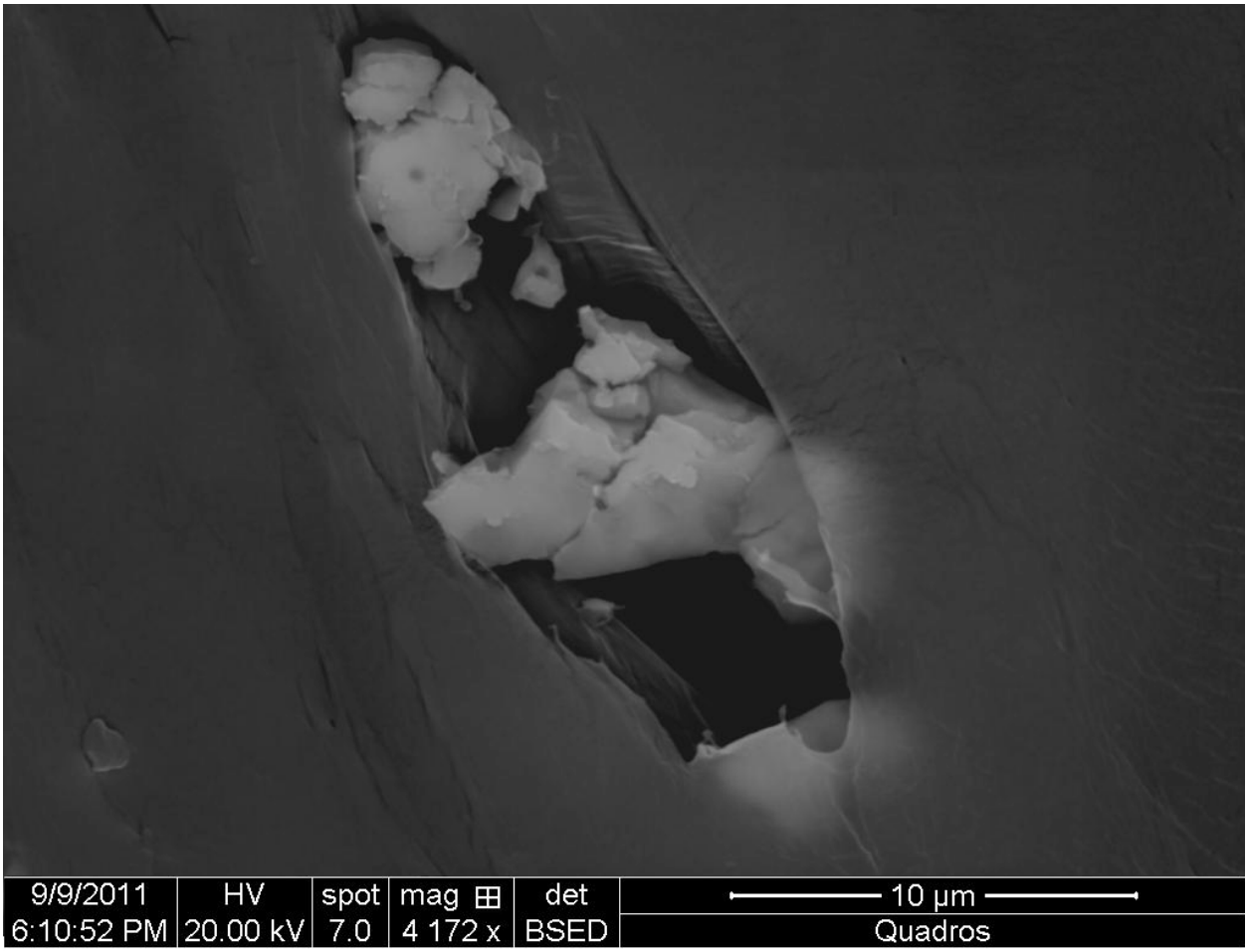

Figure 4.22. SEM micrograph of the transparent blue cap from Sippy Cup #1 showing silver particles lodged within the plastic matrix (backscattered mode).

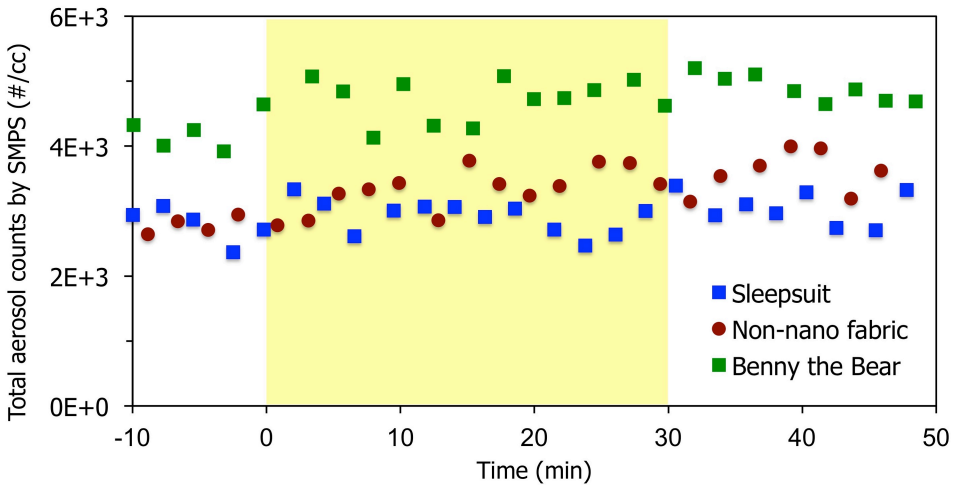

Figure 4.23. Time series of concentrations of aerosols between 14 nm and 750 nm before, during, and after handling of stuffed toy and fabrics. The yellow shaded area indicates the period when the products were handled.

Figure 4.24 and Figure 4.25 show the size distributions of aerosols in the room before and during the shaking procedure.

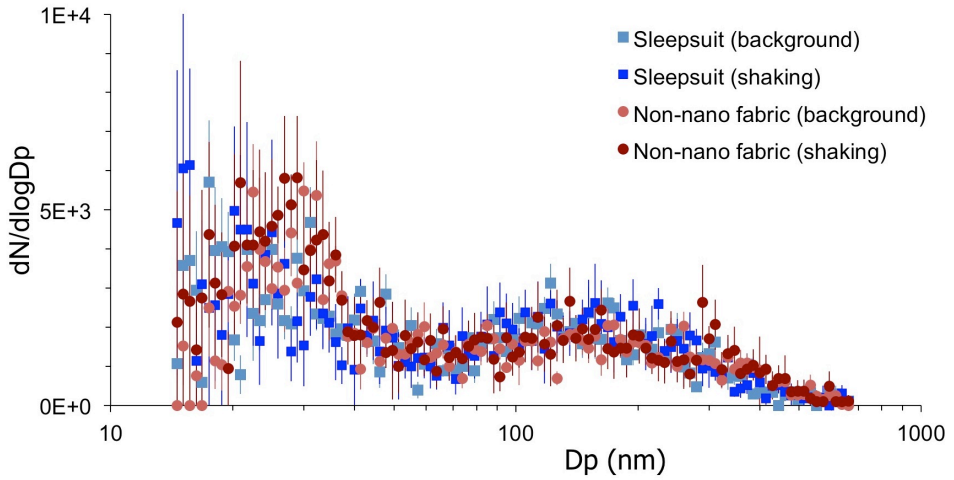

Figure 4.24. Size distributions of aerosols before (background) and during shaking.

As with the spray product, handling of the nanosilver fabric (Precious Protechtor Sleepsuit) and the stuffed toy (Benny the Bear) did not result in elevated aerosol concentrations in the 14 – 750 nm size range.

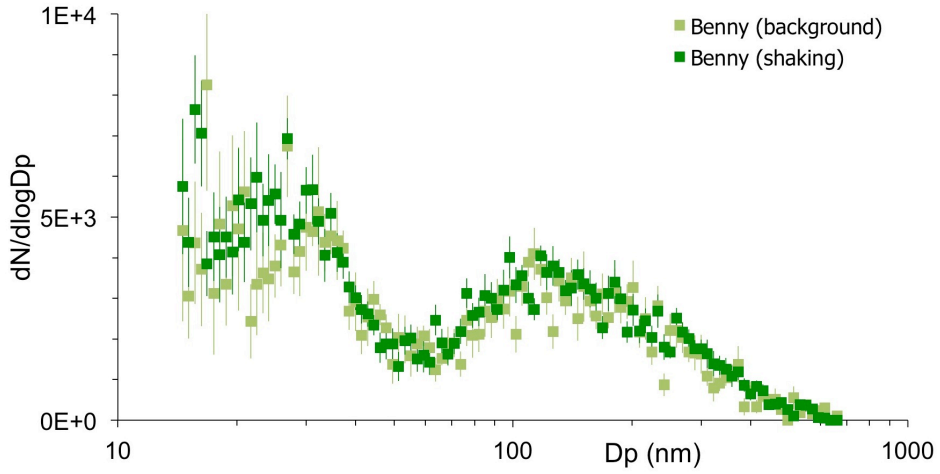

Figure 4.25. Size distributions of aerosols before (background) and during shaking.

Figure 4.26 shows the aerosol concentrations, ranging from 300 nm to 10 μm in diameter, measured by the optical particle counter. The concentrations of larger aerosols seemed to decrease over time during the experiments with Benny the Bear and the nanosilver sleepsuit. Aerosol concentrations increased during shaking of the non-nano cotton t-shirt and then decreased afterwards. Since this behavior was not observed during the shaking of the nanosilver material, we believe it can be attributed to the suspension of particles from the fabric or fluctuations in the background aerosol concentrations.

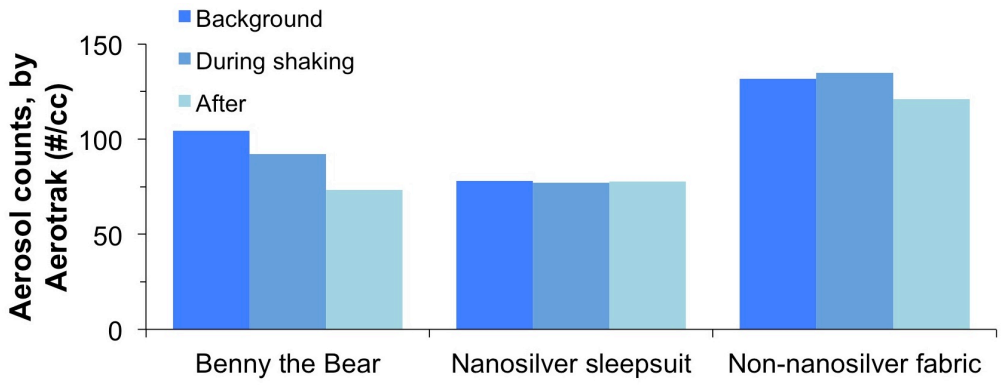

Figure 4.26. Total concentrations of aerosols between 300 nm and 10 μm in diameter, before, during, and after 30 min of handling.

The aerosol emissions testing followed a protocol designed to mimic real-world scenarios in which these products might be used. In these scenarios, none of the products produced aerosol concentrations that were significantly different from background levels. If these products emit any form of silver-containing aerosols, the emission rates are extremely low and could only be measured in a small chamber with very low background aerosol concentrations.

Humidifiers

Table 4.10 shows silver concentrations in water stored in the reservoirs of four different humidifiers. In all cases, silver concentrations were below the ICP-MS' detection limit (1 ppb).

Table 4.10. Silver concentrations in humidifier reservoir water after different periods of soaking.

product	soaking time (days)	silver concentration (ppb)
Germ Guardian H1000 tabletop humidifier	6	ND[1] (0.07 ± 0.01)
	18	ND (0.14 ± 0.01)
Germ Guardian H2000 manual ultrasonic humidifier	6	ND (0.11 ± 0.01)
	18	ND (0.03 ± 0.01)
Stadler Form Ionic Silver Cube (into a non-nano humidifier)	5	0.8 ± 0.2
	17	ND (0.05 ± 0.01)
non-nano humidifier (blank)	5	ND (0.11 ± 0.01)

[1]Not detected. Silver concentrations in the ICP-MS samples were below 0.5 ppb.

Table 4.11 shows silver concentrations in the vapor collected from the humidifiers' output. Only the Germ Guardian Table Top humidifier emitted silver-containing vapors.

Table 4.11. Silver concentrations in humidifier vapor during use.

product	silver concentration (ppb)
Germ Guardian H1000 tabletop humidifier	2.3 ± 0.7
Germ Guardian H2000 manual ultrasonic humidifier	ND[1] (0.16 ± 0.02)
Stadler Form Ionic Silver Cube (into a non-nano humidifier)	ND (0.08 ± 0.01)
non-nano humidifier (blank)	ND (0.17 ± 0.01)

[1]Not detected. Silver concentrations in the ICP-MS samples were below 0.5 ppb.

We performed the aerosol experiments using two Safety 1st humidifiers, one of which contained the Ionic Silver Cube in the water reservoir. Figure 4.27 shows a time series of the concentration of aerosols between 14 and 750 nm in diameter. Background measurements began ~15 min before the humidifiers were turned on at time t = 0 min. The humidifiers ran for 90 min, and this period is represented by the yellow shaded area in Figure 4.27.

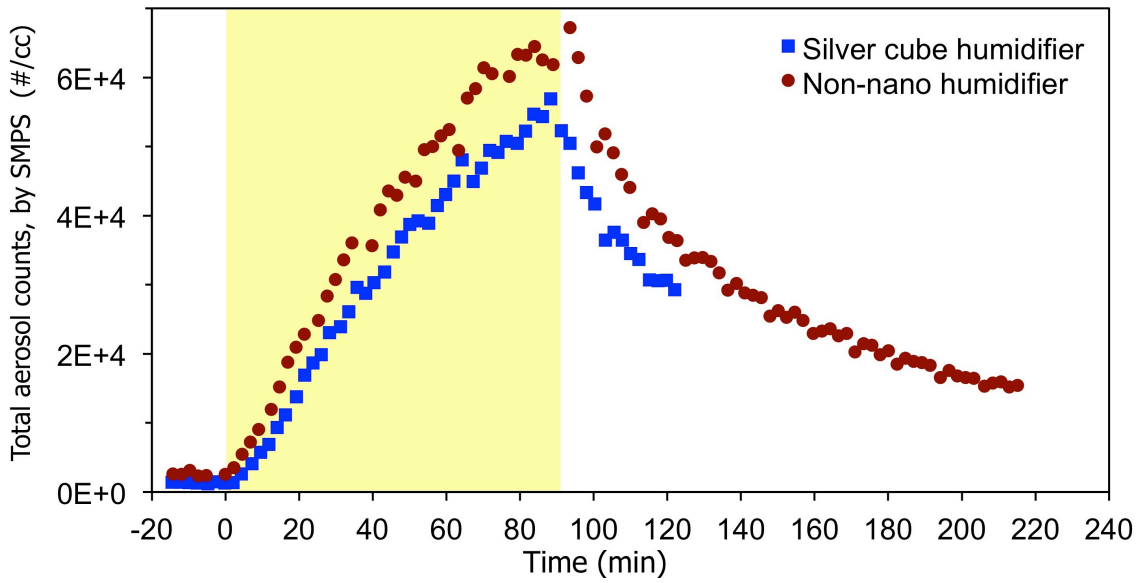

Figure 4.27. Time series of concentrations of aerosols between 14 nm and 750 nm before, during, and after humidifier operation. The yellow shaded area indicates the period when the humidifiers were running.

Ambient aerosol concentrations (14 – 750 nm) were not higher during use of the humidifier that had the silver cube in its water reservoir. The same was also true for larger aerosols, ranging from 300 nm to 10 μm in diameter. Concentrations of these aerosols were 1467 ± 5 and 1593 ± 5 cm^{-3} above background levels for the humidifier containing the Ionic Silver Cube and the one without it, respectively. In fact, higher aerosol concentrations were associated with the non-nano humidifier.

Figure 4.28 shows the size distributions of background aerosols in the room and of those corresponding to the highest concentrations during use of the humidifiers. While concentrations were elevated above background during use of the humidifiers, they were not significantly different for the humidifier with the Ionic Silver Cube compared to the one without it.

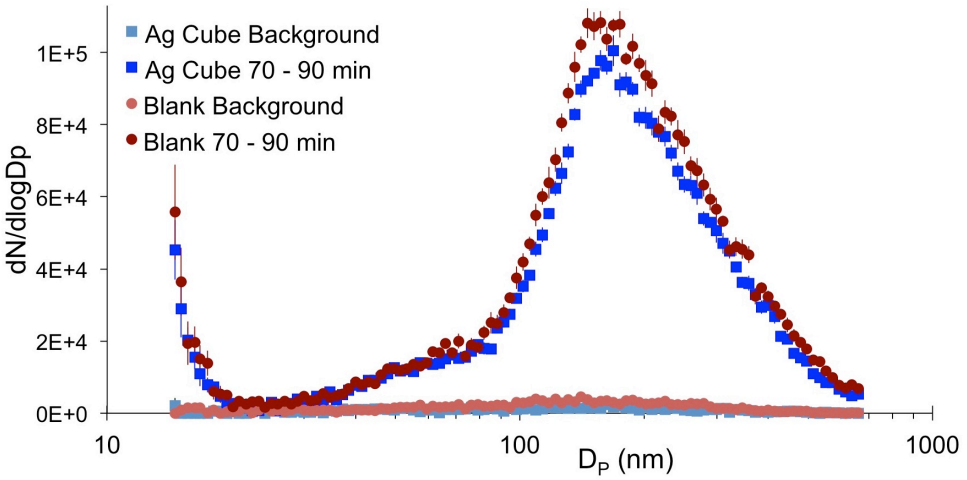

Figure 4.28. Aerosol size distributions during operation of the humidifiers The "blank" humidifier is the one without the Ionic Silver Cube.

These results show that use of the Ionic Silver Cube was not associated with greater potential for exposure to aerosols under these experimental conditions.

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Chapter 5: Release of silver from children's nanotechnology consumer products

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ABSTRACT

The objective of this work was to assess the potential for exposure of children to silver during use of nanotechnology-based consumer products. We measured the release of silver from a plush toy, fabrics, breast milk storage bags, sippy cups, cleaning products, and humidifiers. We determined the amount of silver that leached into tap water, orange juice, and milk formula and synthetic formulations of sweat, urine, and saliva. Sweat and urine yielded the largest release of silver, 6% to 38% of total silver in the products, while tap water yielded the lowest amount, 0.5% to 2.6%. Results suggest that release of silver occurs mainly via dissolution and is facilitated in media with high concentrations of salts and low pH. Leaching from the blanket into sweat plateaued within 5 min, and less silver was released after washing. In assessing the potential for dermal exposure, we found that 0.3 - 23 $\mu g\ m^{-2}$ of silver transferred from products to wipes. Product use did not produce significant amounts of aerosols. The amount of silver to which children would be exposed during normal use of these products is likely to be low, and the silver released is expected to be in ionic rather than particulate form.

KEYWORDS: children, consumer products, dissolution, leaching, nanoparticle, silver, nanosilver.

INTRODUCTION

Silver nanoparticles (nanosilver) are known for their broad-spectrum antimicrobial properties,[1-4] which have led to many applications in consumer products, such as disinfecting sprays, cosmetics, fabrics, and household appliances.[5,6] Such widespread use has led to concerns because exposure to silver is associated with chronic health effects, such as argyria,[7,8] and the safety of nanosilver has not been established. Ingested nanosilver has been shown to accumulate in various organs and to cause slight liver damage in rats.[9] Inhalation exposure to nanoparticles can lead to respiratory inflammation and cardiovascular disease,[8,10,11] and nanosilver has been shown to be more toxic than chrysotile asbestos fibers to lung cells.[12] Inhaled nanosilver has also been shown to translocate to the brain and other organs in rats.[13]

Compared to adults, children are at a higher risk for exposure because (1) they have a higher metabolism and surface-area-to-mass ratio, (2) their organs and tissues are still under development, (3) they have more years ahead of them to develop health conditions from chronic exposure to emerging materials,[14] and (4) they have a higher tendency to place hands and objects in the mouth.[15] Guney and Zagury[16] call for the development of regulations concerning toxic substances in toys and children's products based on risk assessment rather than just total contaminant content.

Although there have been studies of the toxic effects of silver nanoparticles to human cells and the environment, there still exists a large gap in knowledge about realistic human exposure scenarios during the use of nanosilver consumer products. Previous studies have shown that silver leaches from consumer products into water,[17-21] but more information is needed to describe the release of silver into biologically relevant liquid media in exposure scenarios that are relevant to real-world use. The objective of this work is to determine the potential for exposure of children to silver from consumer products that employ silver nanotechnology. Specific objectives are to quantify the silver released into media relevant to each product's expected use and to determine how differences in liquid media composition control dissolution of silver from products.

EXPERIMENTAL METHODS

Products and release scenarios

We compiled an inventory of 82 consumer products that claim to contain nanosilver and that may be used by or around children.[22] From that inventory, we selected 13 products for testing, including one plush toy (i.e., a teddy bear), three fabric products (one baby blanket, one sleepsuit, and one pair of baby mitts), one set of breast milk storage bags, two sippy cups, three cleaning products (a spray, a surface wipe, and a kitchen scrubber), two humidifiers, and one humidifier accessory, a cube that was to be placed in a humidifier's reservoir. Because some of the 13 products consisted of multiple pieces, they generated a total of 21 items for testing, of which 15 were shown to contain silver.[22] We analyzed these 15 items for their potential to release silver under conditions of real-world use. We determined release scenarios for each item based on the product's intended use and whether it might come into contact with liquids such as water, milk, saliva, sweat, or urine, might directly touch the skin, or might release aerosols (Table 5.1).

Table 5.1. Release scenarios for each product.

product	scenario
plush toy: exterior (new)[a]	leaching: tap water, saliva, sweat, urine dermal assay aerosol emissions
plush toy: exterior (aged)	leaching: tap water, saliva, sweat, urine
plush toy: interior[a]	leaching: tap water, saliva, sweat, urine
baby blanket[b] (new)	leaching: tap water, saliva, sweat, urine dermal assay aerosol emissions
baby blanket (aged)	leaching: tap water, saliva, sweat, urine
breast milk storage bags	leaching: milk formula
sippy cup #1: rubber ring	leaching: milk formula, orange juice[c]
sippy cup #1: transparent cap	leaching: milk formula, orange juice
sippy cup #2: spout cover	leaching: milk formula, orange juice
disinfecting spray	dermal assay aerosol emissions
surface wipes	dermal assay
kitchen scrubber	dermal assay

Table 5.1. (Continued) Release scenarios for each product.

product	scenario
tabletop ultrasonic humidifier	leaching: tap water
manual ultrasonic humidifier[d]	leaching: tap water
humidifier accessory cube[e]	leaching: tap water aerosol emissions

[a]The interior foam and exterior fur of the plush toy were tested for leaching separately.

[b]Since all three fabric products (baby blanket, sleepsuit, and scratch mitts) had approximately the same silver concentration, we concluded the fabric used in all three was the same and examined release scenarios with only one product.

[c]The sippy cup leaching experiments were restricted to two leaching media because of the small sample mass available for use.

[d]Described by the manufacturer as "manual," probably because it lacks a hydrostat and must be turned on and off by hand.

[e]Tested in a conventional, non-nanosilver humidifier.

Silver release into liquid media

The leaching assays consisted of soaking product samples in relevant liquid media under various conditions related to normal use. The leaching media included tap water from the town of Blacksburg, Virginia (2.9 mg/L total chlorine, pH 7.6); synthetic saliva,[23] sweat,[21] and urine,[24] which were prepared and stored in a refrigerator at ~5°C before use; milk formula (Gerber Good Start Infant Formula) prepared using tap water, according to the manufacturer's instructions; and orange juice (Kroger brand from concentrate, no pulp). A detailed description of all leaching media is included in the Supporting Information. We weighed ~0.5-g pieces of each product, placed each of them in a 100-ml beaker, and added enough liquid media to achieve a 1:50 ratio between the product mass and leaching media.[25] For saliva, to simulate chewing, we placed 3 – 5 samples of 0.1 – 0.2 g of product into 2-ml bead-beating vials and added ~0.3 g of 1-mm glass beads and 1 – 1.5 ml of synthetic saliva at ~37°C. We beat samples for 30 s in a bead beater at 2500 rpm and then combined them into one composite sample. We repeated this procedure twice more to produce three replicates. To each replicate, we added more saliva to obtain a 1:50 ratio of product mass to leaching media.

The soaking time depended on each product's intended use and type of liquid media:

- *Tap water and milk formula:* Heated in a microwave oven until lukewarm (~38°C), let sit in a refrigerator for 24 h, and then re-heated in a microwave oven until lukewarm (~38°C).
- *Orange juice*: Placed in refrigerator at ~5°C for 3 days.
- *Artificial saliva, sweat, and urine*: Incubated in a water bath at body temperature (37°C) for 2 h.

When soaking was completed, we removed 10-ml aliquots from the leachate, added 10% nitric acid (HNO_3 69%, reagent grade, Sigma-Aldrich), and analyzed the leachate for silver content using inductively coupled plasma mass spectrometry (ICP-MS, Thermo Electron X-Series ICP-MS, Waltham, MA, detection limit of 0.1 ppb). Because fabric samples released fibers into the leaching media, we filtered the media through 0.45-µm PTFE syringe filters (Millipore Millex, Billerica, MA) before adding HNO_3. We digested the orange juice and milk formula samples using a modified version of the methods described by Benn and Westerhoff.[20] We added 10 mL of HNO_3 to 25-mL samples and heated them on a hot plate at ~90°C for ~30 min. After the samples had cooled, we added 5-10 mL of hydrogen peroxide (H_2O_2) and returned samples to the hot plate until effervescence was minimal and sample volume was reduced to below 25 mL. We then completed the sample volumes to 25 mL using ultrapure water and analyzed them by ICP-MS. To determine whether silver in the leachate was in ionic or particulate form, we filtered samples that proved to contain silver using 3-KDa centrifugal filtering units (Millipore Amicon Ultra), acidified them with HNO_3, and analyzed the filtrate for silver by ICP-MS. This cutpoint is recommended for concentrating proteins ~1 nm or larger.[26]

We also compared our leaching media with two that have been used by the Consumer Product Safety Commission (CPSC): a saline solution (9 g/L NaCl), as a surrogate for saliva, and a solution of hydrochloric acid (HCl, 0.07 M), to simulate ingestion.[25] We soaked samples from the blanket in these media under the same conditions previously described for sweat and urine.

Leaching kinetics and chemistry

We examined the kinetics of leaching and effects of product aging with the baby blanket, chosen for its high silver content and high repeatability in terms of silver concentration upon digestion, which is indicative of a homogenous spatial distribution of silver within the product. We

performed most additional experiments with the synthetic sweat formulation, due to its higher silver leaching yield compared to other biologically relevant media. To study the kinetics of leaching, we soaked triplicate samples of the baby blanket in synthetic sweat (37°C) at a mass ratio of 1:50, for six periods of time: 5, 15, 30, 60, 120, and 240 min. At the end of each period, we filtered samples to remove suspended fabric fibers (0.45-µm filter), acidified them with 10% HNO_3, and analyzed them by ICP-MS.

In the interest of determining how differences in liquid media composition affect dissolution of silver from a consumer product, we soaked blanket samples in various formulations of synthetic sweat, modified to omit one ingredient at a time (Table 5.2). We soaked samples for a period of 1 h at 37°C in a water bath. We also soaked samples in saline (185 mM of NaCl) and in ultrapure water. We filtered, acidified, and analyzed samples as described above.

Product use and aging

To understand the release of silver during consecutive product usage cycles, we soaked samples from the baby blanket in synthetic sweat (37°C) for 2 h, rinsed them with ultrapure water, and air-dried them. We repeated this procedure three times, and after each soaking period, we extracted, filtered, acidified, and analyzed leachate samples as described above. We measured pH and dissolved oxygen (DO) before and after each soaking period.

Table 5.2. Sweat formulation descriptions, where • indicates the presence of an ingredient.

sweat formulation	pH	urea 1.3 g/L	NaCl 10.8 g/L	lactic acid, 88% 1.2 g/L	NH_4OH[a]
original	6.5	•	•	•	•
no urea	6.5		•	•	•
no NaCl	6.5	•		•	•
no lactic acid	7	•	•		
saline	7		•		
ultrapure water	7				

[a] Ammonium hydroxide, added to adjust the pH to 6.4 – 6.6 after the addition of lactic acid.

To simulate product aging in the baby blanket and the plush toy, we placed one piece of each product under a UV lamp (GE G875, 8W, 254 nm) for 1 – 2 weeks and then hung these samples on a clothes line outdoors to expose them to natural weathering for ~1 week. We then rubbed the samples against a concrete block for ~1 min and subjected them to the same leaching experiments as for the new, un-aged products.

Characterization by electron microscopy

To determine the size and location of nanosilver in products, we characterized selected ones using an environmental scanning electron microscope (SEM) with electron dispersive X-ray spectrometry (EDS) capabilities (FEI Quanta 600 FEG, Hillsboro, OR), operated under high vacuum using backscattered and secondary electron detectors.

Silver release onto skin

We assessed products that may come in contact with skin—the plush toy, baby blanket, disinfecting spray, surface wipes, and kitchen scrubber—for the potential for dermal exposure though transfer of particles from product's surface onto skin.[27] On the basis of prior studies and recommendations, we identified wipes as the most reliable and feasible method for assessing the potential for dermal exposure to nanosilver.[27-28] We followed NIOSH Method 9102: Elements on Wipes[30] that specifies the use of benzalkonium chloride moist towelettes. For the baby blanket and the kitchen scrubber, we delimited three different areas for wiping. Since we only had one plush toy whose available surfaces had been mostly consumed by other experiments, we repeated wiping on the same areas (front and back of the toy) three times. For the disinfecting spray and surface wipes, we mimicked a situation in which a child could touch a surface after it has been cleaned using one of these products. We applied the spray and wipes onto three individual 1 ft × 1 ft vinyl floor tiles (Armstrong) and then wiped the surfaces with towelettes using horizontal and vertical strokes. Towelettes were digested in HNO_3 and H_2O_2 and analyzed for silver content by ICP-MS.

Silver release from humidifiers

We filled the water reservoir of each humidifier with tap water and let it sit at room temperature for 5 – 6 days. Then, we collected water samples, acidified them with 10% HNO_3, and analyzed them by ICP-MS. We also collected new samples after ~17 days of soaking. To assess the potential for inhalation exposure to silver, we measured the silver concentration in the vapor

produced by each humidifier. We filled the humidifier reservoirs with tap water and let them sit for 3 days. Using PVC reducing pipe and tubing, we routed the outlet of each humidifier through a sealed beaker, submerged in ice to promote condensation inside the beaker. The condensate was then acidified with 10% HNO_3 and analyzed by ICP-MS.

Aerosol release

We simulated product use in a room that had a floor area of 13.6 m^2 and a volume of 36 m^3. It had carpeted floors, one door, and one window. The room was furnished with two desks, two cushioned chairs, a bookcase, and a wooden wardrobe. Prior to each experiment, an air conditioning unit (AC) was switched on until the temperature dropped below 25°C and the relative humidity dropped below 40%. The AC was then turned off, and background measurements were performed for at least 10 min before products were used.

We placed the humidifiers in a corner of the testing room and ran each one for 90 min, during which ~300 mL of water from the reservoirs were vaporized. We sprayed the disinfectant onto a surface located 1 m above the floor and at a horizontal distance of 0.3 m from the aerosol instrument inlets, once per minute for 30 min. We handled the fabric in a repetitive fashion for 30 min: picking it up, shaking it, folding it, and setting it down on a surface located 0.3 m horizontally from the sampling inlets. We performed a similar procedure with the stuffed toy, by repeatedly picking it up, shaking it, and lightly beating it on the surface.

We measured concentrations and size distributions of aerosols 14 – 750 nm in diameter using a Scanning Mobility Particle Sizer (SMPS 3936, TSI). We measured larger aerosols (300 nm – 10 μm in diameter) using an optical particle counter (Aerotrak, TSI).

RESULTS AND DISCUSSION

Silver release into liquid media

Table 5.3 shows the total silver content in each product component[22] and the amount that leached into liquid media. Most fabric and plush toy samples released 1 – 6% of their silver, although there were some outliers at <1% and >35% (plush toy's interior). Results for the fabric were in agreement with Kulthong et al.'s[21] findings of non-detectable levels to 4% for silver leaching from commercial fabrics into different sweat formulations. Even though the baby blanket had a higher silver concentration than did the plush toy, the fraction released was lower

than the plush toy's interior in all media (Figure 5.1). The methods used to incorporate silver into the baby blanket's polyester fibers and the plush toy's interior low-resilience polyurethane "memory" foam likely differ. It appears that silver is more strongly impregnated into or bound to the blanket fibers than the plush toy's foam. Silver release was generally low for the plastic samples (breast milk storage bags and sippy cups).

Table 5.3. Amount of silver in each product and amount leached into relevant liquid media (standard errors).

product	silver content (mg ag/kg product) [22]	liquid media	amount of silver leached	
			mg ag/kg product	%
plush toy: exterior	0.6 ± 0.1	tap water	nd[a] (0.02 ± 0.01)	-
		saliva	0.03 ± 0.001	5.6 ± 0.2
		sweat	0.14 ± 0.002	2.6 ± 0.6
		urine	nd (0.04 ± 0.003)	-
plush toy: interior	48.2 ± 5.0	tap water	0.24 ± 0.02	0.5 ± 0.0
		saliva	1.77 ± 0.03	3.7 ± 0.1
		sweat	18.5 ± 1.1	38.3 ± 2.4
		urine	17.4 ± 0.8	36.1 ± 1.6
baby blanket	109.8 ± 4.1	tap water	1.6 ± 0.3	1.5 ± 0.3
		saliva	1.2 ± 0.1	1.1 ± 0.1
		sweat	4.8 ± 0.3	4.8 ± 0.3
		urine	3.7 ± 0.3	3.4 ± 0.3
		HCl	4.7 ± 0.0	4.4 ± 0.0
		saline	4.00 ± 0.0	3.7 ± 0.0
breast milk storage bags	0.9 ± 0.6	milk formula	nd (0.88 ± 0.03 ppb)[b]	-
sippy cup #1: rubber ring	24.3 ± 2.9	milk formula	nd (0.10 ± 0.01)	-
		orange juice	0.41 ± 0.01	1.7 ± 0.0
sippy cup #1: transparent cap	9.4 ± 1.0	milk formula	nd (0.02 ± 0.00)	-
		orange juice	0.07 ± 0.01	0.7 ± 0.1
sippy cup #2: spout cover	2.1 ± 1.5	milk formula	0.93 ± 0.02	43.8 ± 0.9
		orange juice	nd (0.05 ± 0.001)	-

[a]Not detected. Silver concentrations in the leaching media were below 0.5 ppb.

[b]Since the milk formula was placed inside the bags, the product was not immersed into the leaching medium as with other products, so the amount reported is the silver concentration in

the milk formula, not the amount leached per mass of product.

The amount of silver leached from a product varied with the type of media. Figure 5.1 shows the amount of silver leached from the baby blanket and the plush toy into six different leaching media. The plush toy was not tested in HCl and saline. The release of silver from the plush toy's interior foam into sweat and urine was much higher than that of the other product components and liquid media tested. Across all products, synthetic sweat and urine yielded the largest amount of silver (between 6% and 38% of the amount present in the products), while tap water yielded the lowest amount (0.5% to 2.6%). This was likely due to the high concentration of Cl^- (185 mM) and lower pH (6.4 – 6.6) in sweat than in most other liquid media. Synthetic urine also contained a high amount of Cl^- (210 mM), but it had a pH of 7.8, which may account for its slightly lower silver yield than sweat. Silver dissolution is catalyzed by chloride (Cl^-) and can be capped at high pH and at low DO conditions because hydrogen ions and oxygen are consumed during the silver dissolution reaction.[31-33]

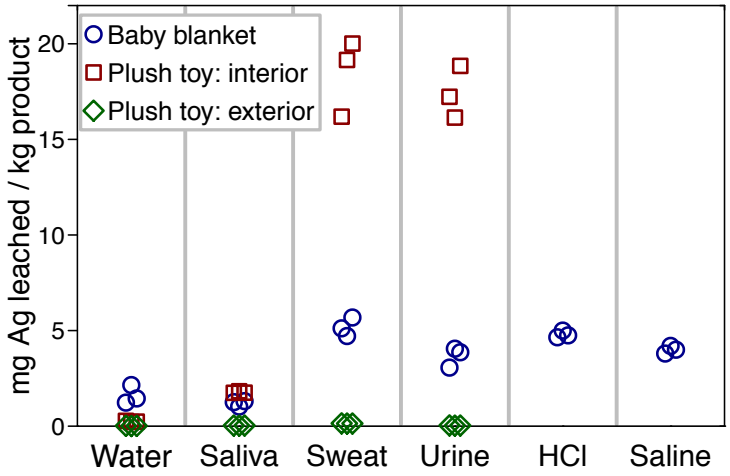

Figure 5.1. Amount of silver released into different leaching media (all data points shown). Data points are slightly offset to improve legibility.

Figure 5.2 shows the amount of silver released from fabric into various formulations of synthetic sweat. There was no direct relationship between the liquid media's pH and the amount of silver leached. Moreover, since neither the pH nor the DO level changed during the soaking period, these are not likely to have capped the dissolution reaction. The fraction of silver leached was lower in the formulation lacking NaCl, indicating the importance of Cl^- for silver dissolution. The absence of urea and lactic acid individually did not affect leaching, but the absence of both (saline), resulted in less leaching. This suggests the possibility that amine groups in urea and

the carboxyl group in lactic acid might be in competition for leaching. Citrate, which contains three carboxyl groups, is a widely-used silver nanoparticle stabilizing agent.[33-35]

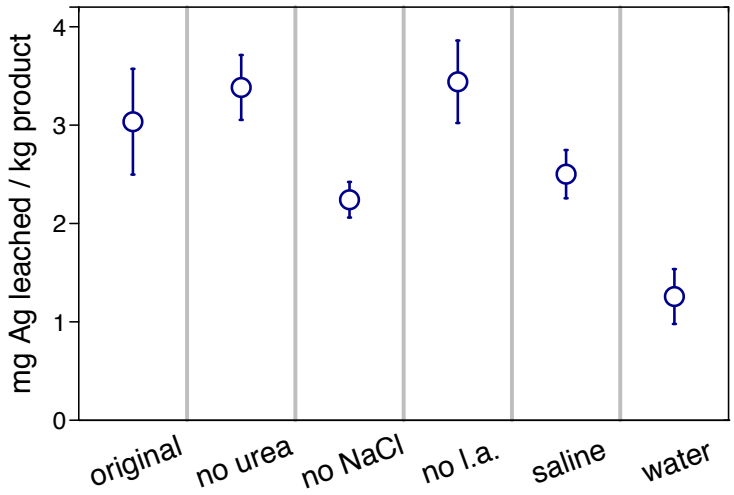

Figure 5.2. Silver release from fabric in various formulations of synthetic sweat. "l.a." is lactic acid (median and standard errors shown, n=5).

The average ionic fraction of silver in urine, sweat, and saliva for the plush toy's interior foam and the baby blanket was 87.1 ± 2%. This was not assessed for other products due to their low silver concentration in the leachate. We assessed silver ion sorption (i.e., loss) to filter media by repeating the same procedure with an ionic silver solution, obtained by dissolving ~30 mg l^{-1} silver nitrate (Fisher Scientific) in ultrapure water. Loss of ionic silver to PTFE filters was negligible, but loss to the 3-KDa centrifuge filtering membrane was 40 ± 1%.[17] Results indicate that the percentage of ionic silver lost due to sorption to this filtering membrane is not easily transferable to more complex silver-containing solutions, such as the product tested. Although we cannot quantify the exact amount of silver ions in the leachate lost to the filter, it is reasonable to infer that most, if not all, of the silver released from these products was in dissolved form.

Leaching kinetics during product use and aging

Figure 5.3 shows the release of silver from the baby blanket into sweat over time. The amount of silver released reached a maximum by the first data point (5 min) and then remained relatively constant; thus, extended exposure to sweat does not result in the release of more silver. Blanket samples that were soaked in synthetic sweat, rinsed with ultrapure water, and dried, with this cycle repeated three times, released less silver each time they were exposed to sweat. In each consecutive cycle, the samples released 4 ± 1, 0.39 ± 0.02, and 0.14 ± 0.01

mg/kg, totaling ~5% of the silver originally present in the fabric. In contrast, Benn and Westerhoff[20] found that socks that were exposed to ultrapure water in four 24-h wash cycles released silver in all washes, in some cases, releasing almost all of their silver content by the fourth wash. Dissolution was not the only mechanism for silver release, as silver particles that became dislodged from the fabric were observed in the wash water. Their experiment did not aim to simulate the product being used by a consumer, but to estimate how much silver would be released into wash water.

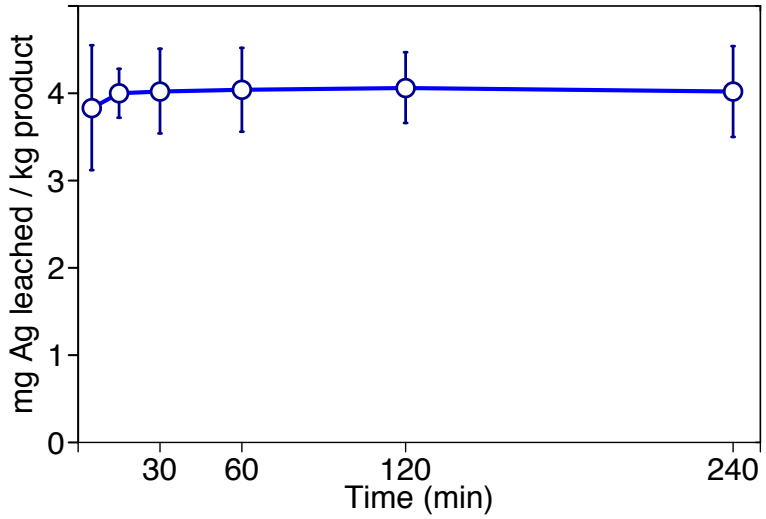

Figure 5.3. Silver release from fabric over time (median and standard errors shown, n=5).

Blanket samples that had been previously soaked in synthetic sweat were coated with gold and visualized using SEM/EDS at 20 KeV, and ~500-nm silver particles were observed in backscattered mode (Figure 5.4) but not using the secondary electron detector. Since backscattered electrons travel from deeper within the sample then secondary electrons, it is clear that these particles were inside the blanket's polyester fibers. A silver chloride aggregate was observed on the surface of a blanket fiber that had not been exposed to sweat.[22] Thus, it seems likely that silver dissolution was limited to the particles present on the surface or near the surface of the blanket fibers. Silver particles have been successfully grown within polyester fibers[36] or applied to polyester fibers after the removal of the fibers' surface finish with heat and caustic soda and before dying and finishing.[37]. One implication of this result is that after an initial period of use, fabric products may no longer release measurable amounts of silver because all silver on the surfaces of fibers may be depleted. Whether such products maintain their antimicrobial properties after extended use has not been assessed.

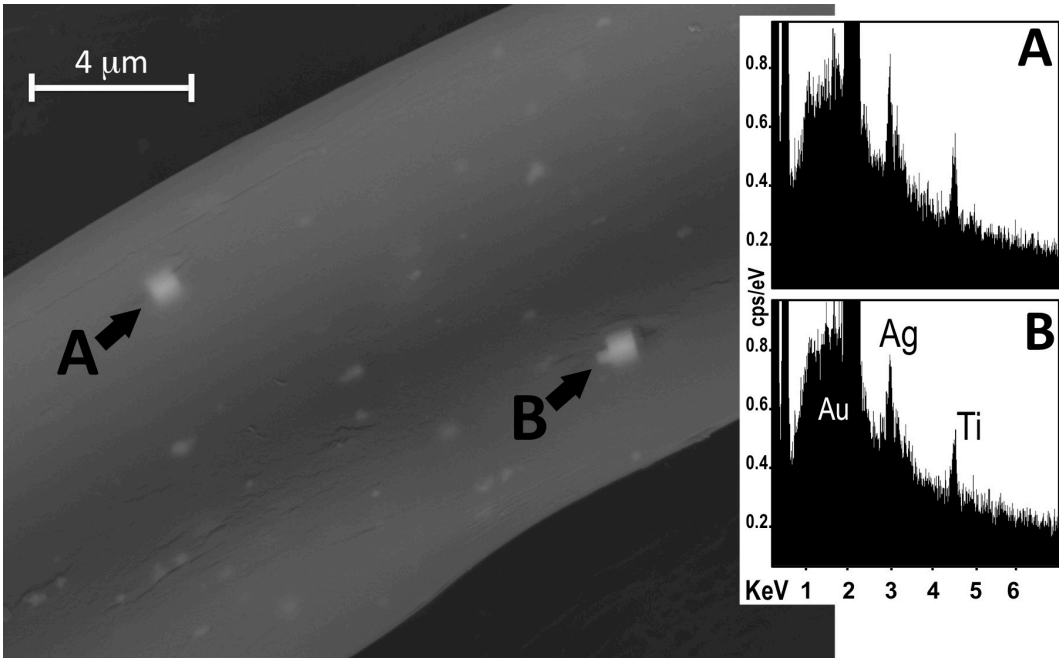

Figure 5.4. Silver particles (A and B) inside polyester fibers from the baby blanket, observed by SEM (left) and EDS spectra from the particles A and B (right).

Figure 5.5 compares the silver released by new products v. those subjected to simulated aging. While no general trend is apparent, the aged samples soaked in sweat released more silver than did new samples from the same products.

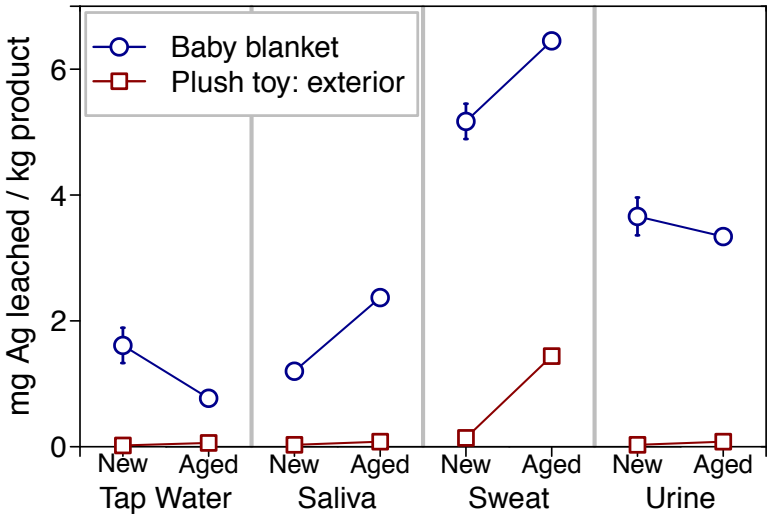

Figure 5.5. Comparison of silver leaching between new and aged products. (median and standard errors shown, n=3).

Silver release onto wipes

Table 5.4 shows the amount of silver that was transferred from products onto wipes, intended to assess the potential for dermal exposure. Values are shown in units of mass of silver transferred per area of product. Percentages of silver transferred were not calculated because that necessitates assuming a depth within the product from which silver leaches, which would assign too much uncertainty to the results. The amount of silver transferred loosely follows the same rank as the total amount of silver present in the products, except for the plush toy. It is likely that silver from the interior of this product migrated to the exterior and contributed to silver release onto wipes.

Table 5.4. Amount of silver transferred from surfaces onto dermal wipes.

product	total silver in product (µg/kg)	silver transferred (µg/m^2)
baby blanket	110 ± 4	23.0 ± 1.4
plush toy: exterior	0.6 ± 0.1	13.8 ± 8.4
disinfecting spray	27.1 ± 0.6	9.0 ± 2.8
surface wipes	4.5 ± 3	2.3 ± 0.2
kitchen scrubber	4.6 ± 0.3	0.3 ± 0.1

Silver release from humidifiers

Table C.1 shows silver concentrations in the reservoir water and in the vapor collected from each humidifier's output. We detected silver only in the reservoir water of the humidifier with the silver-releasing accessory, at a very low concentration of 0.8 ± 0.2 ppb after 5 days of soaking, and not in the nanotechnology-based humidifiers. After 17 days of soaking, however, we no longer detected silver in this sample. It is possible that over time, silver sorbed onto the humidifier's plastic surfaces. The tabletop humidifier emitted 2.3 ± 0.7 ppb of silver in the condensed vapor, while the manual humidifier did not emit detectable levels of silver. Benn and Westerhoff[19] reported concentrations in this range for two humidifiers: 0.19 (standard deviation not reported) and 1.1 ± 0.4 ppb.

Aerosol release

Ambient aerosol concentrations were not significantly elevated above background levels during product use. If these products emit any form of silver-containing aerosols, the emission rates are extremely low and could only be measured in a small chamber with very low background aerosol concentrations.

Bioavailability of nanosilver in children's consumer products

Bioavailability of nanomaterials in environmental studies is a concept that does not yet have standard testing methods.[38-40] We assessed the bioavailability of silver to children by considering the concentration, size distribution, and location of silver within products,[22] as well as whether silver is released from the products into air, biological liquid media, or upon skin contact. Based on these results and the intended use for each product, we ranked product categories according to their potential for silver bioavailability, from most likely to least likely to be a source of bioavailable silver: plush toy and fabric products (e.g., teddy bear, clothing, blanket), cleaning products (e.g., disinfecting spray and surface wipes), sippy cups, humidifiers, breast milk storage bag, and kitchen scrubber. The levels of silver to which children would be exposed during the normal use of these consumer products is likely to be low, and bioavailable silver is expected to be in ionic rather than particulate form. Given the current state of knowledge, the implications of this finding cannot be predicted at this time because the health effects of nanosilver and mechanisms of toxicity are not yet fully understood.

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Chapter 6: Conclusions

Marina E. Quadros and Linsey C. Marr

There is a large gap in knowledge regarding human and environmental exposure associated with nanotechnology, including silver nanoparticles. Hundreds of new products containing silver nanoparticles have been introduced, and laboratory studies have shown that silver nanoparticles can be toxic, but the potential for human exposure has not been assessed.[1-10]

The main contribution of this work is new information on potential exposure associated with the use of silver nanotechnology products. This work has answered fundamental questions about the physico-chemical properties as well as the dose-metrics of silver that may be released from products containing silver nanoparticles. The characteristics of silver to which humans may be exposed depend on a combination of the product's properties and how it is used. Furthermore, exposure may occur through multiple routes. We demonstrated that consumer spray products emit silver-containing particles capable of depositing throughout the respiratory system, especially in the nasopharyngeal region. On the other hand, exposure to nanosilver via biologically relevant liquid media occurs in ionic form and is facilitated in media with high concentrations of chloride and low pH.

Our results indicate that the release of silver cannot be predicted solely on the basis of silver concentration in the product. How and where the silver nanoparticles are incorporated into the product also control the potential for exposure. Consumer products composed of similar materials (e.g., the polyester fibers in a blanket versus polyurethane foam inside a plush toy) may release silver at very different rates, even if those products contain similar amounts of nanosilver. Silver release is also controlled by the location of particles within the product (surface versus interior of fibers or plastic matrix) and by how easily the product's surface is abraded during use.

We demonstrated that exposure levels for individual consumer products are expected to be low but rarely zero. If silver nanotechnology becomes widespread and multiple consumer products are unknowingly used in combination, the minimal dose for argyria may be reached within the lifetime of a consumer, especially for children. These results substantiate a need for the establishment of labeling requirements and concentration limits in nanosilver consumer

products. As consumer products containing silver nanoparticles grow in numbers and regulatory concerns increase, the value of this research will become even more apparent.

OUTCOMES OF RESEARCH OBJECTIVE 1:
Review of the environmental and human health risks of airborne nanoparticles.

This work has provided the scientific community with a summary of the state of the art in airborne nanosilver, including its uses and applications, aerosolization scenarios, fate and transport in the environment, and toxicological effects. This work has helped guide researchers toward identifying the most pressing questions for future studies.

The review has also equipped the public and policy makers with information to assess the potential risk associated with the popularization of silver nanoparticle consumer products, specifically products that may release aerosols (e.g., sprays, humidifiers, and hair dryers). This work may help guide policy makers toward defining airborne nanosilver safety guidelines for industrial and outdoor environments.

OUTCOMES OF RESEARCH OBJECTIVE 2:
Characterization of silver emissions from spray products.

This work has addressed the need for understanding particle emissions from nanotechnology-based spray products under realistic conditions. We demonstrated there is a risk for respiratory exposure to silver from the use of nanotechnology spray products. Results from this study can be used to guide the selection of relevant particle doses in nanotoxicity testing and the estimation of inhalation exposure to nanoparticles. By characterizing the size, state of agglomeration, morphology, and surface area of these aerosols, we provided critical data needed to predict the fate and transport of silver nanoparticles in the environment. Since aerosol deposition in the human respiratory system is largely size-dependent, showing that aerosol emissions were sub-micron indicated that there is a realistic inhalation risk from the use of these products. This information can be used to help develop regulations to ensure consumer safety.

OUTCOMES OF RESEARCH OBJECTIVE 3:
Characterization of nanosilver released from children's consumer products.

This work has provided the scientific community with an inventory of silver nanotechnology-related consumer products specifically concerning children. Methods were developed to assess the amounts and physical characteristics of silver nanoparticles that are likely to be released from products during use in real-world scenarios. Products were tested comprehensively for silver release into water, air, synthetic formulations of sweat, urine, and saliva, commercially available orange juice and milk formula, and onto skin. The methods developed and data obtained can be used for evaluating potential exposures to nanomaterials and for predicting fate and transport in the environment. We demonstrated that silver in nanotechnology-based products leaches more into sweat and urine than into other liquid media such as saliva and water, and that most, if not all of the released silver is in ionic form.

Information from this work has been provided to the EPA and the CPSC for regulatory purposes. The results provide a scientific basis for decisions on concentration limits and manufacturer compliance in the consumer product industry. Knowledge of the amount and physical characteristics of nanosilver released from products will enable an improved risk assessment and life-cycle analysis of nanotechnology.

RECOMMENDATIONS FOR FUTURE WORK

We recommend that future cytotoxicity studies be developed using silver nanoparticle doses, size distributions, and chemical compositions identified in the present body of work in order to assess realistic health effects associated with silver nanotechnology. Since relevant doses will invariably be lower than in existing toxicity studies, which mostly use high doses of pure, uncoated, silver nanoparticles,[7,11-15] a genetic toxicology approach focusing on changes in molecular mechanisms must be used rather than median lethal dose (LD50) metrics.[11,16,17]

This work provides the necessary information for exposure assessment to certain silver nanotechnology consumer products. Once sufficient toxicological information becomes available, a full risk characterization can be completed.

The research methods developed during the completion of Research Objectives 2 and 3 can be extended to consumer products containing other types of emerging nanomaterials, such as titanium dioxide, cerium oxide, fullerenes, and gold nanoparticles for emissions into air or liquid media.

Results indicate that the release of silver cannot easily be predicted on the basis of knowledge about the quantity and size of nanosilver within the product alone. It is likely that the method used for impregnating particles into products greatly affects the potential for silver release. Further work is recommended to elucidate the release of silver as a function of (1) the nanosilver characteristics (i.e., size, composition, aggregation state, coating composition), (2) the product's composition, (3) the method used for impregnating particles into the product and depth of impregnation, and (4) the product's intended use and most likely exposure routes.

We recommend that the emissions of aerosols from humidifiers be further investigated. It is likely that the small droplets of misting humidifiers quickly shrink and evaporate as they reach equilibrium with the surrounding air and form nanoscale particles consisting of the droplets' solutes. These nanoscale particles can remain suspended in indoor air for long periods of time.[18] If small amounts of dissolved silver, or other metals are present in water, it is likely that they are present in the resulting aerosols.

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APPENDIX A.
SUPPLEMENTAL INFORMATION TO CHAPTER 3

This appendix contains the following information:

- Description of the experimental setup.
- Physical characteristics of aerosols generated by an atomizer.
- DLS size distributions, UV-VIS absorbance spectra, TEM and SEM micrographs, micrograph-based primary particle size distributions, and EDS spectra of the liquid products.

DESCRIPTION OF THE EXPERIMENTAL SETUP

- Chamber dimensions: ~80 cm × ~80 cm × ~60 cm (since the chamber is an inflatable bag, sides are curved rather than straight, so these measurements are approximate).
- Position of fan: bottom center.
- Position of glove access: left bottom corner.
- Distance between product bottles and furthest chamber wall: ~ 1 m (diagonally).

Table A. 1. Chamber flow settings under different experimental conditions.

measurement condition	instruments used	Q_{in} (ml min^{-1})	Q_{out} (ml min^{-1})
Concentration and size distribution of aerosols 0.3 – 10 μm	CPC and OPC	2830	3130
Concentration and size distribution of aerosols < 0.7 μm	SMPS	0	300
Collection of aerosols onto TEM grids	Thermophoretic precipitator	0	12.2 ± 0.2

DESCRIPTION OF AEROSOLS GENERATED BY ATOMIZER

Table A. 2. Physical characteristics of aerosols generated by an atomizer (mean ± standard error).

product	median diameter[a] (nm)	number concentration < 750 nm ($\times 10^5$ cm^{-3})	number concentration 300 – 10,000 nm (cm^{-3})
Ultrapure water	17.8 ± 0.3	2.5 ± 0.1	1.12 ± 0.02
Hunter spray 2	78.4 ± 1.1	41 ± 1	1,151 ± 4
Disinfecting spray	85.0 ± 1.1	550 ± 11	10,811 ± 43
Throat spray	83.0 ± 0.2	15 ± 0.2	1,485 ± 2

[a] As measured by SMPS.

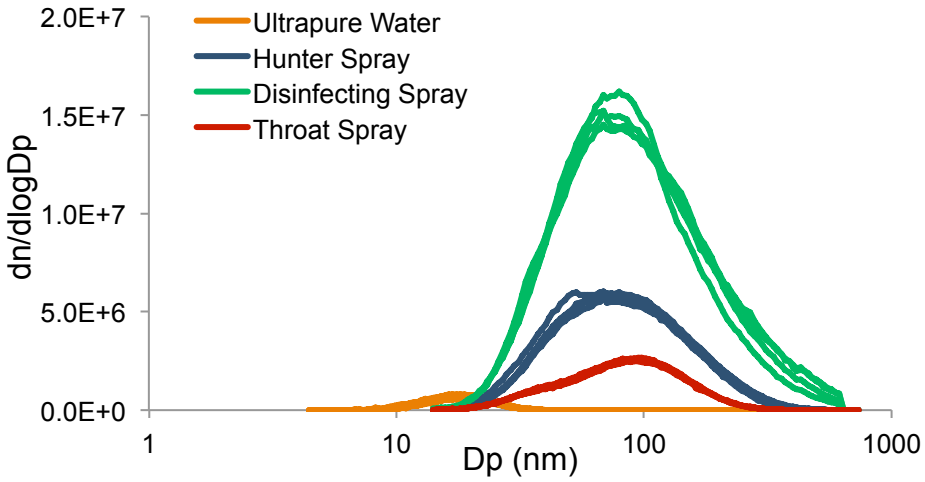

Figure A. 1. Size distribution of aerosols generated by an atomizer, as measured by SMPS.

Because the disinfecting spray generated a high concentration of aerosols from the atomizer, we diluted the aerosol stream with particle-free air at a 1:4 ratio to minimize coagulation. The results shown in Figure A.1 have been corrected for this dilution.

CHARACTERIZATION OF LIQUID PRODUCTS

Throat spray

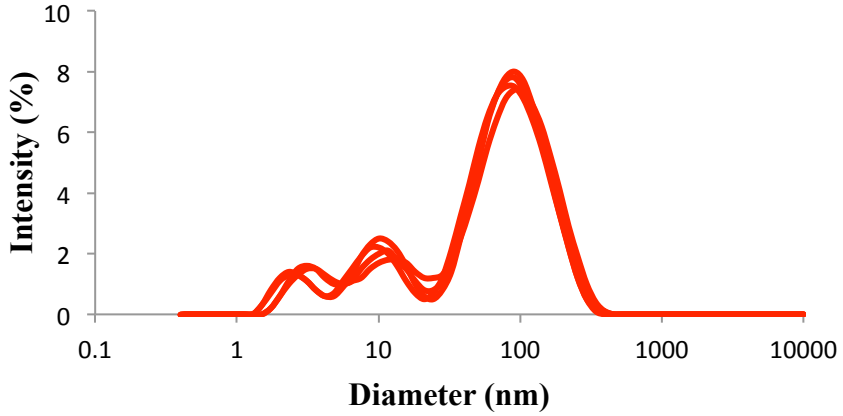

Figure A. 2. DLS size distribution for the throat spray (polydispersity index = 0.63).

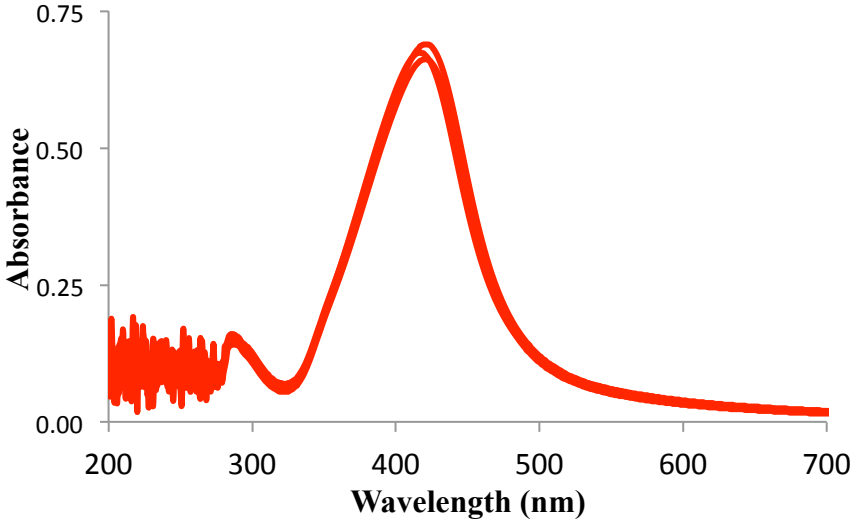

Figure A. 3. UV-VIS absorbance for the throat spray (peak at 419 nm).

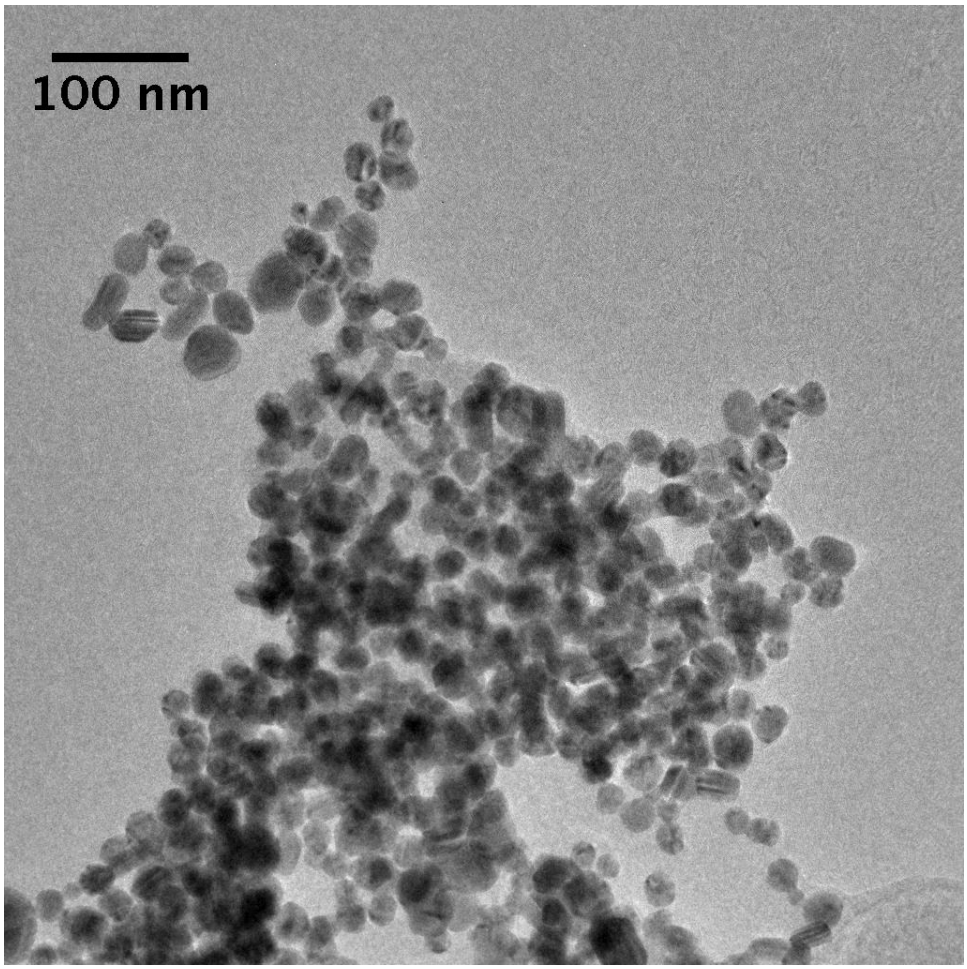

Figure A. 4. TEM micrograph of the throat spray, showing a large, compact aggregate.

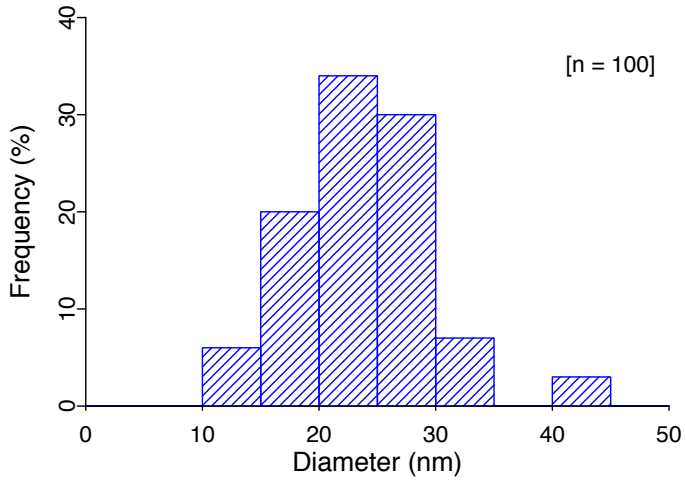

Figure A. 5. Size distribution of the primary particles comprising the throat spray aggregates (average diameter = 23.5 ± 0.7 nm).

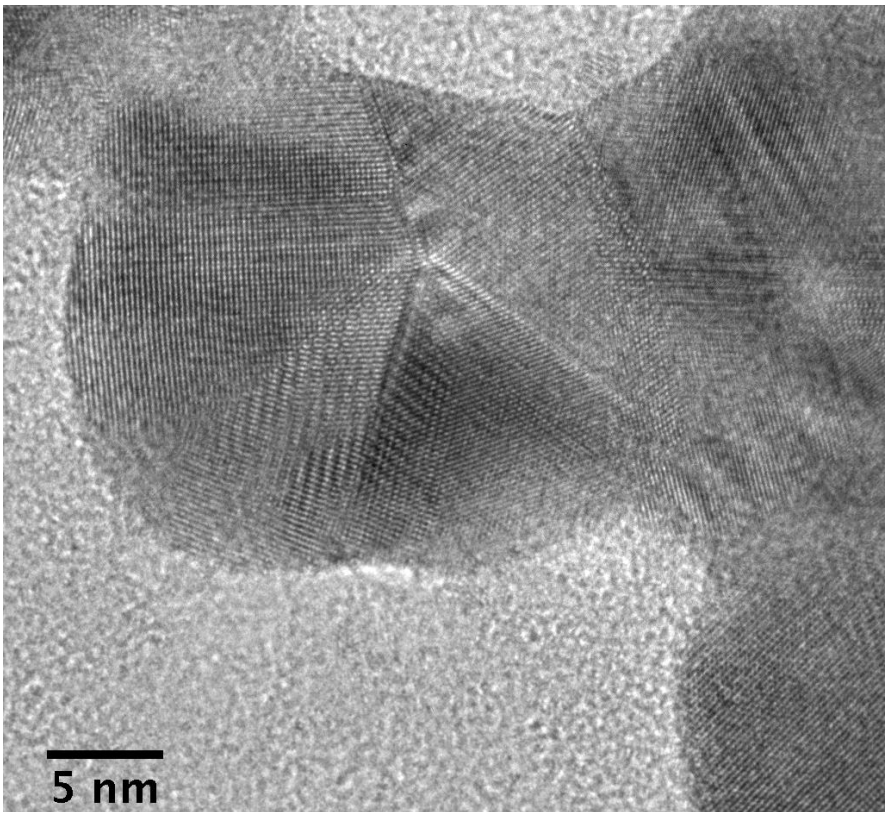

Figure A. 6. High-resolution TEM micrograph of a throat spray aggregate, showing that particles are multifaceted, crystalline, and have internal defects.

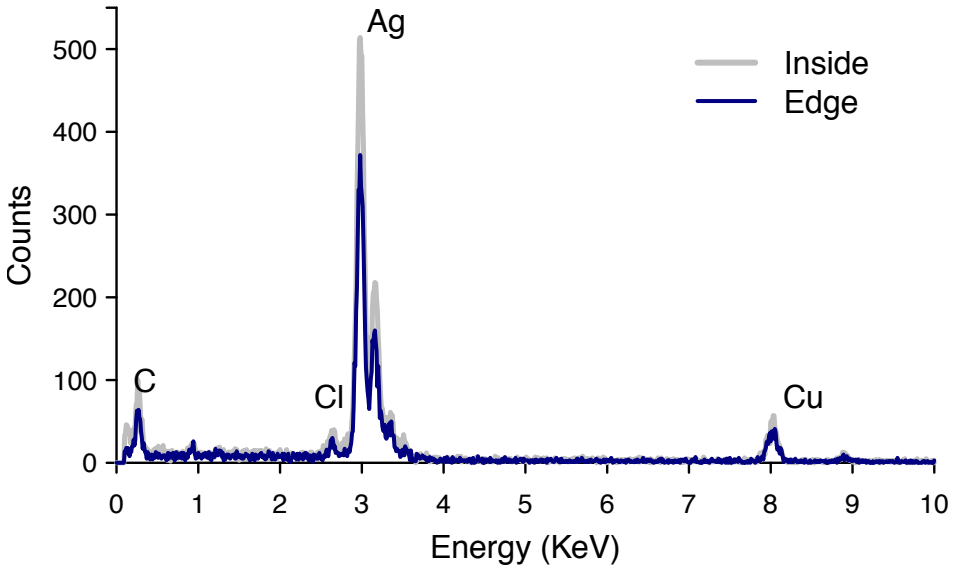

Figure A. 7. EDS spectrum of edge and interior of the aggregate from Figure A.6, showing the presence of silver and small amounts of chlorine. A quantitative analysis of the spectra revealed that in the interior of the particle, silver and chlorine atoms contributed to ~42% and 1 - 2% of the spectrum, respectively. On the edge of the particle, silver atoms contributed to ~40% of he spectrum, and chlorine was not present. Other elements were also present: carbon at 47 - 52%,

copper at ~6%, oxygen at 0 - 1.4%, and magnesium at 0 - 0.6%. Copper and carbon are likely from the TEM grid and carbon film, respectively. Uncertainty levels were very low (< 1% for silver in interior spectra, < 1.7% for edge spectra).

Hunter spray 1 (first bottle)

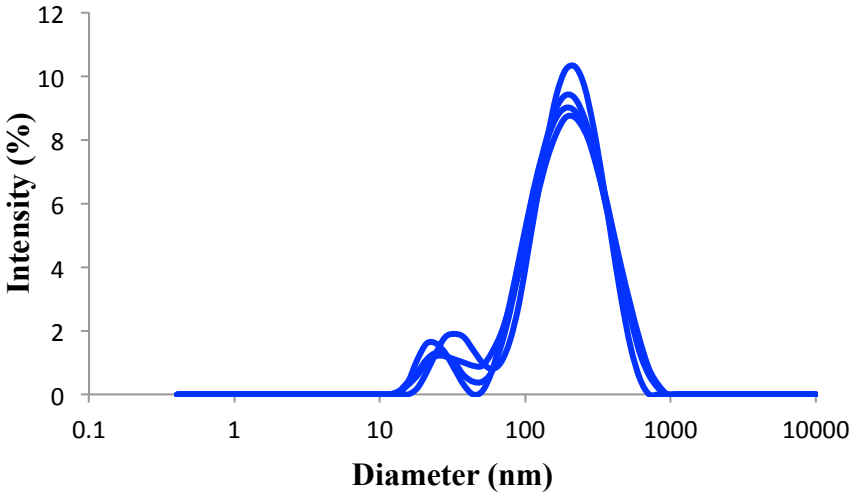

Figure A. 8. DLS size distribution for the hunter spray (polydispersity index = 0.43).

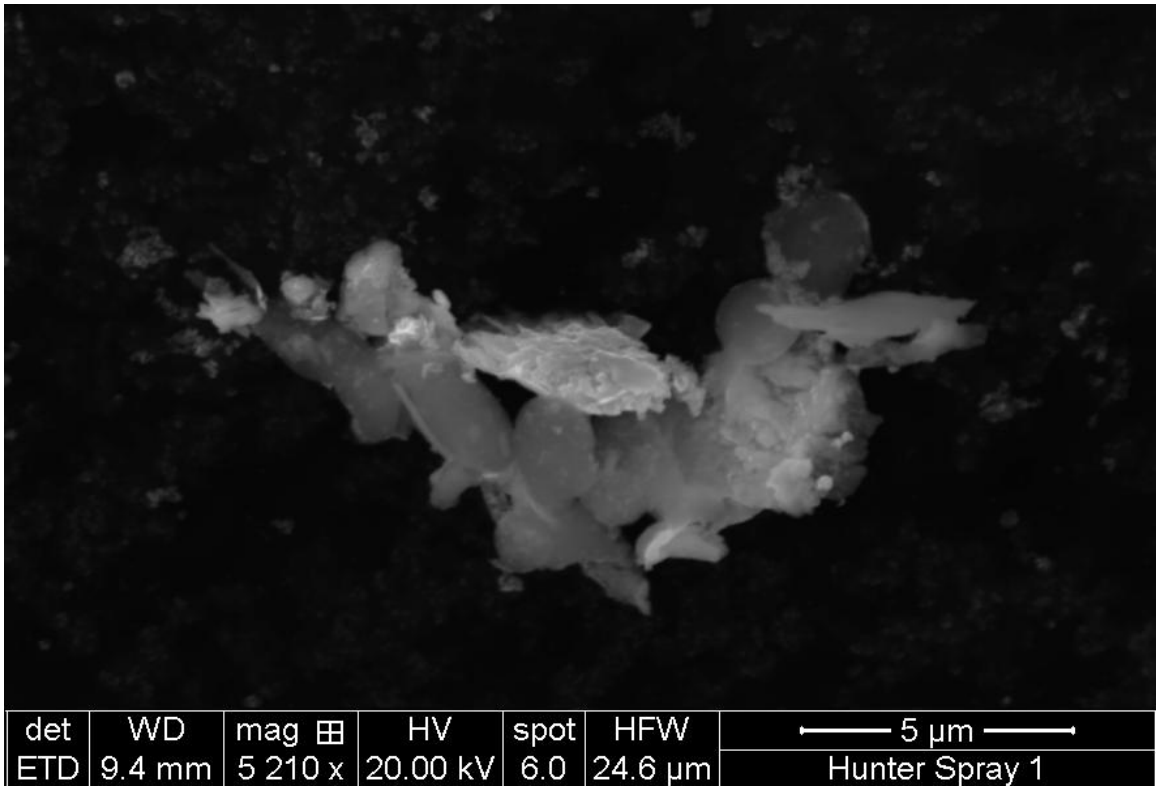

Figure A. 9. SEM micrograph of the hunter spray, showing a large aggregate with scale-shaped primary particles.

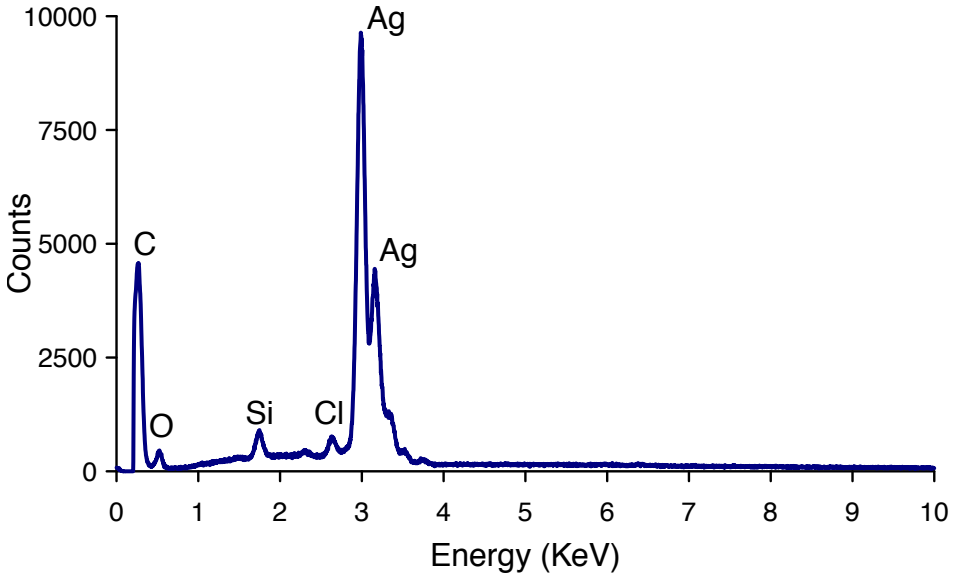

Figure A. 10. EDS spectrum of the aggregate shown in Figure A.9, showing the presence of silver, chlorine, silicone, and oxygen (the sample was fixed on carbon tape, so we cannot affirm whether or not this aggregate contains carbon).

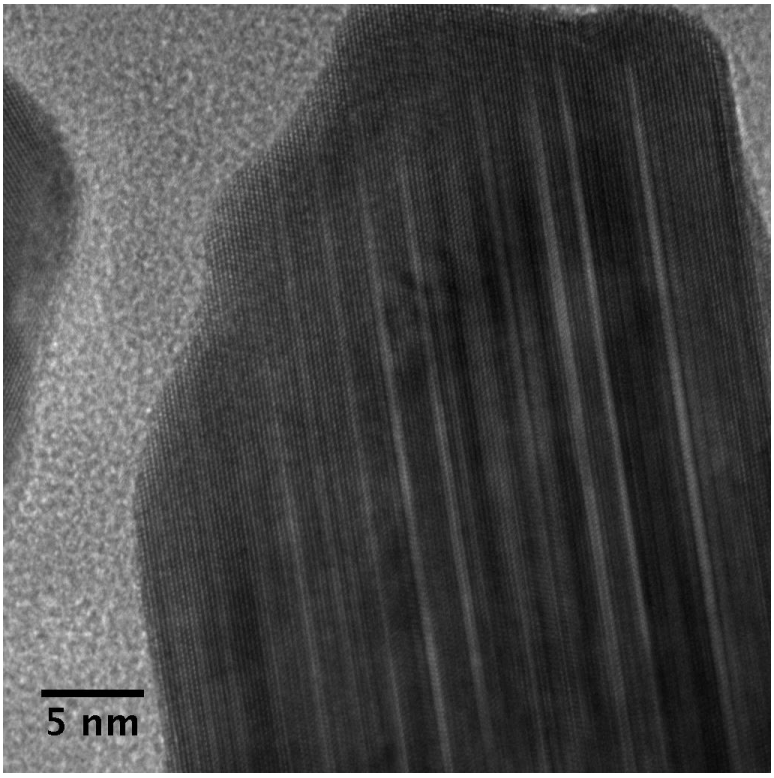

Figure A. 11. High-resolution TEM micrograph of a hunter spray particle, showing a large single crystal.

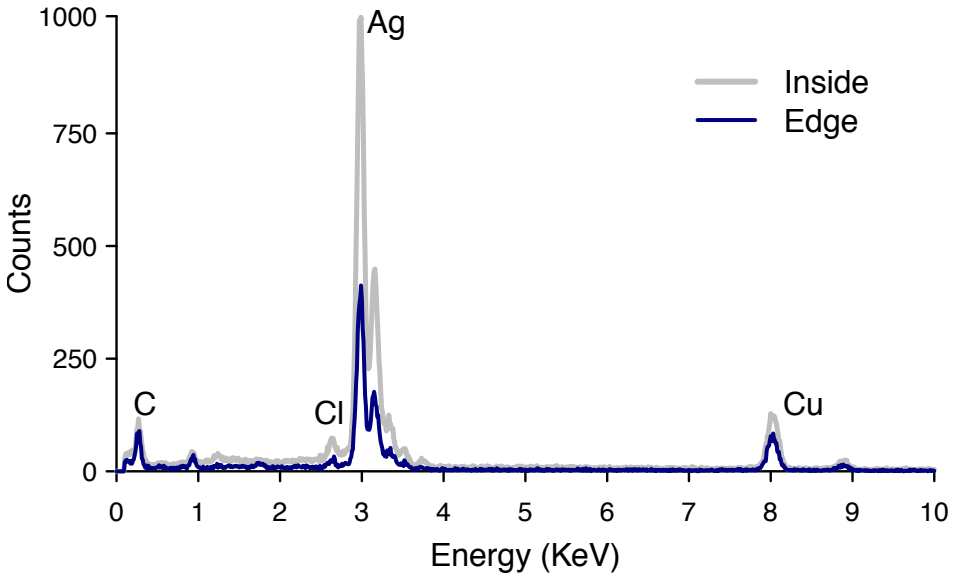

Figure A. 12. EDS spectra of tip and interior of the particle from Figure A11, showing the presence of silver and small amounts of chlorine. A quantitative analysis of the spectra revealed that in the interior of the particle, silver and chlorine atoms contributed to 45 - 51% and 2 - 3.5% of the spectrum, respectively. On the edge of the particle, silver and chlorine atoms contributed to 32 - 37% and 0.5 - 1.5% of the spectrum, respectively. Other elements were also present: carbon at ~35 - 57%, copper at ~10%, magnesium at 0.6 - 1%, and silicone at 0.5 - 1%. Copper and carbon are likely from the TEM grid and carbon film, respectively. Uncertainty levels were very low (< 1% for silver).

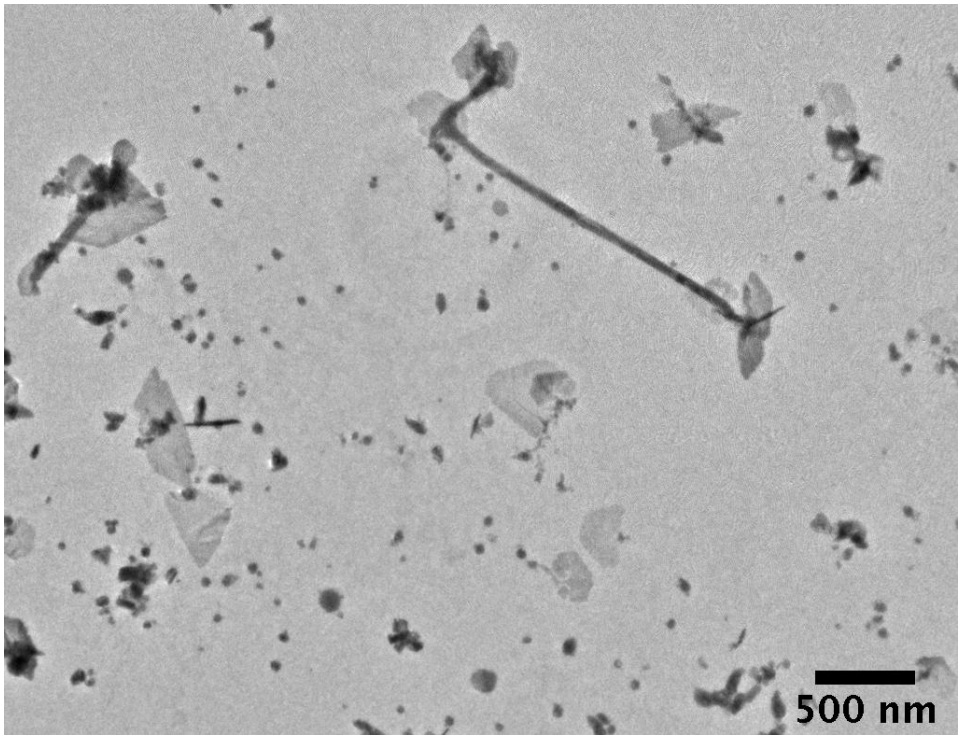

Figure A. 13. TEM micrograph of the hunter spray, showing a wide range of particle sizes and shapes.

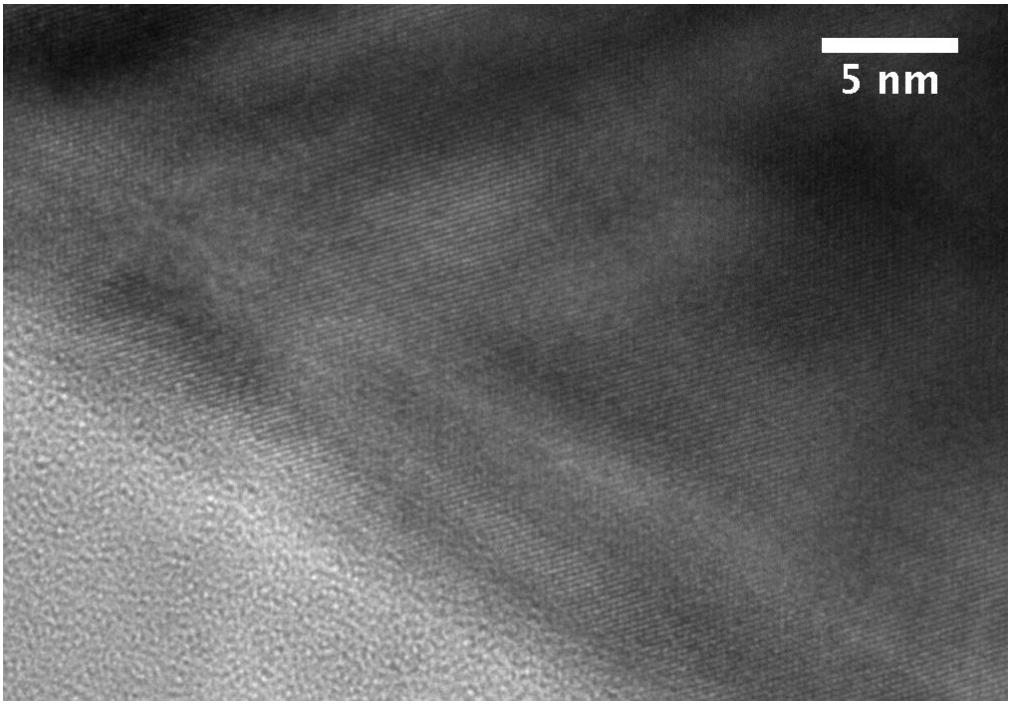

Figure A. 14. High-resolution TEM micrograph of the large fiber shown in Figure A13, showing that the fiber has a single crystal structure.

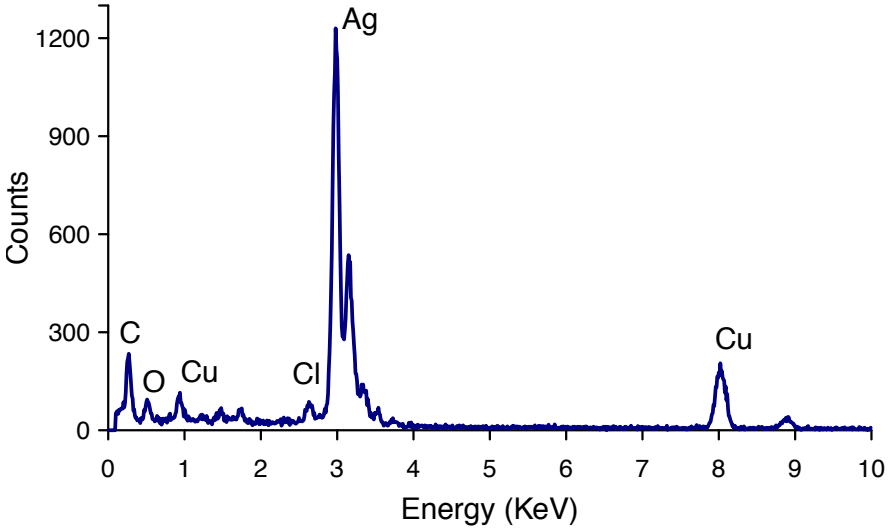

Figure A. 15. EDS spectrum of the fiber shown in Figures A13 and A14, showing the presence of silver, chlorine, and oxygen (copper and carbon are likely from the TEM grid and carbon film, respectively).

Hunter spray 2 (second bottle)

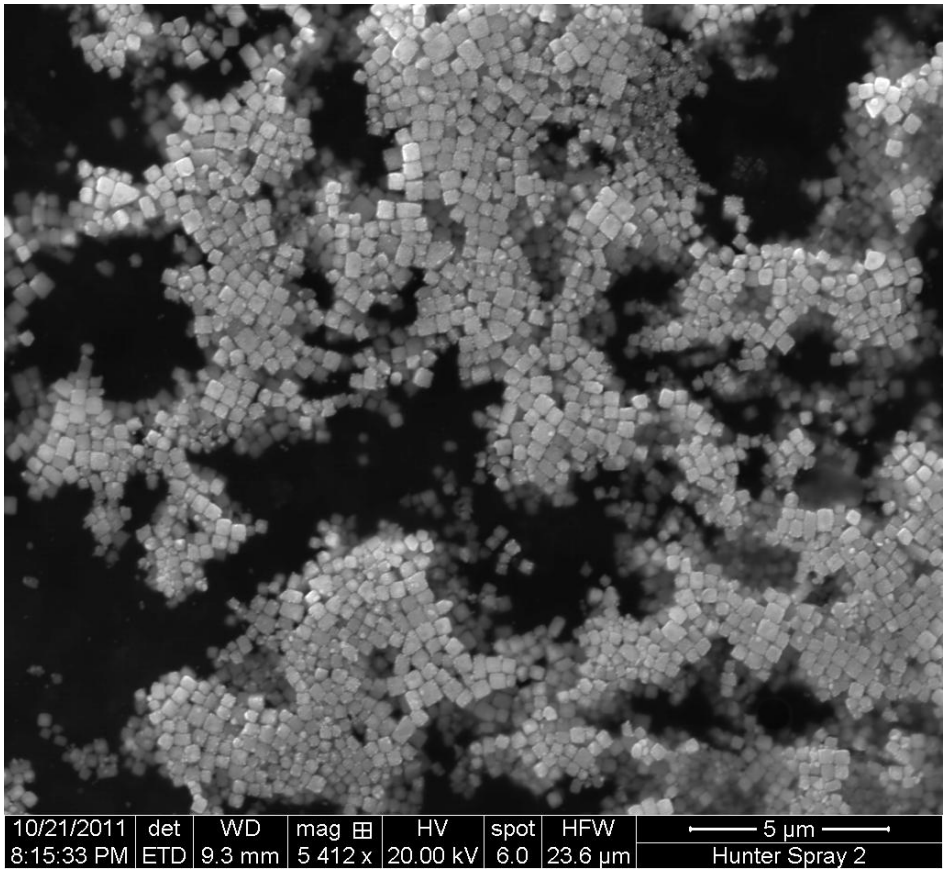

Figure A. 16. SEM image of the second bottle of hunter spray, showing large aggregates consisting of cubic primary particles.

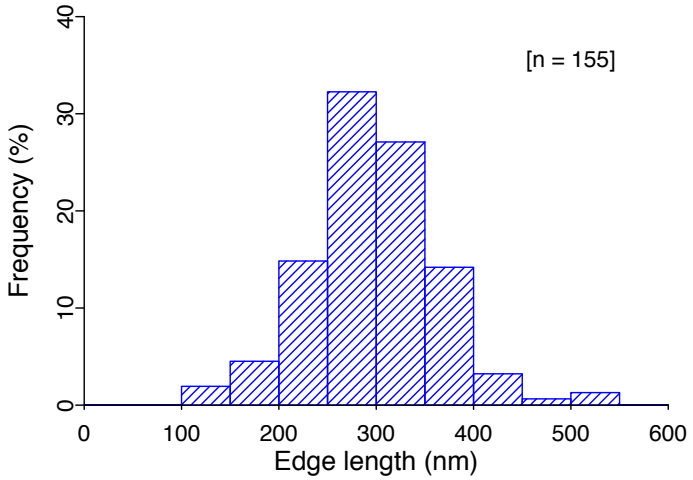

Figure A. 17. Size distribution of the cubic particles shown in Figure A16 (average edge size = 298 ± 6 nm).

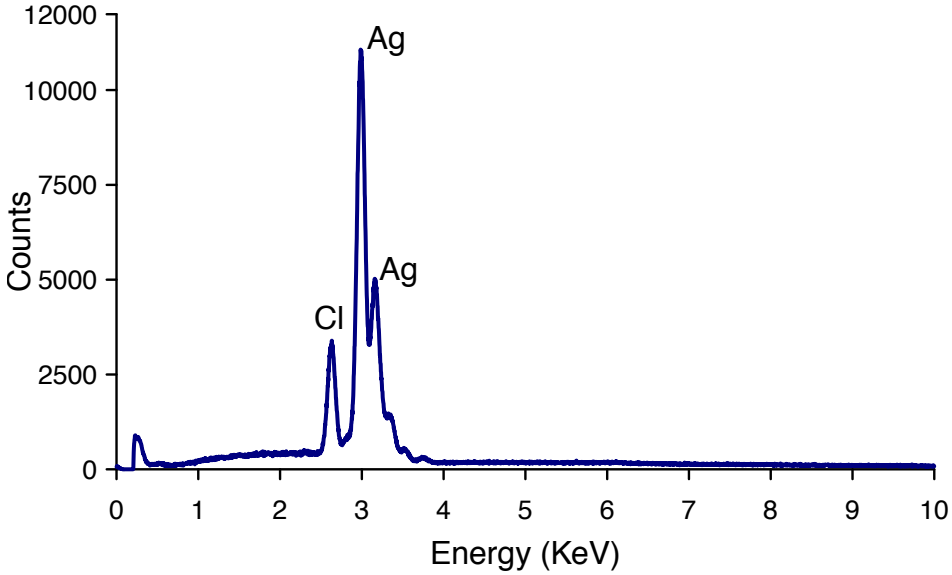

Figure A. 18. EDS spectrum of the aggregate shown in Figure A16, showing the presence of silver and chlorine.

Disinfecting Spray

Although the disinfecting spray claimed to contain silver in ionic form, we were able to find particles and aggregates after repeatedly centrifuging the product in 2-ml vials at 18,000×g, removing the 1.5 ml of supernatant, and adding more product to complete the volume to 2 ml.

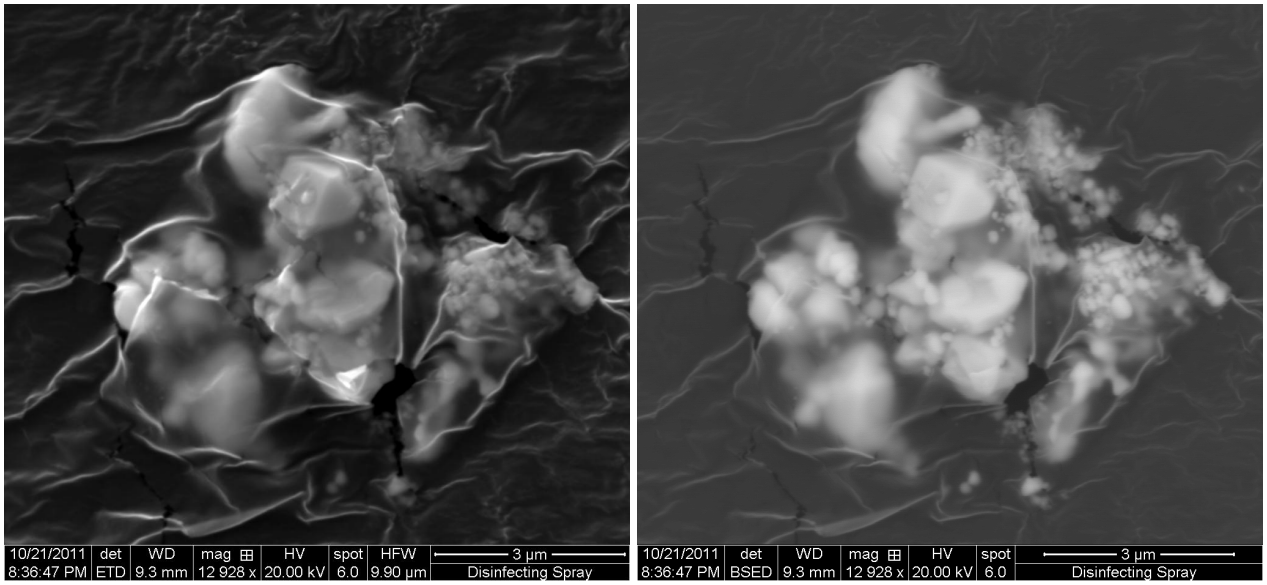

Figure A. 19. SEM image of an aggregate present in the disinfecting spray. The image on the left was obtained using the ETD (secondary electron) detector, and it shows a film of dried detergent covering the sample. The image on the right was obtained using the backscattered electron detector, in which heavy elements—such as silver—shine brighter. This loose aggregate contains particles ranging from ~30 to ~1400 nm.

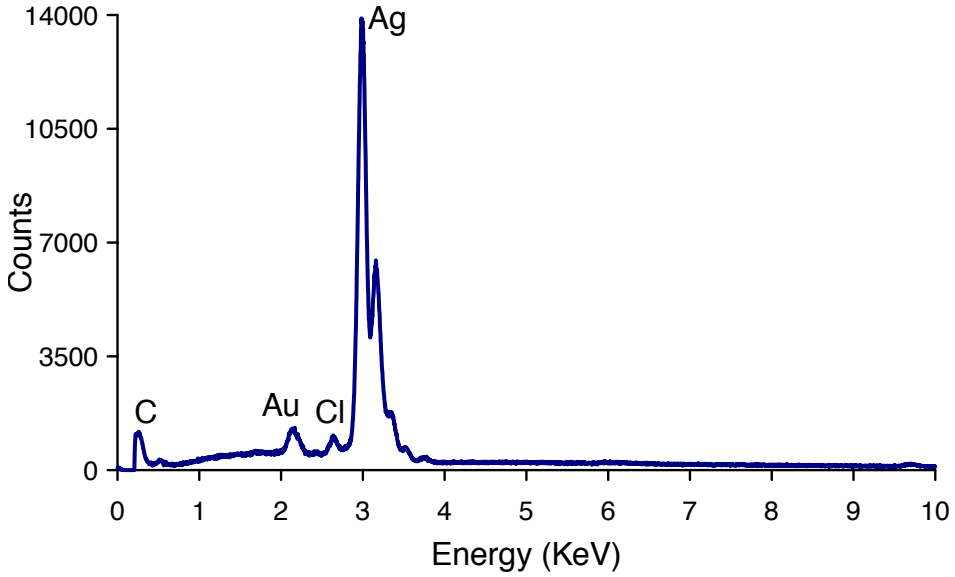

Figure A. 20. EDS spectrum of the aggregate shown in Figure A.19, showing silver and chlorine. This sample was sputter-coated with gold to make the surfactant film conductive. We did not detect aluminum or silicate—which would indicate the presence of a zeolite—in any samples from this product.

APPENDIX B
SUPPLEMENTAL INFORMATION TO CHAPTER 4

This appendix contains the following information:

- Children's nanosilver consumer product inventory.
- SOP # AirVT-Nanosilver-001: Preparation of consumer product samples for silver analysis by ICP-MS.
- SOP # AirVT-Nanosilver-002: Analytical methods for silver nanoparticle characterization via electron microscopy in complex media.
- SOP # AirVT-Nanosilver-003: Silver leaching assays from solid materials into various liquid media.
- SOP # AirVT-Nanosilver-004: Characterization of aerosols generated from nanosilver consumer products.
- Supplemental microscopy data

CHILDREN'S NANOSILVER CONSUMER PRODUCT INVENTORY

(1)	**Product Name:** Benny the Bear Antimicrobial Toy	**Product Type (likely exposure route):** Toy (ingestion, dermal)
Manufacturer (Country): Pure Plushy (country unknown)		**Retailer:** Overstock.com
Retailer website: http://www.overstock.com/Baby/Benny-the-Bear-Antimicrobial-Stuffed-Toy/2876408/product.html		
Advertised form of silver: "Patented animal toy blends memory foam with silver nanotechnology"		
Comments: $27.99. Product sold out, according to Overstock.com. We have one bear that was purchased by EPA. "Donny the Dog" was also advertised to contain silver nanotechnology.		

(2)	**Product Name:** Silver Nano Disco Party Fashion Bracelet	**Product Type (likely exposure route):** Toy (dermal)
Manufacturer (Country): A (USA)		**Retailer:** eBay.com
Retailer website: http://cgi.ebay.com/Silver-Nano-DISCO-PARTY-Fashion-Bracelet-6-pieces-set-/120409060364?pt=LH_DefaultDomain_0&hash=item1c08f0740c		
Advertised form of silver: "silver nano"		
Comments: $7.99 for a 6-pc set.		

(3)	**Product Name:** Nano-Silver Pacifier	**Product Type (likely exposure route):** Pacifier (ingestion)
Manufacturer (Country): Babydream Co., Ltd. (Korea)		**Retailer:** Alibaba.com
Retailer website: http://www.alibaba.com/product-free/104513104/Nano_silver_pacifier.html		
Advertised form of silver: "Nano-silver";		
Comments: Bulk vendor: minimum quantity of 3000 pc. Buyer must contact supplier to receive ordering information. Price not advertised.		

(4)	**Product Name:** Mouth Watchers Nano Silver Toothbrush - Youth	**Product Type (likely exposure route):** Toothbrush (ingestion)
Manufacturer (Country): Mouth Watchers (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Mouth-Watchers-Nano-Silver-Toothbrush/dp/B003IQVDWC/ref=sr_1_5?ie=UTF8&s=hpc&qid=1301412539&sr=8-5		
Advertised form of silver: "Nano-silver bristles."; "(…) proprietary nano-silver technology (…)"		
Comments: $4.99 each, available in pink and yellow.		

(5)	**Product Name:** Nano Silver Children Toothbrush with name tag	**Product Type (likely exposure route):** Toothbrush (ingestion)
Manufacturer (Country): B O K CO LTD (Korea)		**Retailer:** Alibaba.com
Retailer website: http://www.alibaba.com/product-tp/107537527/Nano_silver_Children_toothbrush_with_name.html		
Advertised form of silver: "Nano silver anti bacterial effects"		
Comments: Bulk vendor. Price not advertised. Buyer must contact supplier to receive ordering information.		

(6)	**Product Name:** Nano Dental Care Premium Toothbrush Set Yellow	**Product Type (likely exposure route):** Toothbrush (ingestion)
Manufacturer (Country): SHS (Hong Kong)		**Retailer:** eBay.com
Retailer website: http://cgi.ebay.com/ws/eBayISAPI.dll?ViewItem&item=300539884537		
Advertised form of silver: "Nano is bacteriostatic" "Nano is small and soft which clean and protect your teeth better than normal toothbrush"		
Comments: $3.10 for a set of 4.		

(7)	**Product Name:** Bio-Care Silver Anti Microbial Toothbrush	**Product Type (likely exposure route):** Toothbrush (ingestion)
Manufacturer (Country): Bio-Care (Korea)		**Retailer:** Unknown
Retailer website: Not Applicable		
Advertised form of silver: "silver particle(s)"		
Comments: Product purchased in Seoul by EPA staff.		

(8)	**Product Name:** Nano-silver Baby Toothbrush	**Product Type (likely exposure route):** Toothbrush (ingestion)
Manufacturer (Country): Babydream Co., Ltd. (Korea)		**Retailer:** Alibaba.com
Retailer website: http://www.alibaba.com/product-free/104513226/Nano_silver_Baby_Tooth_Brush.html		
Advertised form of silver: "Nano-silver"		
Comments: Bulk vendor: minimum quantity of 3000 pc. Buyer must contact supplier to receive ordering information. Price not advertised.		

(9)	**Product Name:** Set of 4 toothbrushes	**Product Type (likely exposure route):** Toothbrush (ingestion)
Manufacturer (Country): Unknown (Korea)		**Retailer:** Unknown
Retailer website: Not Applicable		
Advertised form of silver: Unknown. The packaging had the following text, in Korean: "Sensitive"; "Antibacterial, fine, bristle toothbrush"; "Special deal"		
Comments: Product purchased in Seoul by EPA staff (set of 4).		

(10)	**Product Name:** SilvaFresh Anti-Bacterial Toothpaste Soothing Mild Mint	**Product Type (likely exposure route):** Toothpaste (ingestion)
Manufacturer (Country): Trimedica (USA)		**Retailer:** Spring Valley Herbs
Retailer website: http://www.springvalleyherbs.com/catalog/item/3454/SilvaFresh-Anti-Bacterial-Toothpaste-Soothing-Mild-Mint-3-oz.		
Advertised form of silver: "homeopathic Argentum metallicum (silver)", "100 ppm"		
Comments: $5.59 for a 3-oz tube. Directions for children were not mentioned.		

(11)	**Product Name:**	**Product Type (likely exposure route):**
	White Touch Nano Silver Toothpaste Mint	Toothpaste (ingestion)
Manufacturer (Country):		**Retailer:**
The Face Shop (Korea)		eBay.com
Retailer website:		
http://cgi.ebay.com/Face-Shop-White-Touch-Nano-Silver-Toothpaste-Mint-/370329851113?pt=US_Skin_Care&hash=item56396114e9		
Advertised form of silver:		
"Nano Silver"		
Comments:		
$4.85 for a 120-g tube. Directions for children were not mentioned.		

(12)	**Product Name:**	**Product Type (likely exposure route):**
	Nano-silver microfiber baby diapers	Cloth diapers (dermal)
Manufacturer (Country):		**Retailer:**
Gerunzi (China)		DHgate.com
Retailer website:		
http://www.dhgate.com/nano-silver-microfiber-baby-diapers/p-ff808081292a026001293474320f795d.html		
Advertised form of silver:		
"absorbent layer of zeolite containing silver nano-antibacterial microfiber"		
Comments:		
Bulk vendor: $75.24 per lot of 50 diapers. End-consumers can also purchase from this website. Manufacturer country is not clear, probably Chinese since that is the language used in figures.		

(13)	**Product Name:**	**Product Type (likely exposure route):**
	Baby Pink or Blue Precious Protechtor Dribble Drier	Fabric (dermal)
Manufacturer (Country):		**Retailer:**
Precious Protechtor (UK)		Olivers Babycare
Retailer website:		
http://www.olivers-baby-care.co.uk/baby-pink-or-blue-precious-protechtor-dribble-drier.html		
Advertised form of silver:		
"designed using so-called 'nano technology' which incorporates the mineral [sic] silver" "Silver has natural antibacterial properties which kill viruses, and it is one of the safest substances for babies and has no side effects"		
Comments:		
Cost: £8.65. UK vendor website, shipping to the US is available. Product found through the PEN online inventory.		

(14)	**Product Name:**	**Product Type (likely exposure route):**
	Baby Pink or Blue Precious Protechtor Sleepsuit or Babygro	Clothing (dermal)
Manufacturer (Country):		**Retailer:**
Precious Protechtor (UK)		Olivers Babycare
Retailer website:		
http://www.olivers-baby-care.co.uk/baby-pink-or-blue-precious-protechtor-sleepsuit-or-babygro.html		
Advertised form of silver:		
"designed using so-called 'nano technology' which incorporates the mineral [sic] silver" "Silver has natural antibacterial properties which kill viruses, and it is one of the safest substances for babies and has no side effects"		
Comments:		
Cost: £12.00. UK vendor website, shipping to the US is available. Product found through the PEN online inventory.		

(15)	**Product Name:** Baby Pink or Blue Precious Protechtor Baby Blanket	**Product Type (likely exposure route):** Blanket (dermal)
Manufacturer (Country): Precious Protechtor (UK)		**Retailer:** Olivers Babycare
Retailer website: http://www.olivers-baby-care.co.uk/baby-pink-or-blue-precious-protechtor-baby-blanket.html		
Advertised form of silver: "100% nano-impregnated tricot polyester"		
Comments: Cost: £15.00. UK vendor website, shipping to the US is available. Product found through the PEN online inventory.		

(16)	**Product Name:** Precious Protechtor Baby Scratch Mitts by Baby Pink and Blue	**Product Type (likely exposure route):** Clothing (dermal)
Manufacturer (Country): Precious Protechtor (UK)		**Retailer:** Olivers Babycare
Retailer website: http://www.olivers-baby-care.co.uk/precious-protechtor-baby-scratch-mitts-by-baby-pink-and-blue.html		
Advertised form of silver: "designed using so-called 'nano technology' which incorporates the mineral [sic] silver" "Silver has natural antibacterial properties which kill viruses, and it is one of the safest substances for babies and has no side effects"		
Comments: Cost: £5.00. UK vendor website, shipping to the US is available. Product found through the PEN online inventory.		

(17)	**Product Name:** Baby Pink or Blue - Precious Protechtor Baby Vest	**Product Type (likely exposure route):** Clothing (dermal)
Manufacturer (Country): Precious Protechtor (UK)		**Retailer:** Olivers Babycare
Retailer website: http://www.olivers-baby-care.co.uk/baby-pink-or-blue-precious-protechtor-baby-vest.html?utm_source=Google-Base&utm_medium=Web&utm_campaign=Gbase%5BPage visited on 20 September 2010%5D		
Advertised form of silver: "designed using so-called 'nano technology' which incorporates the mineral [sic] silver" "Silver has natural antibacterial properties which kill viruses, and it is one of the safest substances for babies and has no side effects"		
Comments: Cost: £8.00. UK vendor website, shipping to the US is available. Product found through the PEN online inventory.		

(18)	**Product Name:** Kiddy C-Cool Summer Cover	**Product Type (likely exposure route):** Clothing (dermal)
Manufacturer (Country): Kiddy (Germany)		**Retailer:** Olivers Babycare
Retailer website: http://www.olivers-baby-care.co.uk/kiddy-b-cool-summer-cover.html		
Advertised form of silver: "The clever all natural fibre uses 'Nano' technology the structure moves moisture away from the body"		
Comments: Cost: £22.99 UK vendor website, shipping to the US is available.		

(19)	**Product Name:** Skip Hop Pacifier Pocket	**Product Type (likely exposure route):** Textile (ingestion)
Manufacturer (Country): Skip Hop (USA)		**Retailer:** Babies R Us
Retailer website: http://www.toysrus.com/product/index.jsp?productId=3769274&CAWELAID=428235883		
Advertised form of silver: "The Agion® antimicrobial-protected lining helps reduce odor-causing bacteria"		
Comments: Price: $7.99.		

(20)	**Product Name:** Loving Home Bath Towel	**Product Type (likely exposure route):** Bath towel (dermal, ingestion)
Manufacturer (Country): Hangukta Mina (Korea)		**Retailer:** Unknown
Retailer website: http://www.emartmall.com/webapp/wcs/stores/servlet/ Product4_10151_8809026320121?ctgId=3002857&shop_id=&from1=03&from2=&emid=search		
Advertised form of silver: Unknown		
Comments: Product purchased in Seoul by EPA staff.		

(21)	**Product Name:** BreastMilk Storage Bags	**Product Type (likely exposure route):** Food storage (ingestion)
Manufacturer (Country): Perfection (South Korea)		**Retailer:** eBay.com
Retailer website: http://cgi.ebay.com/BreastMilk-Storage-Bags-BPA-Free-30-90-Packs-NanoSilver-/320652895280		
Advertised form of silver: "Nano-Silver Process"		
Comments: $11.00 for 30 200-ml bags, $17.00 for 60 200-ml bags.		

(22)	**Product Name:** Sippy cups	**Product Type (likely exposure route):** Sippy cups (ingestion)
Manufacturer (Country): Baby Dream Co., Ltd (Korea)		**Retailer:** EC21.com
Retailer website: http://babydream.en.ec21.com/		
Advertised form of silver: "silver nano poly system"		
Comments: Bulk vendor. Buyer must contact supplier to receive ordering information. Price not advertised.		

(23)	**Product Name:** Sippy cups, 2 ct, blue	**Product Type (likely exposure route):** Sippy cups (ingestion)
Manufacturer (Country): Unknown (UK)		**Retailer:** Unknown
Retailer website: Not Applicable		
Advertised form of silver: Unknown		
Comments: These two sippy cups were purchased in the UK and provided by EPA.		

(24)	**Product Name:** Idea Village Always Fresh Food Containers	**Product Type (likely exposure route):** Food storage (ingestion)
Manufacturer (Country): Idea Village (Unknown, shipped from USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Idea-Village-Always-Fresh-Containers/dp/B002672G8E/ref=sr_1_1?s=home-garden&ie=UTF8&qid=1301413804&sr=1-1		
Advertised form of silver: "Made with nanotechnology, containers include silver nanoparticles (…)"		
Comments: $5.99 each set of 5 containers plus lids, microwave and dishwasher safe.		

(25)	**Product Name:** Go Green Premium Nano Silver 7 Piece Food Storage Container Set	**Product Type (likely exposure route):** Food storage (ingestion)
Manufacturer (Country): Kinetic (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Kinetic-Premium-Silver-Storage-Container/dp/B002PDOC68/ref=sr_1_1?ie=UTF8&s=home-garden&qid=1301415003&sr=1-1		
Advertised form of silver: "Micro-particles of antimicrobial silver are infused into the polyproylene to protect the containers against mold, fungus and other microorganisms [sic]"		
Comments: $8.71 for a 3-piece set, or $21.42 for a 7-piece set.		

(26)	**Product Name:** Peak Fresh Re-usable Produce Bags	**Product Type (likely exposure route):** Food storage (ingestion)
Manufacturer (Country): Peak Fresh (Australia)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Peak-Fresh-Re-Usable-Produce-Bags/dp/B001CPGYM0/ref=pd_bxgy_k_text_c		
Advertised form of silver: "This low-density polyethylene film is impregnated with a natural occurring mineral that significantly increases gas permeability (…)"; "The mineral also has good deodorizing properties."		
Comments: $5.79 for a set of 10. Does not advertise silver nanoparticles, but silver might be the additive that has "good deodorizing properties".		

(27)	**Product Name:** New Chi Nail Lacquer Pink Shimmer w/ Nano Silver Techno	**Product Type (likely exposure route):** Nail polish (ingestion, dermal)
Manufacturer (Country): Farouk Systems (USA)		**Retailer:** eBay.com
Retailer website: http://cgi.ebay.com/ws/eBayISAPI.dll?ViewItem&item=280417456804 - vi-content		
Advertised form of silver: "Nano Silver"		
Comments: $7.50 for a 0.5-oz bottle.		

(28)	**Product Name:** Nano Silver Antibacterial Skin Gel	**Product Type (likely exposure route):** Skin care (dermal)
Manufacturer (Country): Henan Huier Nano Technology Co. Ltd. (China)		**Retailer:** Alibaba.com
Retailer website: http://www.alibaba.com/product-gs/427548050/Nano_Silver_Antibacterial_Skin_Gel.html		
Advertised form of silver: "nano silver"; "99.99 purity [sic]"; "Nano zinc, nano silver complex compound"; "10mg/l"		
Comments: Bulk vendor: minimum quantity of 1 kg. Buyer must contact supplier to receive ordering information. Price not advertised.		

(29)	**Product Name:** Ultra Colloidal Silver Gel	**Product Type (likely exposure route):** Skin care (dermal)
Manufacturer (Country): Silver Mountain Minerals (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Ultra-Colloidal-Silver-Gel-113-3/dp/B003LNRKII/ref=sr_1_24?s=hpc&ie=UTF8&qid=1301521345&sr=1-24		
Advertised form of silver: "Colloidal Silver Topical Gel with Aloe, Copper, Zinc and Essential Oils"; "Provides you with 240 ppm of pure colloidal silver"		
Comments: $14.49 for a 4-oz tub. Recommended uses fit with common skin care needs of children: "May be used as a skin conditioner, moisturizer and deodorant. Suitable for use on cuts, grazes, minor burns, dry skin, sun-burn, rashes, eczema and psoriasis."		

(30)	**Product Name:** Sovereign Silver First-Aid Pump Gel	**Product Type (likely exposure route):** Skin care (dermal)
Manufacturer (Country): Natural-Immunogenics Corp. (USA)		**Retailer:** Spring Valley Herbs
Retailer website: http://www.springvalleyherbs.com/catalog/item/4491/Sovereign-Silver-First-Aid-Gel-Pump		
Advertised form of silver: "Kid-Friendly"; "metallic silver"		
Comments: $23.98 for two 2-oz bottles, $57.58 for two 8-oz bottles.		

(31)	**Product Name:** Silverbiotics ASAP Ultimate Skin & Body Care Gel	**Product Type (likely exposure route):** Skin care (dermal)
Manufacturer (Country): American Biotech Labs (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/ASAP-Ultimate-Skin-Body-Ounces/dp/B0016SJLD2/ref=sr_1_fkmr0_1?ie=UTF8&qid=1301509926&sr=8-1-fkmr0		
Advertised form of silver: "24 ppm product [sic]"		
Comments: $14.59 for a 4-oz bottle.		

(32)	**Product Name:** Silver Shield Gel	**Product Type (likely exposure route):** Skin care (dermal)
Manufacturer (Country): Nature's Sunshine (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Natures-Sunshine-Silver-Shield-tube/dp/B001JKIFPM/ref=sr_1_3?s=hpc&ie=UTF8&qid=1301519605&sr=1-3		
Advertised form of silver: "Uses only the finest particle-size colloids to ensure maximum bioavailability and efficiency" "safe for children."		
Comments: $17.70 for a 3-oz tube.		

(33)	**Product Name:** SilvaSolution Lotion	**Product Type (likely exposure route):** Skin care (dermal)
Manufacturer (Country): Trimedica (USA)		**Retailer:** Spring Valley Herbs
Retailer website: http://www.trimedica.com/SilvaSolution-Lotion-4oz/productinfo/0144/		
Advertised form of silver: "contains homeopathic Argentum metallicum (silver) to help soothe minor, temporary itching or skin irritation." , "50 ppm"		
Comments: $13.50 for a 4-oz tube. Directions for children were not mentioned.		

(34)	**Product Name:** Colloidal Silver Cream with UMF16+ Manuka Honey	**Product Type (likely exposure route):** Skin care (dermal)
Manufacturer (Country): Skybright (New Zealand)		**Retailer:** Shop New Zealand
Retailer website: http://www.shopnewzealand.co.nz/en/cp/Colloidal_Silver_Cream_with_UMF16_Manuka_Honey_100g		
Advertised form of silver: "Colloidal Silver"		
Comments: $25.40 for a 100-g tub.		

(35)	**Product Name:** Ultra Colloidal Silver Salve	**Product Type (likely exposure route):** Skin care (dermal)
Manufacturer (Country): Source Naturals (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Source-Naturals-Ultra-Colloidal-Silver/dp/B000GFJJJC/ref=pd_sim_hpc_2		
Advertised form of silver: "Produced using a unique electrical process that creates homogeneity, minute particle size, and stability of the silver particles"; "10 ppm"		
Comments: $4.58 for a 0.5-oz tub, $7.90 for 1 ounce, $20.56 for 2 ounces.		

(36)	**Product Name:** SilvaSolution Salve	**Product Type (likely exposure route):** Skin care (dermal)
Manufacturer (Country): Trimedica (USA)		**Retailer:** Spring Valley Herbs
Retailer website: http://www.trimedica.com/SilvaSolution-Salve-2oz/productinfo/0101/		
Advertised form of silver / directions: "contains homeopathic Argentum metallicum (silver) to help soothe minor, temporary itching or skin irritation." "silver (0.01 - 0.0001 microns)" "Use as needed for minor cuts, abrasions, rashes, and other minor skin irritations (…)"		
Comments: $13.50 for a 4-oz tube. Directions for children were not mentioned.		

(37)	**Product Name:** Silver Herbal Ointment	**Product Type (likely exposure route):** Skin care (dermal)
Manufacturer (Country): Natural Path Silver Wings (USA)		**Retailer:** Spring Valley Herbs
Retailer website: http://www.springvalleyherbs.com/catalog/item/3747/Silver-Herbal-Ointment		
Advertised form of silver: "Colloidal Silver (250 ppm)";		
Comments: $ 9.59 for a 1-oz tub.		

(38)	**Product Name:** Liv's Natural Nano Silver Shampoo	**Product Type (likely exposure route):** Shampoo (dermal, ingestion)
Manufacturer (Country): Liv's Natural (USA)		**Retailer:** Livsnatural.com Also on eBay.com
Retailer website: http://www.livsnatural.com/servlet/the-Shampoo/Categories		
Advertised form of silver: "nano silver"		
Comments: $8.00 for a 2-oz bottle, $23.00 for 8 ounces. No directions for children.		

(39)	**Product Name:** Shampoo With Colloidal Silver + Lemongrass	**Product Type (likely exposure route):** Shampoo (dermal, ingestion)
Manufacturer (Country): Skybright (New Zealand)		**Retailer:** Shop New Zealand
Retailer website: http://www.shopnewzealand.us/en/cp/Colloidal_Silver_Benefits?currency=USD		
Advertised form of silver: "(…) Skybright added their own Colloidal Silver to give them the added ability to help rid the scalp of infection, dandruff or itchiness if they are present"; "It is also safe to use on baby's hair"		
Comments: $7.00 for a 60-ml bottle. This NZ-based retailer ships to the US and advertises prices in US dollars.		

(40)	**Product Name:** No Tears Natural Baby Shampoo	**Product Type (likely exposure route):** Shampoo (dermal, ingestion)
Manufacturer (Country): Nature's Boundaries (USA)		**Retailer:** MaeNatural.com
Retailer website: http://www.maenatural.com/content-product_info/product_id-2026/no_tears_natural_baby_shampoo.html		
Advertised form of silver: "The product that you order may be preserved with the highly effective silver citrate solution"		
Comments: $9.75 for one bottle.		

(41)	**Product Name:** Natural Hair Conditioner	**Product Type (likely exposure route):** Conditioner (dermal, ingestion)
Manufacturer (Country): Skybright (New Zealand)		**Retailer:** Shop New Zealand
Retailer website: http://www.shopnewzealand.co.nz/en/cp/Information_On_Colloidal_Silver		
Advertised form of silver: "(…) Skybright added their own Colloidal Silver to give them the added ability to help rid the scalp of infection, dandruff or itchiness if they are present"; "It is also safe to use on baby's hair"		
Comments: $21.40 for a 250-ml bottle. This NZ-based retailer ships to the US and advertises prices in US dollars.		

(42)	**Product Name:** Wellness Colloidal Silver Throat Spray	**Product Type (likely exposure route):** Throat spray (ingestion and inhalation)
Manufacturer (Country): Source Naturals (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Source-Naturals-Wellness-Colloidal-Silver/dp/B001GCTZ2U/ref=sr_1_3?s=hpc&ie=UTF8&qid=1301513598&sr=1-3		
Advertised form of silver: "Fine silver particles suspended in deionized water"		
Comments: $14.65 for a 2-oz bottle. This product is currently under study by our group for silver aerosol release.		

(43)	**Product Name:** SilvaSolution Spray	**Product Type (likely exposure route):** Throat spray (ingestion, inhalation)
Manufacturer (Country): Trimedica (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Trimedica-Pro-50-Spray-liquid/dp/B00014TRDG		
Advertised form of silver: "contains Argentum metallicum (silver)" "Spray for sore throat"		
Comments: $9.53 for a 2-oz bottle.		

(44)	**Product Name:** SilvaSolution Spray	**Product Type (likely exposure route):** Throat/nasal spray (ingestion, inhalation)
Manufacturer (Country): Trimedica (USA)		**Retailer:** Trimedica
Retailer website: http://www.trimedica.com/SilvaSolution-Pro50-Spray-2oz/productinfo/0262/		
Advertised form of silver / directions: "Contains homeopathic Argentum Metallicum (silver) 4x"; "50 ppm" "Super Strength Colloidal Silver Spray" "a potent form of microscopic silver in a purified, ozonated water base" "to relieve symptoms of colds and flu" "Spray on minor cuts, rashes or skin conditions as needed"		
Comments: $12.95 for a 2-oz bottle.		

(45)	**Product Name:** Silver Nasal Spray	**Product Type (likely exposure route):** Nasal spray (ingestion, inhalation)
Manufacturer (Country): Silver Mountain Minerals (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Silver-Mountain-Minerals-Nasal-2ag-Nasal/dp/B004QK37T6/ref=sr_1_4?s=hpc&ie=UTF8&qid=1301522516&sr=1-4		
Advertised form of silver: "colloidal silver nasal spray"		
Comments: $6.85 per spray bottle.		

(46)	**Product Name:** Ultra Colloidal Silver Nasal Spray	**Product Type (likely exposure route):** Nasal spray (ingestion, inhalation)
Manufacturer (Country): Source Naturals (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Source-Naturals-Ultra-Colloidal-Silver/dp/B000F4YA0W/ref=sr_1_3?s=hpc&ie=UTF8&qid=1301521345&sr=1-3		
Advertised form of silver: "Children: Take one-half the adult usage" "Adults: 2 teaspoons per day for no more than 10 days at a time." "Produced using a unique electrical process that creates homogeneity, minute particle size, and stability of the silver particles"; "10 ppm"		
Comments: $6.86 for a 1-oz bottle, $13.56 for 2 ounces.		

(47)	**Product Name:** SilvaSolution Lozenges	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): Trimedica (USA)		**Retailer:** Trimedica
Retailer website: http://www.trimedica.com/SilvaSolution-Lozenges-30ct/productinfo/0203/		
Advertised form of silver / directions: "Contains Argentum metallicum (silver) 4x for minor coughs"; "50 ppm"		
Comments: $6.95 for a 30-ct bottle, $15.95 for 90 ct.		

(48)	**Product Name:** Wellness Colloidal Silver	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): Source Naturals (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Source-Naturals-Wellness-Colloidal-Silver/dp/B000GFHPP2/ref=sr_1_1?s=hpc&ie=UTF8&qid=1301513598&sr=1-1		
Advertised form of silver: "Produced using a unique electrical process that creates homogeneity, minute particle size, and stability of the silver particles"; "33 ppm"		
Comments: $12.10 for a 2-oz bottle, $17.95 for 4 ounces, $37.46 for 8 ounces.		

(49)	**Product Name:** Silver Biotics	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): American Biotech Labs (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/American-Biotech-Labs-Silver-Biotics/dp/B0013664GE/ref=pd_bxgy_hpc_text_b		
Advertised form of silver: "10 ppm silver solution"		
Comments: $24.29 for a 16-oz bottle, $13.79 for an 8-oz bottle. Suggested use for children: " ¼ to ½ teaspoon once daily"		

(50)	**Product Name:** Colloidal Silver "Ultra 240 ppm"	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): Silver Mountain Minerals (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Colloidal-Silver-Ultra-240-ppm/dp/B0036RM6E2/ref=sr_1_2?s=hpc&ie=UTF8&qid=1301522516&sr=1-2		
Advertised form of silver: "liquid colloidal silver concentrate for natural antibiotic supplement"; "240 ppm"		
Comments: $24.50 for a 16-oz bottle.		

(51)	**Product Name:** "Bio-Silver" Ultra Colloidal Silver 50 ppm	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): Silver Mountain Minerals (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Bio-Silver-Ultra-Colloidal-Silver-ppm/dp/B003LUMDUQ/ref=sr_1_6?s=hpc&ie=UTF8&qid=1301522516&sr=1-6		
Advertised form of silver: ""Bio-Silver" is referenced at 50 ppm consisting of pure silver (99.999%) suspended in steam-distilled water"; "Nanometer Particle Size, High Colloidal/Ionic percentage & High Particle Concentration"		
Comments: $10.99 for an 8-oz bottle, $19.49 for a 16-oz bottle.		

(52)	**Product Name:** SilvaSolution Liquid	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): Trimedica (USA)		**Retailer:** Spring Valley Herbs
Retailer website: http://www.trimedica.com/SilvaSolution-Liquid-4oz/productinfo/0103/		
Advertised form of silver: "Contains Argentum Metallicum 5x (Silver)"; "10 ppm" "For Colds & Flu"		
Comments: $14.95 for a 4-oz bottle, $26.95 for 8 ounces. Directions for children were not advertised.		

(53)	**Product Name:** SilvaSolution Advanced	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): Trimedica (USA)		**Retailer:** Spring Valley Herbs
Retailer website: http://www.trimedica.com/SilvaSolution-Advanced-4oz/productinfo/0685/		
Advertised form of silver / directions: "homeopathic silver (argentum metallicum) 5x"; "10 ppm" "Children: Take 15 drops or 1 dropper full under the tongue up to 2 times daily"		
Comments: $16.95 for a 4-oz bottle, $29.95 for 8 ounces.		

(54)	**Product Name:** SilvaSolution Pro 50 Liquid	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): Trimedica (USA)		**Retailer:** Spring Valley Herbs
Retailer website: http://www.trimedica.com/SilvaSolution-Pro-50-Liquid-4oz/productinfo/0263/		
Advertised form of silver: "Contains homeopathic Argentum Metallicum (silver) 4x"; "50 ppm" "For Colds & Flu"		
Comments: $18.95 for a 4-oz bottle, $34.95 for 8 ounces. Directions for children were not advertised.		

(55)	**Product Name:** Sovereign Silver	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): Natural-Immunogenics Corp. (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Sovereign-Silver-Hydrosol-Liquid-Bottles/dp/B002RG8DOA/ref=sr_1_11?s=hpc&ie=UTF8&qid=1301513598&sr=1-11		
Advertised form of silver: "99.999% PURE & Sovereign Silver Provides the Smallest Sub-Nano Silver Particle Size Ever Seen - 0.8 nm [sic]"; "10 ppm"		
Comments: $ 23.98 for two 2-oz bottles, $57.58 for two 8-oz bottles.		

(56)	**Product Name:** Arise Secure Ionic Silver	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): Oil In My Lamp (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Arise-Secure-Ionic-Silver-200ppm/dp/B002PSBHCA/ref=sr_1_13?s=hpc&ie=UTF8&qid=1301513598&sr=1-13		
Advertised form of silver: "Ionic Silver"; "May be taken as frequently as desired without causing argyria"		
Comments: $25 for an 8-oz bottle, $85.95 for a 32-oz bottle.		

(57)	**Product Name:** Silver Water 365	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): Category Creators (USA)		**Retailer:** eBay.com
Retailer website: http://cgi.ebay.com/Nano-Silver-Water-All-Natural-Water-Supplement-8-oz-/180546657327?pt=LH_DefaultDomain_0&hash=item2a096b5c2f		
Advertised form of silver / directions: "silver nano-particle dietary supplement"; "Prepared with 10 ppm (…) silver in a purified water base" "Children 4 and older: 1/4 to 1/2 teaspoon once daily."		
Comments: $18.95 for an 8-oz bottle.		

(58)	**Product Name:** Colloidal Silver Liquid	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): Skybright (New Zealand)		**Retailer:** Shop New Zealand
Retailer website: http://www.shopnewzealand.co.nz/en/cp/Liquid_Colloidal_Silver		
Advertised form of silver: "Ionic Colloidal Silver at 6ppm"		
Comments: $21.45 for a 200-ml bottle. This NZ-based retailer ships to the US and advertises prices in US dollars.		

(59)	**Product Name:** Nature's Sunshine Silver Shield w/Aqua Sol Support Immune System	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): Nature's Sunshine (USA)		**Retailer:** NaturesSunshine.com Also sold at Amazon.com
Retailer website: http://www.naturessunshine.com/us/product/silver-shield-waqua-sol-18-ppm-4-fl-oz/sku-4274.aspx		
Advertised form of silver: "Uses only the finest particle-size colloids to ensure maximum bioavailability and efficiency" "Take one teaspoon with a meal three times daily" "18 ppm"		
Comments: $32.95 for a 4-oz bottles. Directions for children were not given.		

(60)	**Product Name:** MesoSilver® Colloidal Silver	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): Purest Colloids (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/MesoSilver-Colloidal-Silver-250-8-45/dp/B000ZK1NVK/ref=sr_1_1?s=hpc&ie=UTF8&qid=1301520751&sr=1-1		
Advertised form of silver: "Colloidal Silver" "World's Smallest Particle Size, less than 1 nm on average"		
Comments: $29.97 for an 8.45-oz bottle.		

(61)	**Product Name:** Dietary Mineral Supplement, 500 ppm	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): Natural Path Silver Wings (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Natural-Path-Silver-Wings-Supplement/dp/B000BSZBHS/ref=sr_1_2?s=hpc&ie=UTF8&qid=1301521345&sr=1-2		
Advertised form of silver: "Colloidal Silver"; "500 ppm"; "Our colloidal silver is tested for purity and efficacy"		
Comments: $ 24.13 for a 4-oz bottle.		

(62)	**Product Name:** Dietary Mineral Supplement, 250 ppm	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): Natural Path Silver Wings (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Natural-Path-Silver-Wings-Supplement/dp/B000BT4CFE/ref=sr_1_2?s=hpc&ie=UTF8&qid=1301523914&sr=1-2		
Advertised form of silver: "Colloidal Silver"; "250 ppm"; "Our colloidal silver is tested for purity and efficacy"; "Our mean range is 0.002 microns"		
Comments: $ 23.98 for a 4-oz bottle.		

(63)	**Product Name:** Colloidal Silver 250 ppm Spray	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): Natural Path Silver Wings (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Colloidal-Silver-250ppm-Spray-Liquid/dp/B0049Z27HG/ref=sr_1_8?s=hpc&ie=UTF8&qid=1301524013&sr=1-8		
Advertised form of silver: "Colloidal Silver"; "50 ppm"; "(…) pure, metallic silver, in particles of 15 atoms or fewer, each with a positive electric charge and attached to a molecule of a simple protein"; "0.1% casein"; "(…) extremely small, usually ranging from about 0.001 to about 0.01 microns in diameter"; "(…) suspended in deionized water".		
Comments: $ 13.49 for a 2-oz bottle.		

(64)	**Product Name:** Colloidal Silver 125 ppm	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): Natural Path Silver Wings (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Colloidal-Silver-125ppm-Natural-Wings/dp/B0041VOGRC/ref=sr_1_7?s=hpc&ie=UTF8&qid=1301524013&sr=1-7		
Advertised form of silver: "Colloidal Silver Herbal Tinctures"; "125 ppm"; "Contains Aloe Vera and Tea Tree Oil".		
Comments: $ 12.99 for a 1-oz bottle.		

(65)	**Product Name:** Colloidal Silver 50 ppm	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): Natural Path Silver Wings (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Natural-Path-Silver-Wings-Colloidal/dp/B0049YYYJG/ref=sr_1_9?s=hpc&ie=UTF8&qid=1301524013&sr=1-9		
Advertised form of silver: "Colloidal Silver"; "50 ppm"; "(…) pure, metallic silver, in particles of 15 atoms or fewer, each with a positive electric charge and attached to a molecule of a simple protein"; "0.1% casein"; "(…) extremely small, usually ranging from about 0.001 to about 0.01 microns in diameter"; "(…) suspended in deionized water".		
Comments: $ 10.19 for a 2-oz bottle.		

(66)	**Product Name:** MesoSilver® Antifungal/Antibacterial Disinfecting Spray	**Product Type (likely exposure route):** Surface disinfectant (dermal, ingestion)
Manufacturer (Country): Purest Colloids (USA)		**Retailer:** Amazon.com Also at PurestColloids.com
Retailer website: http://www.amazon.com/MesoSilver-Antifungal-Spray-125-Colloidal/dp/B00106J28O/ref=pd_sim_hpc_5		
Advertised form of silver / Directions: "Colloidal Silver" "World's Smallest Particle Size, less than 1 nm on average" "Use in the kitchen, bathroom and children's room (…) [sic]"		
Comments: $27.99 for a 125-ml bottle.		

(67)	**Product Name:** Nature's Sunshine Silver Shield with Aqua Sol	**Product Type (likely exposure route):** Surface disinfectant (dermal, ingestion)
Manufacturer (Country): Nature's Sunshine (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Natures-Sunshine-Silver-Shield-18ppm/dp/B000G45YG0/ref=pd_bxgy_hpc_img_b		
Advertised form of silver: "Uses only the finest particle-size colloids to ensure maximum bioavailability and efficiency" "18 ppm" "Acts as an EPA-approved surface disinfectant."		
Comments: $26.95 for a 4-oz bottle. Might be used as a toy/surface cleaner near infants.		

(68)	**Product Name:** Nature's Fresh Silver Shield Surface Wipes	**Product Type (likely exposure route):** Surface disinfectant (dermal, ingestion)
Manufacturer (Country): Nature's Sunshine (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Natures-Fresh-Silver-Shield-Surface/dp/B004ST0AQ8/ref=sr_1_4?ie=UTF8&s=hpc&qid=1301519636&sr=1-4		
Advertised form of silver: "Contains: (…) , Silver Shield® (silver: colloidal and ionic)"		
Comments: $18.99 for 30 wipes. Might be used as a toy/surface cleaner near infants.		

(69)	**Product Name:** Easy-Well Perfect Nano Silver Scrubber	**Product Type (likely exposure route):** Surface cleaner (dermal, ingestion)
Manufacturer (Country): Hankook Tamina Co., Ltd (Korea)		**Retailer:** Unknown
Manufacturer website: http://www.hktamina.co.kr/		
Advertised form of silver: "Nano Silver"		
Comments: Product purchased in Seoul by EPA staff (set of 2).		

(70)	**Product Name:** Easy-Well Scrubbers	**Product Type (likely exposure route):** Surface cleaner (dermal, ingestion)
Manufacturer (Country): Hankook Tamina Co., Ltd (Korea)		**Retailer:** Unknown
Manufacturer website: http://www.hktamina.co.kr/		
Advertised form of silver: "Nano Silver"		
Comments: Product purchased in Seoul by EPA staff (set of 4).		

(71)	**Product Name:** Air-O-Swiss	**Product Type (likely exposure route):** Humidifier (inhalation)
Manufacturer (Country): PLASTON International Corporation (Switzerland)		**Retailer:** Bed Bath & Beyond
Retailer website: http://www.bedbathandbeyond.com/product.asp?sku=13876550&utm_source=google&utm_medium=organic&utm_campaign=shopping		
Advertised form of silver: "Includes demineralization cartridge and ionic silver stick"		
Comments: Cost: $169.99		

(72)	**Product Name:** Germ Guardian H1000 TableTop Humidifier	**Product Type (likely exposure route):** Humidifier (inhalation)
Manufacturer (Country): Germ Guardian (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Germ-Guardian-H1000-TableTop-Humidifier/dp/B000ZPKVMM/ref=sr_1_3?s=hpc&ie=UTF8&qid=1301872292&sr=1-3		
Advertised form of silver: "Uses Nano-Silver technology to stop mold and bacteria from growing in the tank"		
Comments: Cost: $49.99		

(73)	**Product Name:** Germ Guardian H1600 Digital Ultrasonic Midsize Humidifier	**Product Type (likely exposure route):** Humidifier (inhalation)
Manufacturer (Country): Germ Guardian (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Germ-Guardian-H1600-Ultrasonic-Humidifier/dp/B002KMILWA/ref=sr_1_2?s=hpc&ie=UTF8&qid=1301872292&sr=1-2		
Advertised form of silver: "Silver clean technology fights mold and bacteria growth in water tank"		
Comments: Cost: $122.37		

(74)	**Product Name:** Germ Guardian H2000 Manual Ultrasonic Humidifier	**Product Type (likely exposure route):** Humidifier (inhalation)
Manufacturer (Country): Germ Guardian (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Germ-Guardian-H2000-Ultrasonic-Humidifier/dp/B001A075IE		
Advertised form of silver: "Uses nano-silver technology to fight the growth of mold and bacteria in the water so germ-free water is vaporized into the room"		
Comments: Cost: $124.78		

(75)	**Product Name:** Germ Guardian H2500 72 Hour Ultrasonic Humidifier with Night Light	**Product Type (likely exposure route):** Humidifier (inhalation)
Manufacturer (Country): Germ Guardian (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Germ-Guardian-H2500-Ultrasonic-Humidifier/dp/B00446IPEO/ref=sr_1_8?s=hpc&ie=UTF8&qid=1301872292&sr=1-8		
Advertised form of silver: "uses silver clean technology to prevent the growth of mold and bacteria in the water tank"		
Comments: Cost: $159.99		

(76)	**Product Name:** Germ Guardian H4500 120 Hour Ultrasonic Humidifier	**Product Type (likely exposure route):** Humidifier (inhalation)
Manufacturer (Country): Germ Guardian (USA)		**Retailer:** Amazon.com
Retailer website: http://www.amazon.com/Germ-Guardian-H4500-Ultrasonic-Humidifier/dp/B00446IPB2/ref=sr_1_1?s=hpc&ie=UTF8&qid=1301872292&sr=1-1		
Advertised form of silver: "uses silver clean technology to prevent the growth of mold and bacteria in the water tank"		
Comments: Cost: $122.99		

(77)	**Product Name:** Swizz Style EMS-171 Humidifier	**Product Type (likely exposure route):** Humidifier (inhalation)
Manufacturer (Country): Stadler Form (USA)		**Retailer:** Macy's
Retailer website: http://www.macys.com/catalog/product/index.ognc?ID=532645&cm_mmc=Google_Feed-_-6-_-77-_-MP677		
Advertised form of silver: "Ionic Silver Cube™ inhibits growth of mold bacteria"		
Comments: Cost: $119.99		

(78)	**Product Name:** Stadler Form AQUILA Ultrasonic Humidifier	**Product Type (likely exposure route):** Humidifier (inhalation)
Manufacturer (Country): Stadler Form (USA)		**Retailer:** Stadler Form
Retailer website: http://swizz-style.myshopify.com/collections/humidifiers/products/aquila		
Advertised form of silver: "patented Ionic Silver Cube ™, inhibiting the growth of mold bacteria"		
Comments: Cost: $79.99		

(79)	**Product Name:** Stadler Form HYDRA Ultrasonic Humidifier	**Product Type (likely exposure route):** Humidifier (inhalation)
Manufacturer (Country): Stadler Form (USA)		**Retailer:** Stadler Form
Retailer website: http://swizz-style.myshopify.com/collections/humidifiers/products/hydra		
Advertised form of silver: "patented Ionic Silver Cube ™, inhibiting the growth of mold bacteria"		
Comments: Cost: $99.99		

(80)	**Product Name:** Stadler Form ANTON Ultrasonic Humidifier	**Product Type (likely exposure route):** Humidifier (inhalation)
Manufacturer (Country): Stadler Form (USA)		**Retailer:** Stadler Form
Retailer website: http://swizz-style.myshopify.com/collections/humidifiers/products/anton		
Advertised form of silver: "Ionic Silver Cube ™"		
Comments: Cost: $139.99		

(81)	**Product Name:** Stadler Form WILLIAM Ultrasonic Humidifier	**Product Type (likely exposure route):** Humidifier (inhalation)
Manufacturer (Country): Stadler Form (USA)		**Retailer:** Stadler Form
Retailer website: http://swizz-style.myshopify.com/collections/humidifiers/products/william		
Advertised form of silver: "The patented Ionic Silver Cube ™ works nicely with William, inhibiting the growth of mold bacteria."		
Comments: Cost: $299.99		

(82)	**Product Name:** Stadler Form Ionic Silver Cube	**Product Type (likely exposure route):** Humidifier (inhalation)
Manufacturer (Country): Stadler Form (USA)		**Retailer:** Stadler Form
Retailer website: http://swizz-style.myshopify.com/collections/accessories/products/ionic-silver-cube		
Advertised form of silver / directions: "Releases silver ions into the water" "For use with most humidifiers on the market"		
Comments: Cost: not advertised		

SOP # AIRVT-NANOSILVER-001

PREPARATION OF CONSUMER PRODUCT SAMPLES FOR SILVER ANALYSIS BY ICP-MS

May 16, 2011

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APPROVED:

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Nicolle Tulve _____ _____
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SUMMARY

SOP description	This SOP describes the laboratory methods for dissolving silver particles from liquid consumer products, acid digesting fabrics and plastics to dissolve silver from these materials, and analyzing silver concentrations in the products by ICP-MS.
Example consumer goods appropriate for testing by this SOP	• Liquid products that claim to contain silver ions or particles, such as throat sprays, disinfecting liquids or gels, deodorant sprays, etc. • Fabric products that claim to contain silver ions or particles, such as clothes, hats, gloves, towels, blankets, etc. • Plastic products that claim to contain silver ions or particles, such as bags, utensils, sippy cups, milk bottles, pacifiers. • Mixed-media products, such as stuffed toys and pillows (fabric shell and foam interior).
Strengths	This SOP describes a simple method for dissolving silver from solid samples. The hot-plate method allows for the researcher to watch as the solid samples digest and easily tailor the digestion time to different products.
Weaknesses	The hot-plate method for digesting samples, though more transparent to the researcher, is very time consuming and can be substituted for microwave digestion if available. Plastic products do not digest in nitric acid, so they must be sliced into small pieces to facilitate silver dissolution.
Unanswered issues	Silver is not homogeneously distributed in consumer products, so the exact number of replicate samples necessary for reporting data with confidence cannot be determined before the first triplicate samples are analyzed.

A. PURPOSE AND APPLICABILITY

This protocol outlines the analytical methods for sample preparation by acid digestion and the determination of total silver content in various matrixes within consumer products via various inductively-coupled plasma (ICP) techniques. The objective of sample preparation for detection by ICP techniques is to convert all silver present in the sample into ionic silver in liquid media. This protocol can be applied to any suitable ICP technique, such as ICP-MS (mass spectroscopy), ICP-AES (atomic emission spectroscopy), and ICP-OES (optical emission spectroscopy). This protocol is intended to be an evolving document that may be improved over time. The structure of this document follows the format recommended by EPA.

B. SUMMARY OF METHOD

Different dissolution methods are used for liquid and solid products. Product samples are digested using aqua regia or a combination of nitric acid and hydrogen peroxide.

C. MATERIALS AND REAGENTS

1. General laboratory equipment

a. Fume hood

b. Centrifuge

c. Ultracentrifuge, if needed

d. Balance capable of weighing 10 mg

e. Hot plate (or microwave-assisted acid digestion system)

2. General laboratory materials

a. 100-ml graduated cylinder

b. 1.0-ml and 5.0-ml glass pipettes

c. 10, 25, 50, and 250-ml volumetric flasks

d. Capped culture vials (polystyrene) or glass vials

e. 100-ml digestion beakers

f. Watch glasses, 65 mm or larger

3. Reagents

a. Reagent-grade hydrogen peroxide (H_2O_2)

b. Reagent-grade nitric acid (HNO_3), concentrated (69 to 71%, w/w)

c. Reagent-grade hydrochloric acid (HCl), concentrated (38%)

4. Water

Ultrapure water should be used. If ultrapure water is not available, deionized (DI) water from laboratory taps can be used after allowing it to run for several minutes before collection.

5. Filters

a. 100, 450, and 1000-nm PTFE (Teflon) filter membrane or syringe filters

b. 3 or 10 KDa cut-off (such as Millipore Amicon or similar)

6. Instrumentation

a. ICP instrument coupled with a spectroscopy technique, such as ICP-MS (mass spectroscopy), ICP-AES (atomic emission spectroscopy), or ICP-OES (optical emission spectroscopy).

D. AQUA REGIA PREPARATION

a. Pour 25 ml of HNO_3 into glass container, in fume hood.

b. Slowly add 75 ml HCl.

c. Place aqua-regia into a glass jar with an acid-resistant cap.

Do not fasten cap, as aqua regia continuously releases fumes with nitrogen dioxide (NO_2) and chlorine (Cl_2), which may increase the pressure inside the jar.
Discard the solution when it loses its characteristic yellow-orange color and becomes clear.

E. SAMPLE PREPARATION PROCEDURES

1. Silver dissolution from liquid products

Silver nanoparticles from liquid products (e.g., throat spray) are dissolved in aqua regia. At least three samples must be obtained for each product.

a. Gently shake the product in its original container.

b. If using pure product, pipette 5.0 ml of liquid product into a 50-ml volumetric flask, complete to volume using ultrapure water, to obtain a 1:10 dilution of original sample.

c. If using a pre-diluted sample, omit step 1.b.

d. In a 10-ml volumetric flask, pipette 1.0 ml of diluted liquid product, 2 ml of nitric acid, and complete to volume with ultrapure water, to obtain a 1:100 diluted, acidified sample.

e. If this solution is clear, it is ready for ICP analysis. If samples are to be stored for more than 2 days, store in glass vials capped with acid-resistant plastic caps.

2. Size fractionation of liquid products

If the ICP analysis performed in step E.1 or E.2 confirms the presence of silver in liquid products, a subsequent filtration step is required to determine whether silver is in ionic or particulate form.

a. Gently shake the product in its original container.

b. Pour 25 ml of liquid product into a 250-ml volumetric flask, complete to volume using ultrapure water, to obtain a 1:10 dilution of original sample.

c. Pass sample through 1000-nm PTFE (Teflon) filter membrane or syringe filter.

d. Take 20 ml of the 1000-nm filtered sample, acid digest using the methods described in step E.1, and analyze through ICP.

e. Take remaining portion (~230 ml) of the 1000-nm filtered sample, pass through a 450-nm PTFE (Teflon) filter membrane or syringe filter.

f. Take 20 ml of the 450-nm filtered sample, acid digest using the methods described in step E.1, and analyze through ICP.

g. Take remaining portion (~210 ml) of the 450-nm filtered sample, pass through a 100-nm PTFE (Teflon) filter membrane or syringe filter.

h. Take 20 ml of the 100-nm filtered sample, acid digest using the methods described in step E.1, and analyze through ICP.

i. Take 4 ml of 100-nm filtered sample, place in a 4-ml centrifuge filter with a 3 or 10 KDa cut-off. Place centrifuge filter in centrifuge for 30 minutes (or time recommended by the filter manufacturer) at the G-force specified by the filter manufacturer. If centrifuge filters of this size-cutoff are not available, 20-nm or 25-nm dialysis membrane filters (such as Millipore type VS) may be used instead.

j. Ultracentrifugation (340,000 g or higher) may also be used as a parallel method to step E.3.i for separating small silver nanoparticles and ions.

k. Acid digest the filtrate according to step E.1.

l. Transfer all digested samples to capped culture vials (polystyrene) for ICP analysis. If samples are to be stored for more than 2 days, store in capped glass vials.

3. Silver dissolution from fabrics and plastics

Fabrics and plastics are digested in nitric acid and hydrogen peroxide at moderately high temperatures (Benn and Westerhoff, 2008; Benn et al., 2010). At least three samples must be obtained for each product. For stuffed toys, one set of samples must come from the outer layer of the product and another set from the outer layer of the stuffing.

a. Cut or slice 500 – 1000 mg (air-dry mass) of representative locations within product. For hard plastics, mechanically scrape off the outer 1-mm of product.

b. Place in a digestion beaker.

c. Submerge sample in 5 ml of reagent-grade nitric acid (concentrated, 69 – 71%, w/w) and 5 ml of ultrapure water.

d. Place a watch glass over the beaker and heat to ~100^{o}C.

e. Add nitric acid in 2-ml aliquots until the material is digested.

f. Allow to cool, and add 3 ml of 30% hydrogen peroxide (H_2O_2).

g. Heat beaker to ~100^{o}C, and add hydrogen peroxide in 1-ml aliquots until effervescence is minimal.

h. Allow for cooling, filter through a glass fiber filter.

i. Transfer to a 100-ml volumetric flask and dilute to volume with ultrapure water.

j. Transfer to vial for ICP analysis.

A microwave-assisted acid digestion method may be used instead of heating samples over a hot plate.

If nitric acid and hydrogen peroxide do not completely digest the product matrix, then the same method should be attempted utilizing aqua regia in its stead. The same volumes may be used.

F. PRECAUTIONS AND GUIDELINES

1. Always wear appropriate personal protective gear (e.g., gloves, lab coat, goggles, etc.).
2. Perform all acid digestion procedures inside an appropriate fume hood.
3. Ensure enough working space inside the fume hood to allow for safe arm movements.
4. Wash all glassware in 10% hydrochloric acid (HCl), 10% HNO_3, or aqua regia. Then triple rinse in ultrapure water and air-dry prior to use.
5. Do not fasten the cap of the aqua regia storage jar, as the hydrochloric and nitric acids in aqua regia react, continuously releasing fumes with nitrogen dioxide (NO_2) and chlorine (Cl_2), which may increase the pressure inside the jar.
6. Discard the aqua regia solution when it loses its characteristic yellow-orange color and becomes clear, indicating that the aqua regia has lost its potency and has turned into mostly water.

G. QUALITY ASSURANCE & QUALITY CONTROL

1. Purchase silver-free consumer products, similar to those to be analyzed, to be used as blank controls. Perform the same silver extraction methods, and report results.
2. Prepare a standard solution of ionic silver (by dissolving silver nitrate in ultrapure water, for example), and subject this solution to the same filtration procedures to assess silver ion sorption (i.e., loss) to filters.
3. Prepare a standard silver nanoparticle (such as Nanocomposix or similar) suspension of known particle size (<100 nm), and subject this solution to the same filtration procedures to assess nanoparticle sorption (i.e., loss) to filters.
4. The calibration curve for the ICP instrument must have at least five points, beginning at zero ppb (blank) and extending to 1000 ppb standard solutions.
5. In the case that an ICP result extends beyond the calibration curve values (e.g., >1000 ppb), new standard solutions must be analyzed on the same day so that the obtained values are encompassed by the calibration curve.

H. REPORTING RESULTS

1. Each product must generate at least three samples, and each sample must be analyzed at least three times by ICP. Average results and standard deviations are reported.
2. Final reported values must be converted to the original volume (for liquid samples) or weight (for fabrics and plastics), such as mg l^{-1} or mg kg^{-1} of silver in product.

I. REFERENCES

Benn, T.M. and P. Westerhoff, *Nanoparticle silver released into water from commercially available sock fabrics.* Environmental Science & Technology, 2008. 42(11): p. 4133-4139.

Benn, T., et al., *The release of nanosilver from consumer products used in the home*. Journal of Environmental Quality, 2010. 39(6): p. 1875-1882.

SOP # AIRVT-NANOSILVER-002

ANALYTICAL METHODS FOR SILVER NANOPARTICLE CHARACTERIZATION VIA ELECTRON MICROSCOPY IN COMPLEX MEDIA

May 16, 2011

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SUMMARY

SOP description	This SOP describes the laboratory methods for preparing samples for imaging with scanning and transmission electron microscopy and analyzing for chemical composition with electron energy dispersive X-ray spectroscopy.
Example consumer goods appropriate for testing by this SOP	• Liquid products that claim to contain silver ions or particles, such as throat sprays, disinfecting liquids or gels, deodorant sprays, etc. • Fabric products that claim to contain silver ions or particles, such as clothes, hats, gloves, towels, blankets, etc. • Plastic products that claim to contain silver ions or particles, such as bags, utensils, sippy cups, milk bottles, pacifiers. • Mixed-media products, such as stuffed toys and pillows (fabric shell and foam interior).
Strengths	This SOP lists microscopy techniques sufficient to provide a full chemical and morphological characterization of silver-containing particles in different types of consumer products. The SEM and TEM techniques are complementary in that SEM can be used for bulk, unaltered products but does not provide image or spectroscopy details for the smallest particles, while TEM requires more sample preparation but enables characterization in greater detail for small (i.e., < 100 nm) particles.
Weaknesses	The cryo-ultramicrotoming technique suggested in this SOP has not been validated in this work because this instrument was unavailable for use during this project.
Unanswered issues	A large number of electron microscopy techniques can be used to assess the presence of silver in consumer products. The specific instrumentation listed here is not mandatory but suggested for the specific product categories that are listed here.

A. PURPOSE AND APPLICABILITY

This protocol outlines the analytical methods for sample preparation and characterization of nanosilver-containing consumer products via transmission or scanning electron microscopy imaging (TEM or SEM, respectively) and electron dispersive x-ray spectroscopy (EDS). The objective of sample preparation for TEM is to spread the product over a TEM grid and/or slice it into electron-transparent samples. Bulk samples can be viewed through SEM, but TEM grids must be propped against a carbon-coated holder to reduce the backscattered signal from the sample holder and stage.

This protocol can be applied to any suitable electron microscopy technique, such as TEM, high-resolution TEM (HR-TEM), and SEM. This protocol is intended to be an evolving document that may be improved over time. The structure of this document follows the format recommended by EPA.

B. SUMMARY OF METHOD

Samples are dispersed onto TEM grids to be viewed via TEM, or sliced to be viewed via SEM. Textile samples are also ashed to be viewed via TEM.

C. MATERIALS AND INSTRUMENTS

1. General laboratory equipment

a. Balance capable of weighing 10 mg

b. Muffle furnace capable of reaching 550°C

2. General laboratory materials

a. Micro syringe capable of measuring 10 µl

b. Reversed tweezers

c. Carbon tape

d. Reagent-grade nitric acid (HNO3), concentrated (69 to 71%, w/w)

e. Reagent-grade hydrochloric acid (HCl), concentrated (38%)

f. Standard commercially available silver nanoparticles (NanoAmor, Nanocomposix, etc.)

3. Water

Ultrapure water should be used. If ultrapure water is not available, deionized (DI) water from laboratory taps can be used after allowing it to run for several minutes before collection.

4. TEM grids and sample holders

a. Carbon-coated TEM grids are to be used.

b. Copper grids are recommended but not mandatory. Silver grids may not be used.

c. Grids must contain markings in the center of the grid or throughout the grid (e.g., “finder grids”), so that particles viewed on SEM can be later found on TEM or HR-TEM.

d. If available, amine-coated TEM grids (Dune Sciences or similar) may be used to promote an even particle distribution over the grid and minimize agglomeration.

e. SEM holders for TEM grids must be used for SEM work.

5. Required instrumentation

a. Transmission electron microscope.

b. Scanning electron microscope equipped with energy-dispersive X-ray spectroscopy.

c. High-resolution electron microscope equipped with energy-dispersive X-ray spectroscopy or other elemental characterization technique.

d. A cryo-ultramicrotome, capable of slicing materials into electron-transparent samples (for TEM viewing).

e. A muffle furnace, capable of reaching 550oC.

D. SAMPLE PREPARATION PROCEDURES

1. Liquid products

Liquid products (e.g., surface disinfectant spray) are evaporated over TEM grids. At least three samples must be obtained from each product. This method is also applied for leachate samples.

a. Use a micro-syringe to place a 10-µl droplet of product over a carbon-coated TEM grid (the droplet should cover the diameter of the grid).

b. Using reversed tweezers, place TEM grid into a desiccator and wait for droplet to evaporate, leaving particles on grid.

c. Repeat these steps until the total sample volume reaches 20 µl.

2. Fabrics

Fabric samples are viewed unaltered by SEM and also ashed to concentrate particles and enable TEM analysis (Benn and Westerhoff, 2008). At least three samples must be obtained from each product.

a. Take a small piece of fabric from representative locations within product.

b. Using carbon tape, adhere fabric to SEM stub.

c. Cut a representative sample from the product (about 1000 mg).

d. Ash the material at <550°C in a muffle furnace.

e. Dust some of the ashed sample onto carbon tape on an SEM stub or onto carbon-coated TEM grids.

f. Also suspend some of the ashed material in distilled water into a scintillation vial, immerse it in a sonicating bath for ~1 min, and subsequently evaporate droplets of it on carbon-coated TEM grids.

3. Plastics

Plastic samples are sliced and viewed unaltered by SEM and, if possible, by TEM. At least two samples must be obtained from each product.

a. Using a cryo-ultramicrotome, slice the material in a tapered fashion that encompasses both the surface and interior of the product. Attempt to obtain thin enough slices (<100 nm) for TEM imaging and chemical characterization.

b. At least five representative slices per sample will be analyzed by high-resolution SEM/EDS.

E. ELECTRON MICROSCOPY PROCEDURES

a. Samples must first be surveyed by SEM. EDS spectra will be obtained whenever particles are observed in backscattered mode (when particles containing higher atomic weight substances, like metals, shine brighter).

b. Images are recorded whenever an EDS spectrum is obtained.

c. One sample per product that has been shown to contain silver nanoparticles will be analyzed using high-resolution TEM to determine the form of silver in more detail.

d. Using HR-TEM, small particles that may not be detected using SEM or other TEM techniques may be imaged. Images are recorded whenever an EDS spectrum is obtained.

e. If HR-TEM is not available, TEM may be used to obtain complementary images to those obtained by SEM.

f. Both HR-TEM and TEM may be used to visualize the crystal state of particles, either by imaging on HR-TEM or by obtaining diffraction patterns on HR-TEM or TEM. The presence of organic surface coatings may be observable with HR-TEM.

g. TEM images may be used to obtain a particle size distribution (for liquid samples). To this end, at least 100 particles must be imaged, and then the diameter of each particle must be measured at least three times and averaged.

F. PRECAUTIONS AND GUIDELINES

1. Always wear appropriate personal protective gear (e.g., gloves, lab coat, goggles, etc.).
2. Wash all glassware in 10% hydrochloric acid (HCl), 10% HNO_3, or aqua regia (solution of 1:3 nitric acid and hydrochloric acid, respectively). Then triple rinse in ultrapure water and air-dry prior to use.
3. If a sample becomes electrically charged during the SEM analysis, it should be removed from the SEM, carbon-coated, and reanalyzed.

G. QUALITY ASSURANCE & QUALITY CONTROL

1. ImageJ software can be used when appropriate to aid in particle counting and sizing.
2. Evaporation of liquid during drying in the desiccator may cause aggregation/agglomeration of the nanoparticles. As a QA/QC measure, standard commercially available nanoparticles will also be dried on a TEM grid to assess nanoparticle aggregation/agglomeration during the drying process.
3. Ashing of nanosilver-containing fabrics may alter the nanoparticles and/or bias microscopy observations. As a QA/QC measure, standard commercially available silver nanoparticles or laboratory-synthesized silver nanoparticles should be added to a piece of fabric that does not contain nanosilver, and this product should also be ashed to assess any alterations that may occur to particles during the ashing, dusting, and resuspension of samples.
4. Standard commercially available nanoparticles will also be used for assessing the potential loss of small particles when dusting materials on carbon tape.

H. REPORTING RESULTS

Results can be reported as in published works (Benn and Westerhoff, 2008; Adachi and Buseck, 2010; Kim at al., 2010):

1. Images and counterpart EDS spectra are reported together.
2. Size distribution graphs are reported for each product.

I. REFERENCES

Adachi, K. and P. R. Buseck. *Hosted and free-floating metal-bearing atmospheric nanoparticles in Mexico City*. Environmental Science & Technology, 2010. 44(7): p. 2299-2304.

Benn, T.M. and P. Westerhoff, *Nanoparticle silver released into water from commercially available sock fabrics.* Environmental Science & Technology, 2008. 42(11): p. 4133-4139.

Kim, B., C.-S. Park, et al. *Discovery and characterization of silver sulfide nanoparticles in final sewage sludge products*. Environmental Science & Technology, 2010. 44(19): p. 4509-4514.

SOP # AIRVT-NANOSILVER-003

SILVER LEACHING ASSAYS FROM SOLID MATERIALS INTO VARIOUS LIQUID MEDIA

May 16, 2011

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SUMMARY

SOP description	This SOP describes the laboratory methods for soaking silver-containing consumer products into application-specific liquid media, such as saliva and sweat, for assessing whether or not silver leaches in real-world scenarios.
Example consumer goods appropriate for testing by this SOP	• Fabric products that claim to contain silver ions or particles, such as clothes, hats, gloves, towels, blankets, etc. • Plastic products that claim to contain silver ions or particles, such as bags, utensils, sippy cups, milk bottles, pacifiers. • Mixed-media products, such as stuffed toys and pillows (fabric shell and foam interior).
Strengths	This SOP lists six different liquid media, application-specific temperatures, and relevant soaking periods, which can be applied to a wide variety of products.
Weaknesses	The liquid media listed in this SOP, though extensive, may not cover all possible use scenarios, so future work may wish to consider liquid media (e.g., coffee and soda) and leaching conditions more specifically customized to the products to be tested and their applications.
Unanswered issues	Variability within different formulations of synthetic liquid media (i.e., saliva, sweat, urine), different brands of purchased media (i.e., milk formula, orange juice), and different characteristics of tap water (pH, ionic strength, etc.) may influence leaching.

A. PURPOSE AND APPLICABILITY

This protocol outlines the steps involved in leaching assays for determining the amount of silver released from commercially available consumer products into various liquid media and for determining whether the silver released is in ionic or particulate form.
This protocol is intended to be an evolving document that may be improved over time. The structure of this document follows the format recommended by EPA.

B. SUMMARY OF METHOD

The leaching assays consist of exposing product samples in relevant liquid media and submitting these samples to various conditions related to normal product use (Benn and Westerhoff, 2008; Benn et al., 2010).
Then, leachate samples are filtered and analyzed using any suitable ICP technique, such as ICP-MS (mass spectroscopy), ICP-AES (atomic emission spectroscopy), or ICP-OES (optical emission spectroscopy). Figure B.1 shows a flow chart depicting this method in detail.

C. MATERIALS

1. General laboratory equipment

a. Balance capable of weighing 10 mg
b. pH meter
c. Conductivity meter
d. Bead beater
e. Thermometer (20 – 100°C)
f. Water bath set to body temperature (37°C)
g. Microwave
h. Refrigerator

2. General laboratory materials

a. Knife or scissors
b. 100-ml beakers
c. 100-ml graduated cylinder
d. Glass beads (about 3 cm × 3 cm × 3 cm), or mortar and pestle
e. Forceps (any material silver)
f. Standard commercially available silver nanoparticles (NanoAmor, Nanocomposix, etc.)

3. Water

a. Ultrapure water. If ultrapure water is not available, deionized (DI) water from laboratory taps can be used after allowing it to run for several minutes before collection.
b. Tap water (analyze and report tap water chemical characteristics, including pH and conductivity).

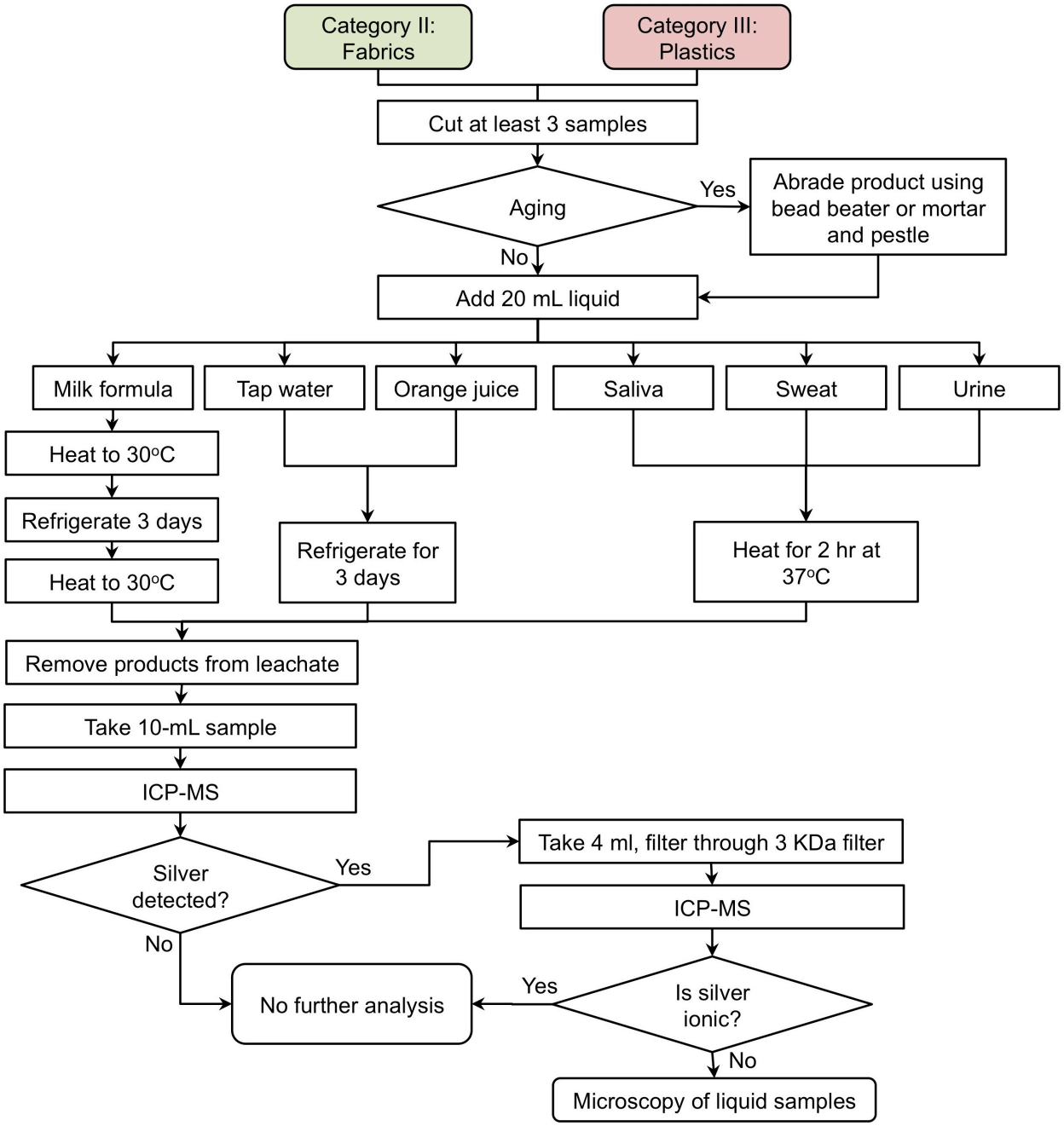

Figure B. 1. Flowchart summarizing the analytical methods for leaching assays of nanosilver consumer products.

4. Reagents

a. Reagent-grade hydrogen peroxide (H_2O_2).

b. Reagent-grade nitric acid (HNO_3), concentrated (69 to 71%, w/w).

c. Reagent-grade hydrochloric acid (HCl), concentrated (38%).

d. Commercially available milk formula (e.g., Nestle, Gerber, etc.), prepared according to product instructions.

e. Commercially available orange juice (e.g., Minute Maid, Tropicana, etc.).

f. Commercially available artificial saliva (e.g., Salivart, Aquoral, etc.).

g. Synthetic sweat (prepared according to ISO 105-E04 standard or similar, following the procedures of Kulthong et al., 2010).

h. Synthetic urine, which can be purchased or prepared in the laboratory (formulation: 25 g urea; 9 g sodium chloride; 2.5 g disodium hydrogen orthophosphate, anhydrous; 3 g ammonium chloride; 2 g creatinine; and 3 g sodium sulfite, hydrated; pH of 7 – 8, according to Mayrovitz and Sims, 2001).

5. Instrumentation

a. ICP instrument coupled with a spectroscopy technique, such as ICP-MS (mass spectroscopy), ICP-AES (atomic emission spectroscopy), or ICP-OES (optical emission spectroscopy).

D. SAMPLE PREPARATION PROCEDURES

1. Sample soaking, wear and tear

a. Cut three 0.5-g pieces of product using a knife or scissors, and place each piece in a 100-ml beaker.

b. Add 25 ml (or enough to cover product completely) of relevant liquid media. If a mass of product different from 0.5 g is used, add enough liquid media to achieve a factor of 50 for the ratio between the leaching media volume and product mass, as recommended by ASTM (ASTM, 2008). If the product floats in liquid media, place glass beads over the product piece to ensure submersion whenever possible. Choose as many options for liquid media as are appropriate according to the product's intended use. These include, but may not be limited to:

- Tap water
- Commercially available milk formula
- Commercially available orange juice
- Commercially available artificial saliva
- Synthetic sweat
- Synthetic urine

c. If product use involves chewing (teething toys) or mechanical wearing (e.g., plush toy), use glass beads (1 mm diameter) in liquid media in a bead beater (for 1 minute) or a mortar and pestle (for 5 minutes) to simulate abrasion. In this case, add liquid media in small aliquots (~5 ml) while abrading the product, and then

d. For the saliva samples, place 3 – 5 samples of 0.1 – 0.2 g of product into 2-ml bead-beating vials, add ~0.3 g 1-mm glass beads and 1 – 1.5 ml of synthetic saliva at ~37°C. Beat samples for 30 s in a bead beater at 2500 rpm. Combine samples into three beakers

and add saliva to obtain the same product-mass-to-leaching-volume ratio as with the sweat and urine samples.

e. Soak products for an appropriate period of time, which varies with liquid media and product type:

- Tap water and milk formula: Heat beaker in microwave until liquid reaches lukewarm temperature (~30°C), let sit in a refrigerator for 24 hours, and then re-heat in a microwave until lukewarm (~30°C).
- Orange juice: Place in refrigerator for 3 days.
- Artificial saliva, sweat and urine: Let sit in beaker for 2 hours at body temperature (37°C).

To simulate product aging in fabrics or plush toys, place one piece of products under a UV germicidal fluorescent lamp for 1 – 2 weeks and then hang these samples on a clothes line outdoors to expose them to weathering for ~ 1 week. After that period, rub products against a concrete block for ~ 1 min and subject these samples to the same leaching experiments as the new, un-aged products.

2. Filtration and acid digestion

a. Using forceps, remove solids from leachate.

b. Take 10 ml of leachate. Acidify tap water, artificial saliva, sweat, and urine using 20% nitric acid. Acid digest orange juice and milk formula samples using nitric acid and hydrogen peroxide, and analyze through ICP according to SOP # AirVT-Nanosilver-001. If silver is detected, proceed to next step. If silver is not detected, report that product does not leach silver under these conditions.

c. Take remaining volume of leachate, filter, acid digest, and analyze through ICP according to SOP # AirVT-Nanosilver-001.

E. PRECAUTIONS AND GUIDELINES

1. Always wear appropriate personal protective gear (e.g., gloves, lab coat, goggles, etc.).
2. Perform all acid digestion procedures inside an appropriate fume hood.
3. Ensure enough working space inside the fume hood to allow for safe arm movements.
4. Wash all glassware in 10% hydrochloric acid (HCl), 10% HNO_3, or aqua regia (solution of 1:3 nitric acid and hydrochloric acid, respectively). Then triple rinse in ultrapure water and air-dry prior to use.

F. QUALITY ASSURANCE & QUALITY CONTROL

1. Purchase silver-free consumer products, similar to those to be analyzed, to be used as blank controls. Perform the same silver extraction methods and report results.
2. Add standard commercially available silver nanoparticles to silver-free products, perform the same silver extraction methods, and report results.

3. Silver sorption to glass beads should be assessed by placing beads in a solution of ionic silver and extracting liquid samples over time for ICP analysis. If glass beads are a source of silver loss, they should be silanized to make them hydrophobic.
4. The calibration curve for the ICP instrument must have at least five points, beginning at zero ppb (blank) and extending to 1000 ppb standard solutions.
5. In the case that an ICP result extends beyond the calibration curve values (e.g., >1000 ppb), new standard solutions must be analyzed on the same day so that the obtained values are encompassed by the calibration curve.

G. REPORTING RESULTS

1. Each product must generate at least three samples, and each sample must be analyzed at least three times by ICP. Average results and standard deviations are reported.
2. Final reported values must be converted to the original volume (for liquid samples) or weight (for fabrics and plastics), such as mg l^{-1} or mg g^{-1} of silver in product.

H. REFERENCES

ASTM, *F963-08. Standard Consumer Safety Specification for Toy Safety*, 2008: West Conshohocken, PA.

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Kulthong, K., et al., *Determination of silver nanoparticle release from antibacterial fabrics into artificial sweat.* Particle and Fibre Toxicology, 2010. 7.

Mayrovitz, H. N.; Sims, N., *Biophysical effects of water and synthetic urine on skin*. Advanced Skin Wound Care, 2001. 14(6): p. 302-308.

SOP # AIRVT-NANOSILVER-004

CHARACTERIZATION OF AEROSOLS GENERATED FROM NANOSILVER CONSUMER PRODUCTS

May 16, 2011

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EPA Project Manager	Date

SUMMARY

SOP description	This SOP describes laboratory methods for the physical and chemical characterization of aerosols generated during the use of nanosilver-containing consumer products under a real-world scenario in a residence.
Example consumer goods appropriate for testing by this SOP	• Liquid products that claim to contain silver ions or particles, such as throat sprays, disinfecting liquids or gels, deodorant sprays, etc. • Fabric products that claim to contain silver ions or particles, such as clothes, hats, gloves, towels, blankets, etc. • Mixed-media products, such as stuffed toys and pillows (fabric shell and foam interior). • Humidifiers.
Strengths	This SOP lists a number of different techniques that, in combination, can produce a very detailed characterization of the aerosols produced (e.g., chemical information, counts in different size ranges, size distributions, etc.).
Weaknesses	These methods are designed to determine whether ambient particle concentrations are elevated above background levels during the use of products, but they do not necessarily address whether small amounts of silver-containing particles are emitted.
Unanswered issues	Whether silver-containing particles are emitted remains unanswered. To determine aerosol emission rates from consumer products, testing in a controlled environmental chamber with very low aerosol background concentrations (less real-world scenario) is required.

A. PURPOSE AND APPLICABILITY

This protocol outlines the steps involved in the physical and chemical characterization of aerosols generated during the normal use of nanosilver-containing consumer products. The method is designed to estimate the potential for children's exposure under conditions of realistic use. This protocol applies to products with the potential to produce aerosolized nanosilver and that have been proven to contain silver.
This protocol is intended to be an evolving document that may be improved over time. The structure of this document follows the format recommended by EPA.

B. SUMMARY OF METHOD

Experiments take place in a test facility (simulated residential room) whose temperature and humidity are brought to specified levels before product use begins. The physical characterization consists of determining the aerosol concentration and size distribution during and after product use. The chemical characterization consists of determining whether there is silver in the aerosol produced and, if so, determining its size-fractionated mass concentration.

C. MATERIALS

1. General laboratory equipment

a. Balance capable of weighing 0.1 – 500 g

b. Thermometer (20 – 100°C)

c. Relative humidity monitor (0 – 100% RH)

d. Space heater, if testing facility lacks a heating system

e. Air conditioner, if testing facility lacks a cooling system

f. Dehumidifier

g. Floor fan, if testing facility lacks a ceiling fan

2. General laboratory materials

a. Teflon filters for Sioutas 4-stage impactor

3. Instrumentation

a. Optical particle counter (Aerotrak, TSI)

b. Condensation particle counter (3025 CPC, TSI)

c. Scanning mobility particle sizer system (3936 SMPS, TSI)

d. Cascade impactor (Sioutas, SKC; Adachi and Buseck, 2008 and 2010; Yokelson et al., 2009)

e. Thermophoretic precipitator (Quadros et al., 2009)

4. Testing facility

Tests will occur in a room prepared for these studies. The room should be similar in size to a typical room in a residence and should be furnished appropriately. The room will have carpet or a rug on the floor, furniture to simulate a child's bedroom, and controlled temperature and humidity. For reference, the American Society of Heating, Refrigerating and Air-conditioning

Engineers (ASHRAE) recommends that indoor temperature be maintained between 20-28°C, and ASHRAE and the EPA recommend that relative humidity (RH) be maintained between 30-60% (ASHRAE, 2010; EPA, 2010), depending on the season.

D. AEROSOL MONITORING DURING AND AFTER PRODUCT USE

1. Place sampling instruments near the center of the room and in the same location each time. Position sampling inlets pointing downward and 1 m above the floor to simulate a child's breathing zone.
2. Close all doors and windows, and if there are any window treatments (e.g., blinds, curtains) note their position, and maintain them in the same position for all experiments. The doors and windows must remain closed for at least 1 hour before simulated product use begins and until all aerosol monitoring is completed.
3. Promote well-mixed conditions by running a ceiling fan or, if not available, a floor fan placed in the corner and pointing upward toward the center of the room at medium speed.
4. Run the air conditioning or heating system until a temperature of 20-25°C and RH of 20-30% are reached. Use the dehumidifier if needed. Once temperature and RH have stabilized, record these parameters, and turn off all temperature and humidity control devices. Attempt to start each experiment at the same temperature and RH.
5. Monitor temperature and humidity inside the room at 1-min intervals throughout the experiment.
6. Monitor total aerosol concentrations and size distributions using the Aerotrak and SMPS for 10 minutes prior to product use to obtain background concentrations:
    a. Set the Aerotrak to collect one data point per minute in 6 size cutoffs (0.3, 0.5, 1, 2.5, 5, and 10 mm).
    b. Set the SMPS to perform at least 3 scans of 120 seconds with 15 seconds of retrace time and a 45 second pause between measurements (for a total time of 3 minutes per measurement).
7. Simulate use of the product. Weigh product before and after using it. If the product is a humidifier, clean it according to instructions, and weigh the water reservoir before and after use.
    a. Liquid products (e.g., disinfecting spray): Spray them from their original bottle using their own spray delivery system at a constant rate for 30 minutes at a distance of 0.3 m from the aerosol sampling inlets.
    b. Textiles or plush toys: Mechanically shake them a constant rate for 30 minutes at a distance of 0.3 m from the aerosol sampling inlets. Options for shaking include vibrating electric shakers, manual action, and others. Agitation should mimic normal consumer use activities such as picking items up and putting them down and pushing them across a flat surface.
    c. Humidifiers: Place them on the floor in the corner of the room. Run the humidifiers at their maximum setting for 2 hours.

8. Monitor total aerosol concentrations using the Aerotrak and aerosol size distributions using the SMPS during product use and for at least 2 hours after product use (or until concentrations return to background levels).
9. Collect samples for electron microscopy:
    a. Place one lacey or holey carbon-coated TEM grid into the thermophoretic precipitator (TP).
    b. Turn on sampling pump. Monitor temperatures on hot side and cold side of the TP, as well as the TP flow rate, for at least 60 minutes.
10. Collect samples for chemical analyses:
    a. Place Teflon filters into cascade impactor as recommended in the instrument manual.
    b. Turn on the pump at 9 l min^{-1}. Run the instrument for at least 60 minutes.
    c. Place Teflon filters into 100-ml digestion beakers, and cover with a watch glass, according to SOP # AirVT-Nanosilver-001. Add 10 ml aqua regia, and heat in hot plate until solution is clear. Remove Teflon filters and watch glass, rinsing them into the beaker with a 5% solution of aqua regia in water. Heat solution until the volume is less than 10 ml. Transfer quantitatively into a 10-ml volumetric flask, and fill to volume with ultrapure water.
    d. Perform ICP analysis on samples.

E. PRECAUTIONS AND GUIDELINES

1. Always wear appropriate personal protective gear (e.g., gloves, lab coat, goggles, etc.).
2. Clean the room before each experiment by wiping surfaces, vacuuming or sweeping the floors and letting the room sit for a night before running experiments.
3. Perform all acid digestion procedures inside an appropriate fume hood.
4. Ensure enough working space inside the fume hood to allow for safe arm movements.
5. Wash all glassware in 10% hydrochloric acid (HCl), 10% HNO_3, or aqua regia (solution of 1:3 nitric acid and hydrochloric acid, respectively). Then triple rinse in ultrapure water and air-dry prior to use.

F. QUALITY ASSURANCE & QUALITY CONTROL

1. Perform blank measurements using a spray bottle filled with ultrapure water, non-nanosilver textiles, and a non-nanosilver humidifier.
2. Perform experimental runs three times for each product.

G. REPORTING RESULTS

1. Each product must generate at least three measurements, and each impactor sample must be analyzed at least three times by ICP. Average results and standard deviations are reported.

H. REFERENCES

Adachi, K. and P.R. Buseck, *Internally mixed soot, sulfates, and organic matter in aerosol particles from Mexico City.* Atmospheric Chemistry and Physics, 2008. 8(21): p. 6469-6481.

Adachi, K. and P.R. Buseck, *Hosted and Free-Floating Metal-Bearing Atmospheric Nanoparticles in Mexico City.* Environmental Science & Technology, 2010. 44(7): p. 2299-2304.

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Environmental Protection Agency (EPA), A Brief Guide to Mold, Moisture, and Your Home, 2010. EPA 402-K-02-003, Office of Air and Radiation, Indoor Environments Division, Washington, DC.

Quadros, M.E., C.T. Faria, and L.C. Marr, *A thermophoretic sampler for collecting airborne nanoparticles (Poster)*, in *1st International Conference on the Environmental Implications of Nanotechnology* 2009, 1st ICEIN: Washington, DC.

Yokelson, R.J., et al., *Emissions from biomass burning in the Yucatan.* Atmospheric Chemistry and Physics, 2009. 9(15): p. 5785-5812.

SUPPLEMENTARY MICROSCOPY DATA

Nanosilver control sample – SEM/EDS

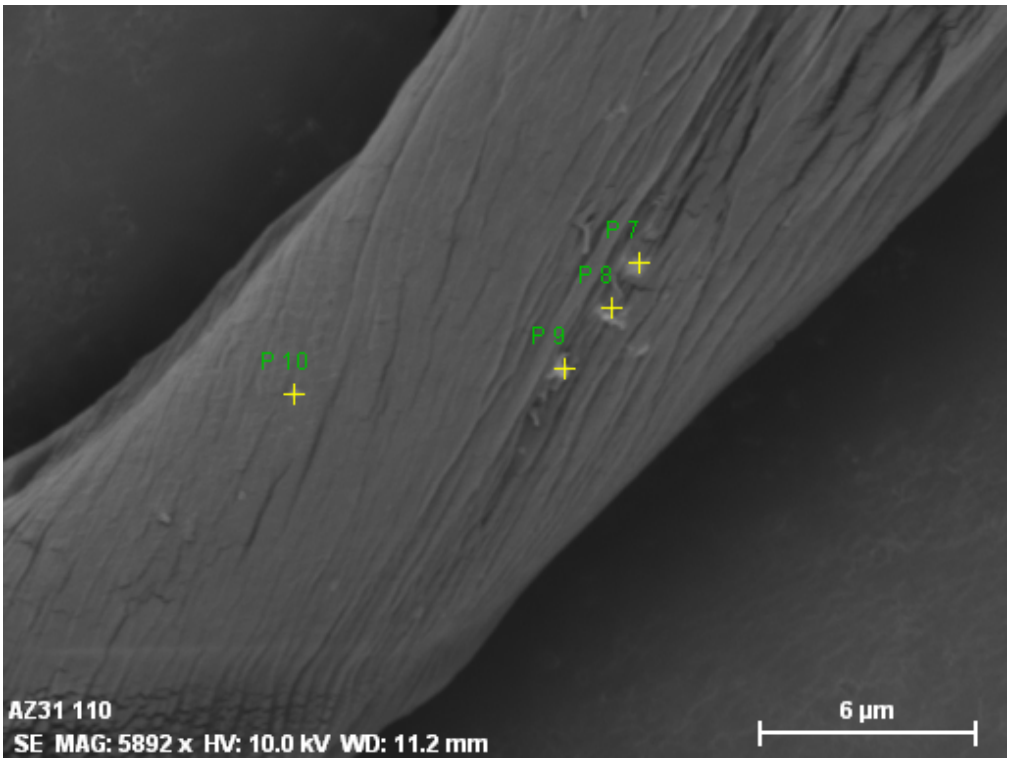

Figure B. 2. SEM image of the control fabric sample showing EDS spectrum positions P7 - P10.

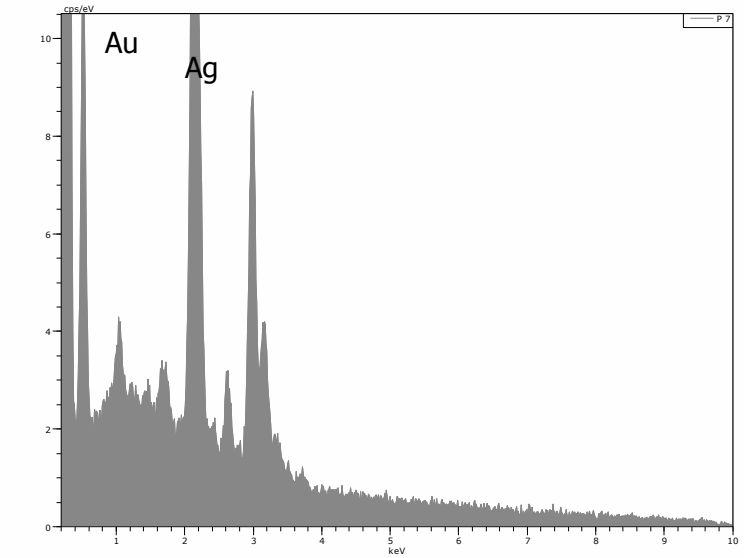

Figure B. 3. Spectrum representative of positions P7, P8, P9, and P10, which yielded identical spectra (gold is from sputter coating).

Nanosilver ash control

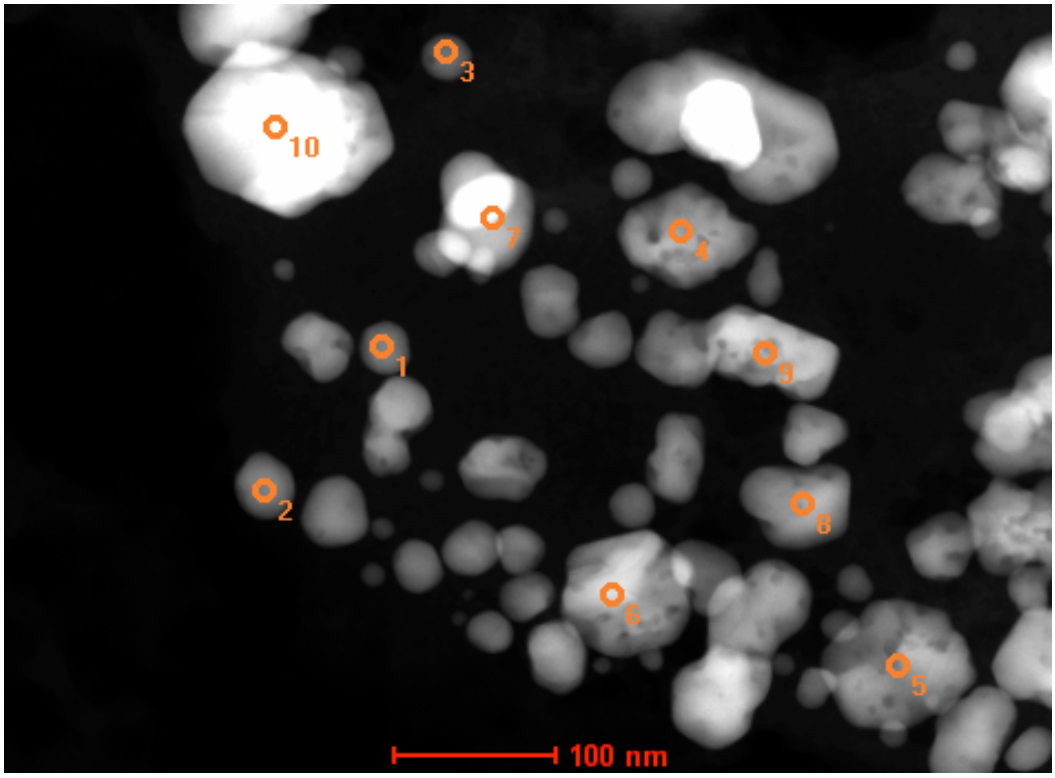

Figure B. 4. TEM image of the ashed fabric control sample in HAADF mode with EDS spectrum positions O1 - O10.

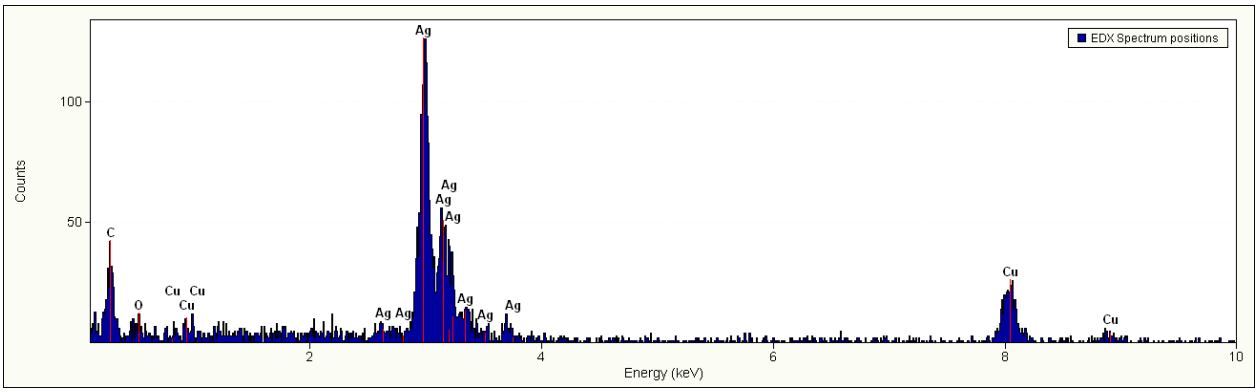

Figure B. 5. Spectrum Position O_1.

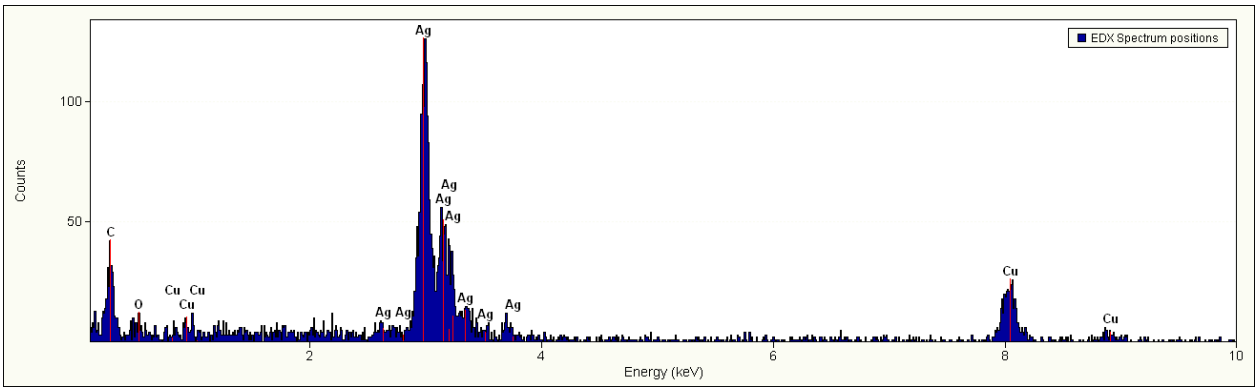

Figure B. 6. Spectrum Position O_2.

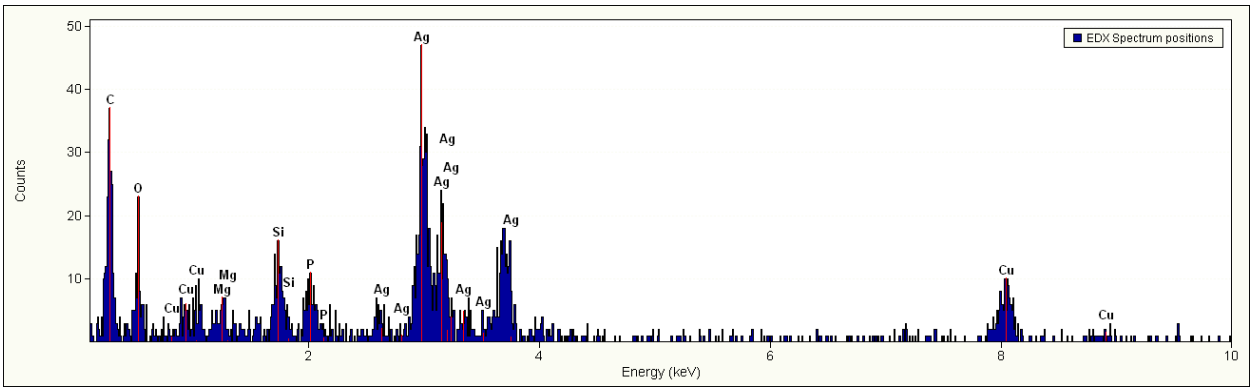

Figure B. 7. Spectrum Position O_3.

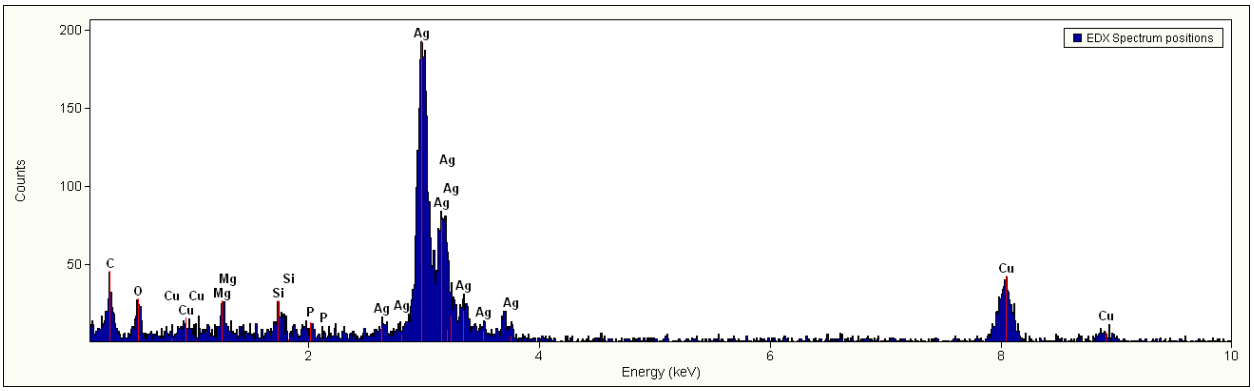

Figure B. 8. Spectrum Position O_4.

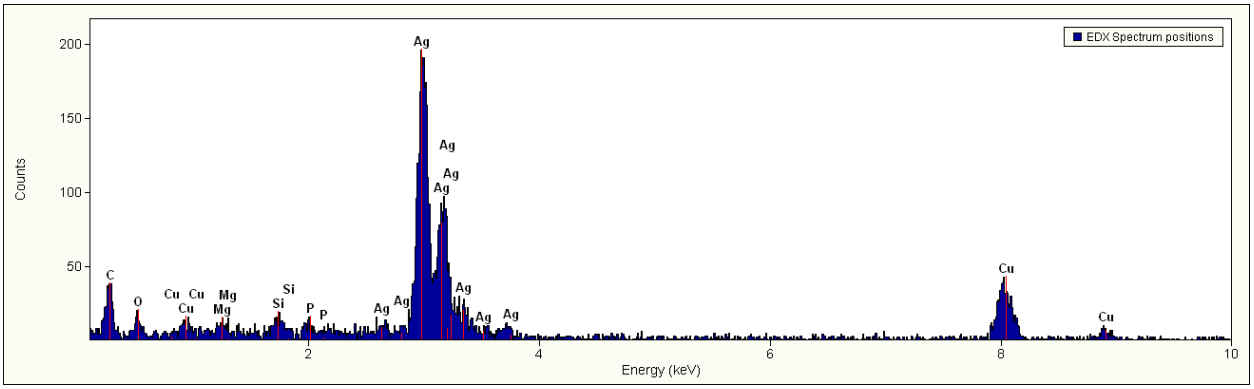

Figure B. 9. Spectrum Position O_5.

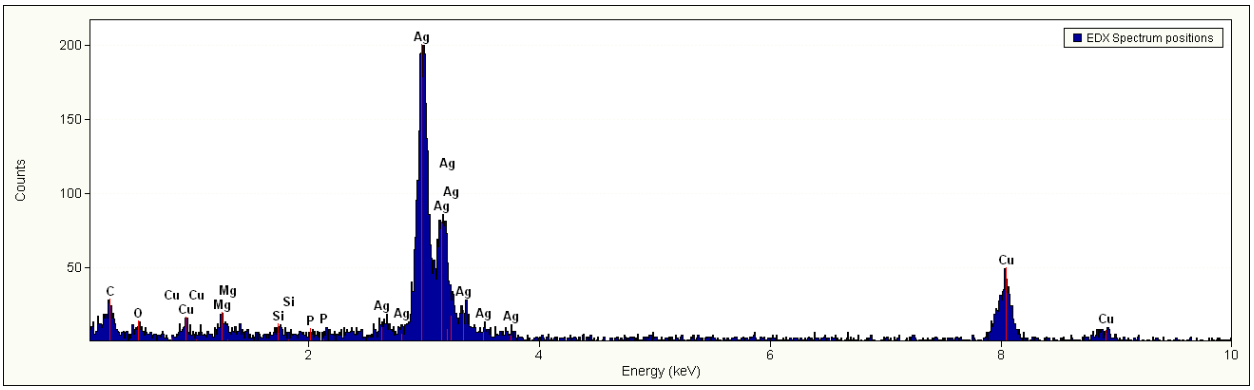

Figure B. 10. Spectrum Position O_6.

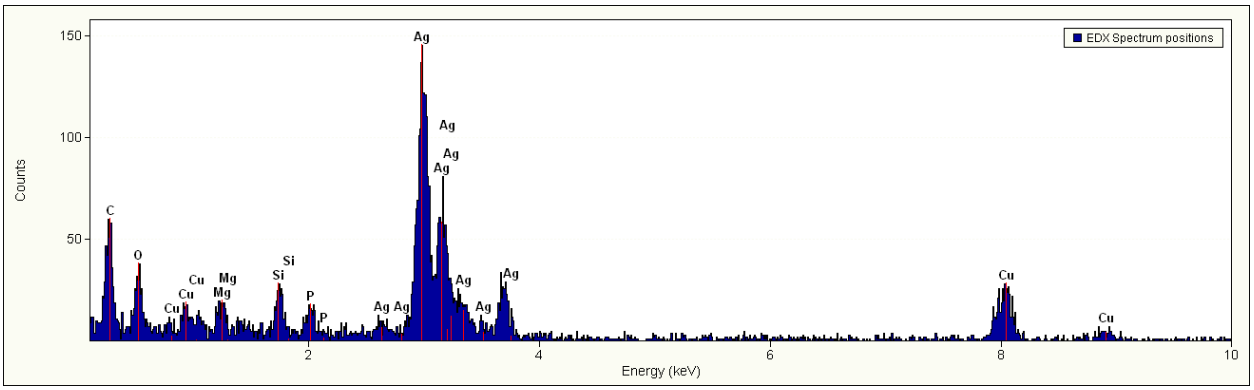

Figure B. 11. Spectrum Position O_7.

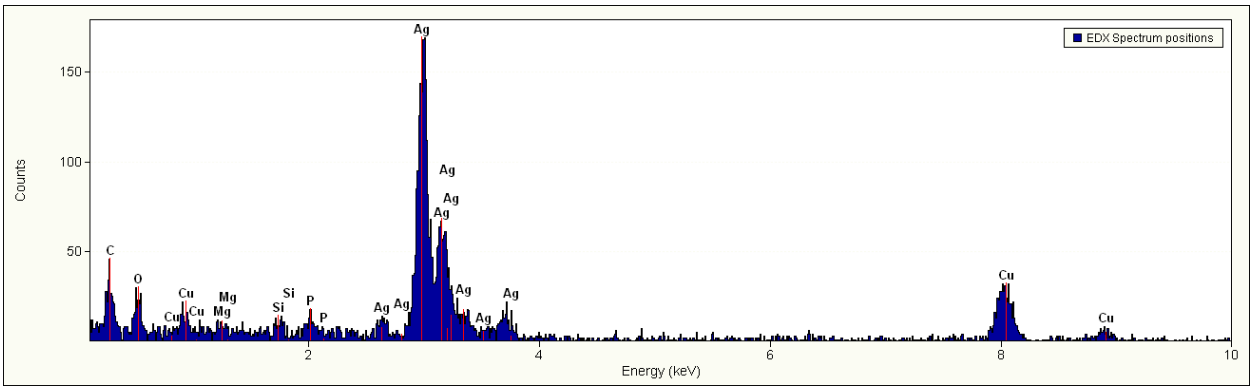

Figure B. 12. Spectrum Position O_8.

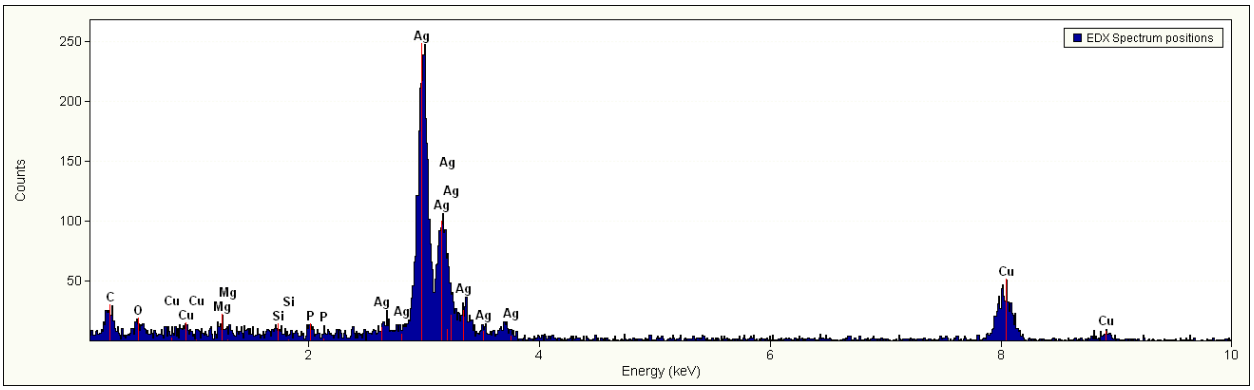

Figure B. 13. Spectrum Position O_9.

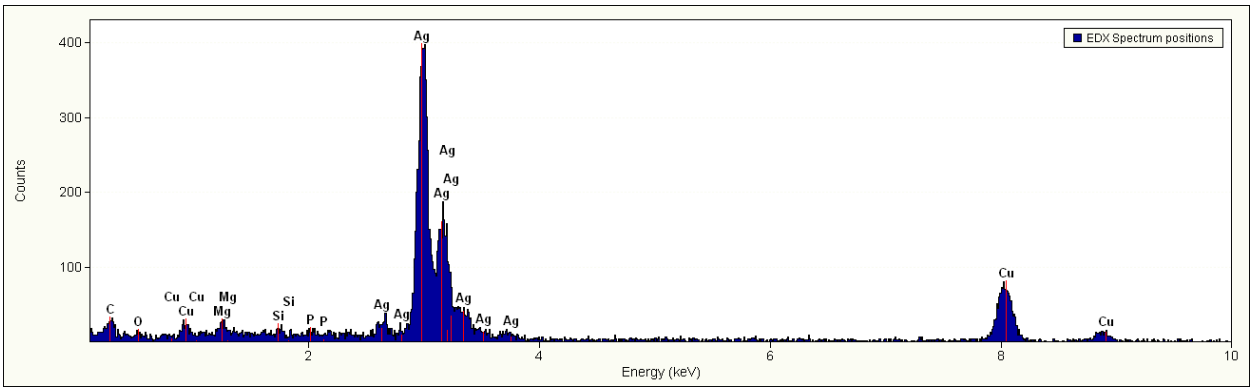

Figure B. 14. Spectrum Position O_{10}.

Baby blanket sample

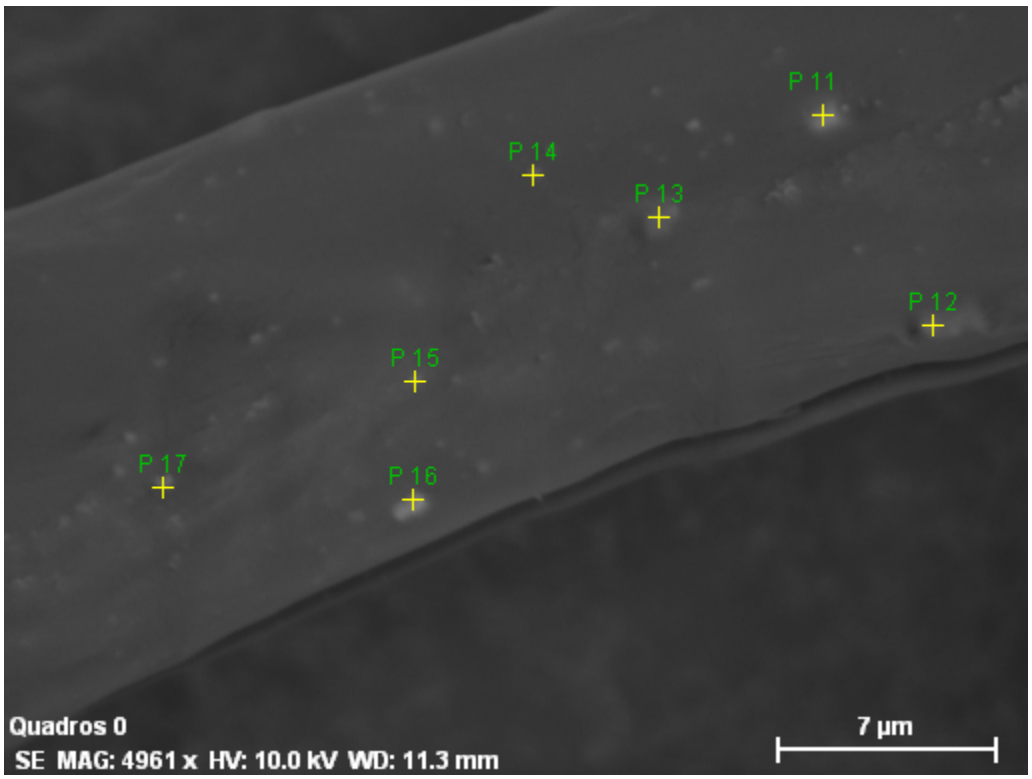

Figure B. 15. SEM image of a sample from the baby blanket, with EDS spectrum positions P11 - P17.

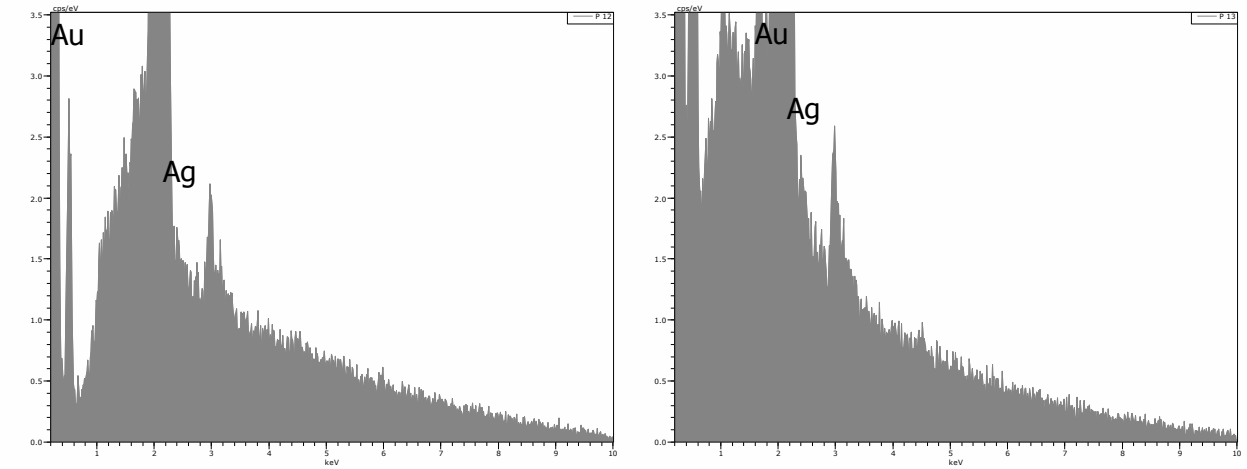

Figure B. 16. Spectrum Positions P12 (left) and P13 (right).

Baby blanket ash sample

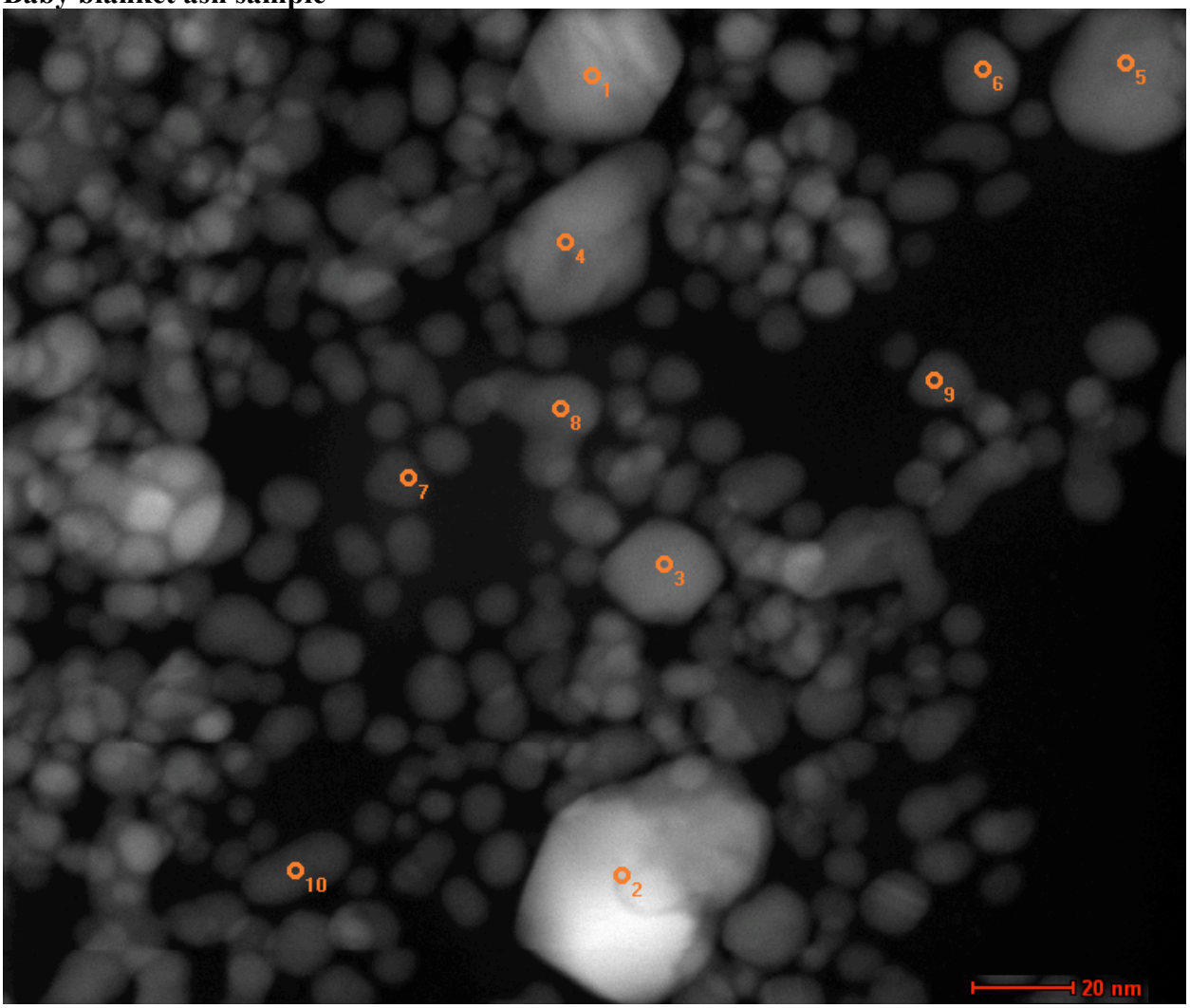

Figure B. 17. TEM image (HAADF mode) of an ashed sample of the baby blanket, with EDS spectrum positions O1 - O10.

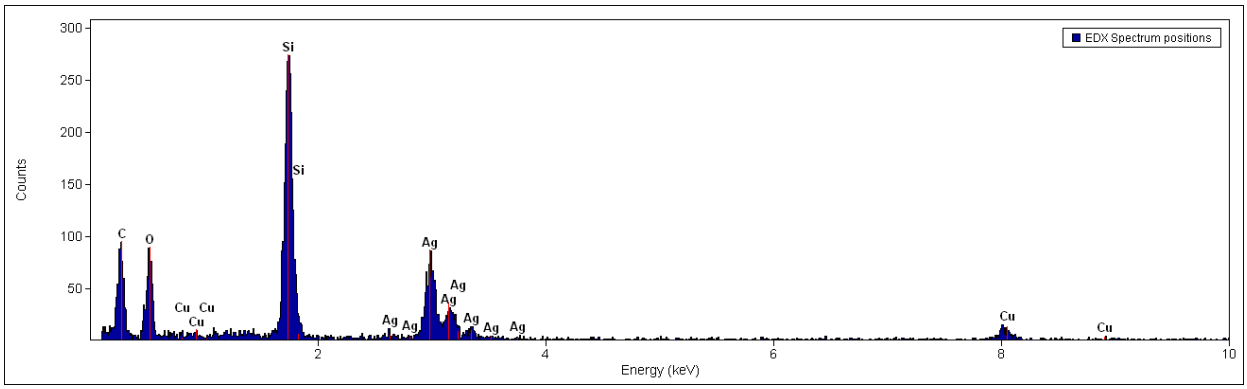

Figure B. 18. Spectrum Position O_1.

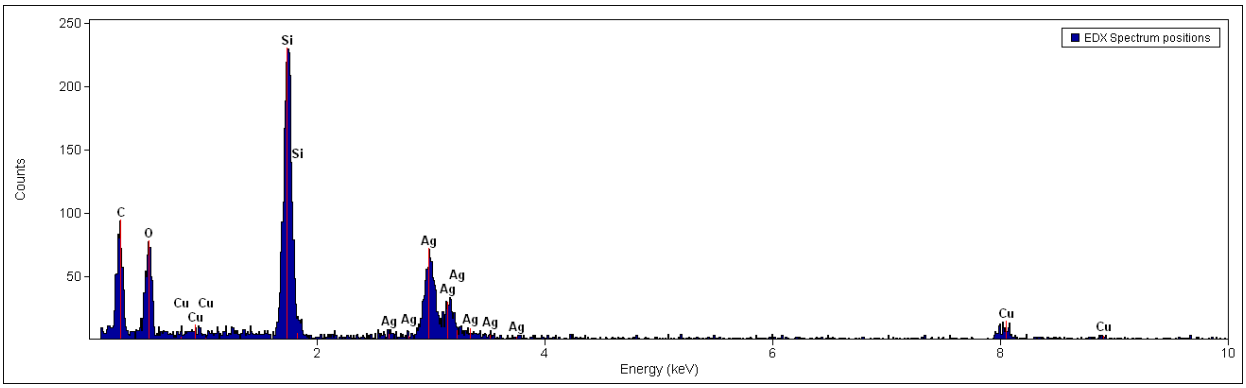

Figure B. 19. Spectrum Position O_2.

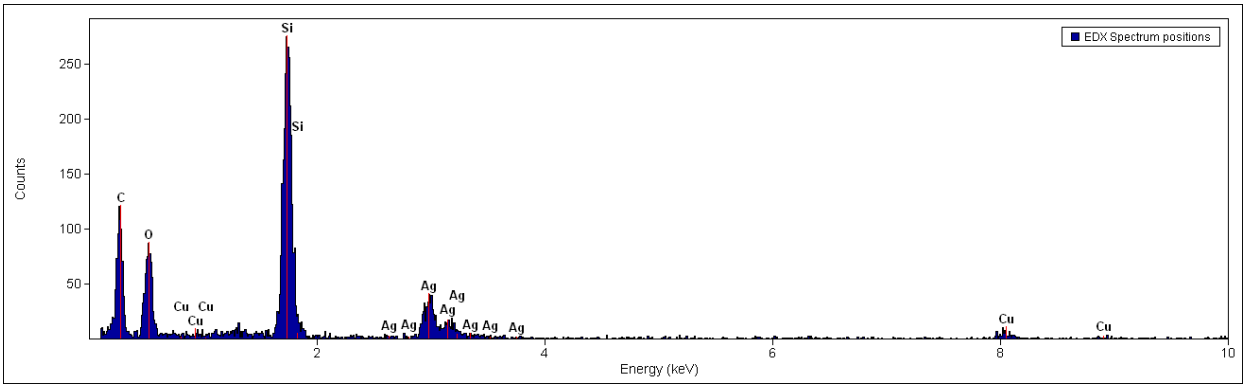

Figure B. 20. Spectrum Position O_3.

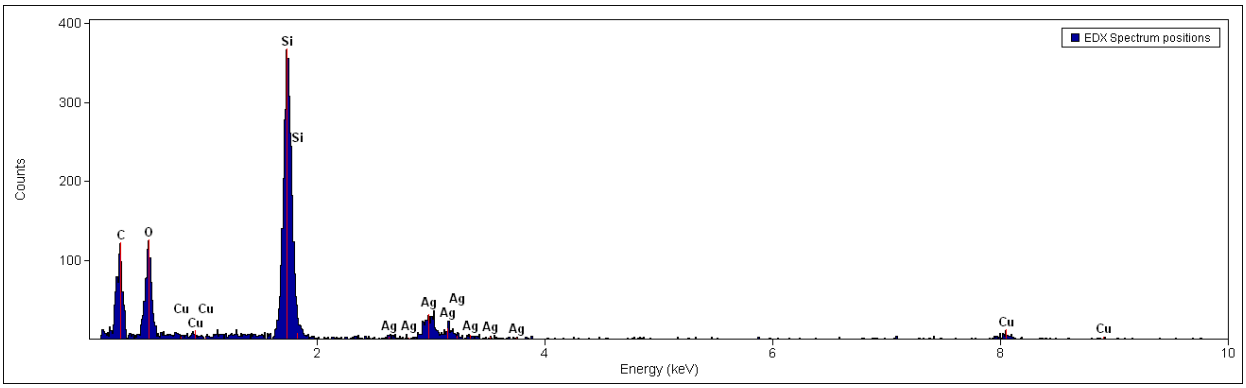

Figure B. 21. Spectrum Position O_4.

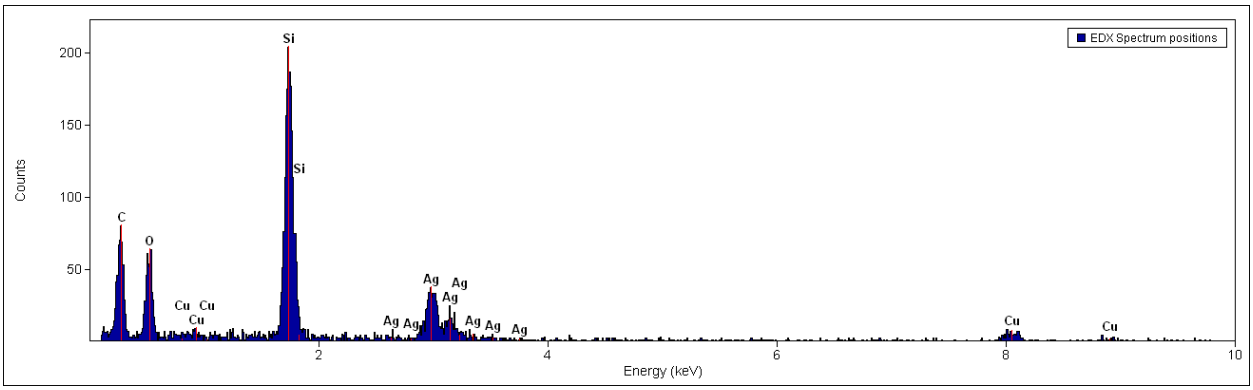

Figure B. 22. Spectrum Position O_5.

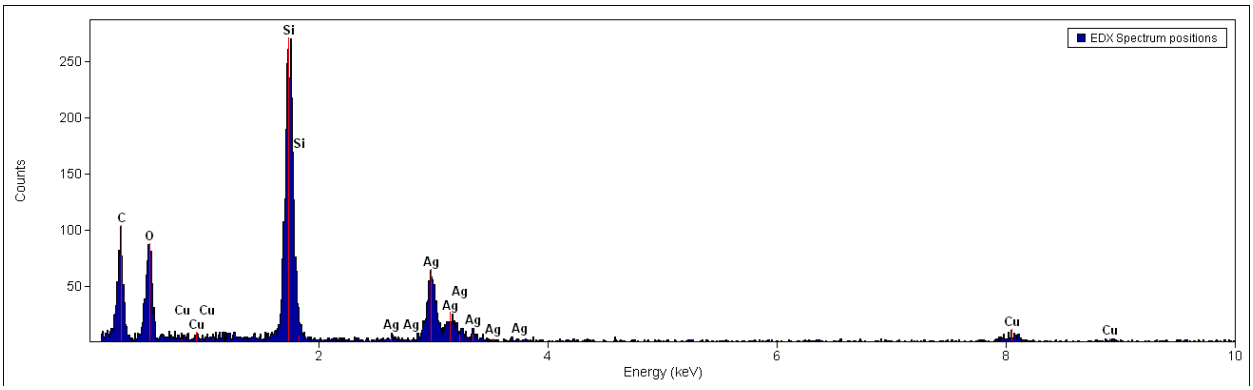

Figure B. 23. Spectrum Position O_6.

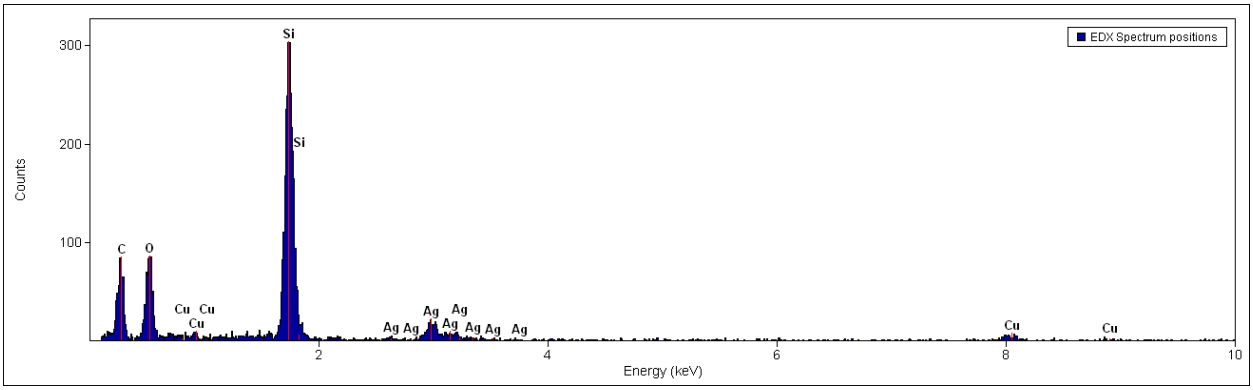

Figure B. 24. Spectrum Position O_7.

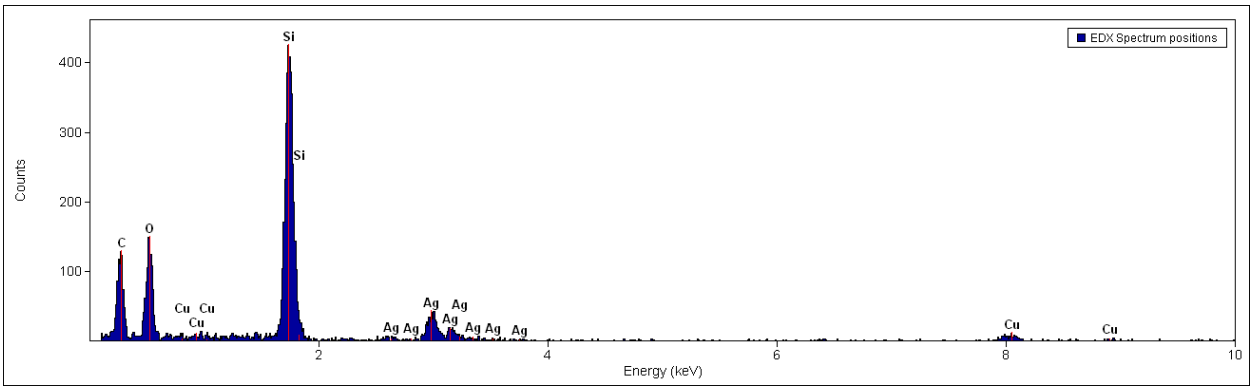

Figure B. 25. Spectrum Position O_8.

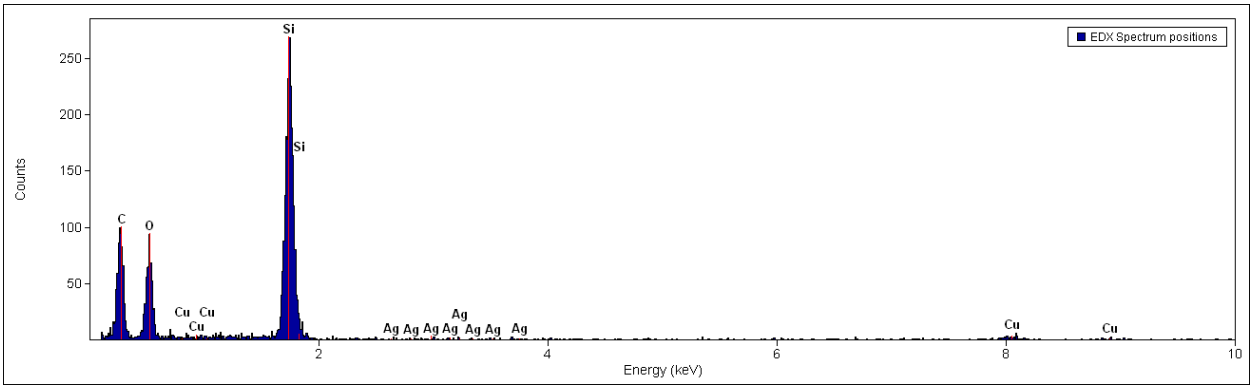

Figure B. 26. Spectrum Position O_9.

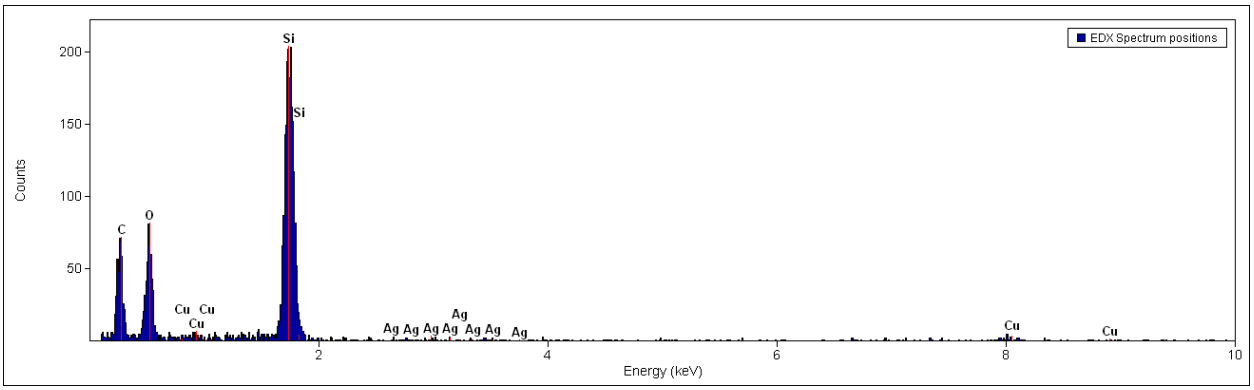

Figure B. 27. Spectrum Position O_{10}.

APPENDIX C
SUPPLEMENTAL INFORMATION TO CHAPTER 5

This appendix contains the following information:

- Description of the leaching media
- Silver concentration in the humidifiers' reservoirs.

Description of Leaching Media

Tap water

We used tap water from the town of Blacksburg, Virginia (2.92 mg/L total chlorine, pH of 7.6). We flushed the faucet for ~5 min before collecting water for use.

Milk formula

We prepared milk formula (Nestlé's Gerber Good Start Infant Formula, 360 g) according to the package instructions, using ultrapure water at 38°C. The milk formula had a pH of 7.0.

Orange juice

We used a store-bought orange juice, Kroger Brand orange juice from concentrate (no pulp, 0.5 gal), with a pH of 4.1.

Synthetic sweat

We used a synthetic sweat formulation suggested by Kulthong et al.,[1] who assessed the release of silver nanoparticles from a fabric into four sweat formulations. We chose the formulation described by the European Standard (EN1811-1999) because it resulted in the highest amount of silver released in the Kulthong study.

The formulation of the synthetic sweat is:

- 1.3 g/L Urea (CH_4N_2O)
- 10.8 g/L Sodium chloride (NaCl)
- 1.2 g/L Lactic acid (88%)

We used ultrapure water to prepare the medium and corrected the pH to 6.4 - 6.6 using a solution of 0.2 N sodium hydroxide (NaOH). This medium was bubbled with air for ~ 2 min before use in order to saturate it with oxygen.

Synthetic urine

We used a synthetic urine formulation described by Mayrovitz and Sims:[2]

- 25 g/L Urea (CH_4N_2O)
- 9 g/L Sodium chloride (NaCl)
- 3 g/L Ammonium chloride (NH_4Cl)
- 2.5 g/l Disodium hydrogen orthophosphate, anhydrous (Na_2HPO_4)
- 2 g/L Creatinine ($C_4H_7N_3O$)
- 3 g/L Sodium sulfite, hydrated (Na_2SO_3, $7H_2O$)

We used ultrapure water to prepare this medium, which had a pH of 7.8. This medium was bubbled with air for ~ 2 min before use in order to saturate it with oxygen.

Synthetic saliva

We used a synthetic urine formulation described by Gal et al.:[3]

- 0.2 g/L Urea (CH_4N_2O)
- 0.126 g/L Sodium chloride (NaCl)
- 0.178 g/L Ammonium chloride (NH_4Cl)
- 0.964 g/L Potassium chloride (KCl)
- 0.189 g/L Potassium thiocyanate (KSCN)
- 0.654 g/L Monopotassium phosphate (KH_2PO_4)
- 0.763 g/L Sodium sulfate (Na_2SO_4, 10 H_20)
- 0.228 g/L Calcium chloride ($CaCl_2$, 2 H_20)
- 0.631 g/L Sodium bicarbonate ($NaHCO_3$)

We used ultrapure water to prepare this medium, which had a pH of 7.0. This medium was bubbled with air for ~ 2 min before use in order to saturate it with oxygen.

Saline

We prepared a simple saline solution (9 g/L NaCl in ultrapure water, pH 7.0) to use as a leaching medium for comparison with the synthetic saliva. We used ultrapure water to prepare

this medium, which had a pH of 7.0. This medium was bubbled with air for ~ 2 min before use in order to saturate it with oxygen.

ASTM F963-08 media (digestion substitute)

As with the saline solution, we prepared a 0.07 M hydrochloric acid solution to use as a leaching medium for comparison with other synthetic media. ASTM4 recommends using this medium "to simulate the situation in which materials stay 4 h in the alimentary tract after swallowing." We used ultrapure water to prepare this medium, which had a pH of 1.4 and bubbled it with air for ~ 2 min before use in order to saturate it with oxygen.

Silver Concentration in Humidifiers' Reservoir

Table C. 1. Silver concentrations in humidifier reservoir water after different periods of soaking.

product	soaking time (days)	silver concentration in reservoir (ppb)	silver concentration in vapor (ppb)
tabletop ultrasonic humidifier	6	ND[a] (0.07 ± 0.01)	2.3 ± 0.7
	18	ND (0.14 ± 0.01)	
manual ultrasonic humidifier	6	ND (0.11 ± 0.01)	ND (0.16 ± 0.02)
	18	ND (0.03 ± 0.01)	
humidifier accessory cube (into a "non-nano" humidifier)	5	0.8 ± 0.2	ND (0.08 ± 0.01)
	17	ND (0.05 ± 0.01)	
non-nano humidifier	5	ND (0.11 ± 0.01)	ND (0.17 ± 0.01)

[a]Not detected. Silver concentrations in the ICP-MS samples were below 0.5 ppb.

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2. Mayrovitz, H. N.; Sims, N., Biophysical effects of water and synthetic urine on skin. *Adv Skin Wound Care* **2001,** *14* (6), 7;
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APPENDIX D
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Publication: Environmental Science & Technology

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