United States
Consumer Product Safety Commission

November 04, 2024

CPSC[1] Staff Statement on: A Preliminary Assessment of the Potential for Exposure to Silver Nanoparticles in Children's Consumer Products

1. Background

The 2012 report titled, "A Preliminary Assessment of the Potential for Exposure to Silver Nanoparticles in Children's Consumer Products" describes studies performed by the United States Environmental Protection Agency (EPA), Office of Research and Development. The study was supported by interagency agreement CPSC-I-10-0009 between the Consumer Product Safety Commission (CPSC) and the EPA. EPA subcontracted the research to Virginia Polytechnic Institute and State University (VT).[2]

The EPA's Office of Research and Development (ORD) is the scientific research arm of the EPA. ORD's research informs EPA decisions and supports the emerging needs of EPA stakeholders.

2. Introduction

Silver nanoparticles (nanosilver) are one of the most frequently used nanomaterials in consumer and medical products due to their anti-microbial and preservative properties. Adverse effects from exposure to nanosilver has not been well understood in humans, including to workers. However, *in vitro* and *in vivo* studies reveal potential toxicity. In 1988 NIOSH developed a recommended exposure limit (REL) as an 8-hour time weighted average (TWA) concentration of 10 µg/m^3 for total elemental silver (non-nano form) to protect workers from developing argyria (bluish-gray pigmentation to the skin, mucous membranes and eyes). In their 2021 Current Intelligence Bulletin on occupational exposure to silver nanomaterials, NIOSH established a TWA of 0.9 µg/m^3 as the recommended exposure limit (REL) for nanosilver. NIOSH based the REL on lung inflammation and liver hyperplasia observed in subchronic inhalation animal studies. *In vivo* results demonstrate that biologic activity and potential adverse health effects are related to particle size, duration of exposure and gender. For example, in a WHO 2017 report on nanomaterial classification, no acute toxicity, by any route of exposure, was observed for 10 nm silver nanoparticles, while subacute and subchronic exposures to 58-60 nm particles resulted in hepatic toxicity. Nanomaterials are materials that range in size from 1 to 100 nm in length.[3]

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[1] This statement was prepared by the CPSC staff, and the attached report was prepared by EPA for CPSC staff. The statement and report have not been reviewed or approved by, and do not necessarily represent the views of, the Commission.

[2] The CPSC nanotechnology program is working to publicly post technical reports produced from interagency agreements and contracts in prior years; limited staff resources prevented posting at the time of the research.

[3] 2019 CPSC Nanomaterial Statement | CPSC.gov

Children may be more vulnerable than adults to nanosilver released from consumer products during foreseeable use due to child behaviors such as direct mouthing of objects and hand-to-mouth activity. At the time of these studies, minimal information on nanosilver release from consumer products was available. Therefore, this body of work was designed to assess the presence of nanosilver in children's products and to quantify its release.

3. Experimental Methods and Results

A literature search was conducted using the Web of Knowledge as described in Appendix A. The Woodrow Wilson Nanotechnology Project (WWNTP) database was used to identify silver-containing consumer products to which children may be exposed. VT identified more than 80 products that claimed to contain nanosilver and which may be used by or near children.

VT evaluated the total silver content of selected consumer products using microwave extraction followed by inductively coupled plasma mass spectrometry (ICP-MS). ICP-MS analysis showed that a liquid disinfecting spray product contained silver at about 27 ppm, greater than its advertised concentration of 20 ppm. Two sippy cups contained silver ranging from 37 to 255 mg; and other children's products, such as a plush toy, sleepsuit, blanket, and mittens had concentrations of approximately 500 mg.

After finding the presence of silver in selected consumer products, electron microscopy was used to determine whether the silver was nanosized. The disinfection spray was evaluated with transmission electron microscopy (TEM) after the liquid was evaporated. Nanosilver presence was confirmed and was mostly in aggregate form, with an average diameter of 25.1 ± 0.6 nm. Scanning electron microscopy (SEM) coupled with energy-dispersive X-ray spectroscopy (SEM-EDX) revealed the presence of nanosilver in a baby blanket, which was confirmed by high resolution-TEM micrograph analysis. A plush toy's interior foam as well as its fur was determined to contain nanosilver by SEM. One of two sippy cup samples evaluated by the SEM-EDX technology showed the presence of micrometer-sized silver particles.

Ingestion and dermal exposures were determined to be the most likely routes through which children are exposed to nanosilver released from consumer products. VT evaluated leaching in application-specific liquids, such as synthetic saliva, synthetic sweat, synthetic urine, orange juice, milk, and tap water. Synthetic sweat and synthetic urine produced the largest amount of leached silver (maximum 38% of the total silver), while tap water yielded the lowest amount (maximum 2.6%). Among the tested products, the plush toy interior foam leached 9.5% of the toy's total silver contents.

To determine whether silver from the leachate was in ionic or particulate form, the samples were filtered using a 3 KDa filter and analyzed by ICP-MS. For the plush toy and baby blanket, VT identified that most of the silver leached from these products is in dissolved ionic form, indicating that dissolution was the main leaching mechanism.

To evaluate exposure via dermal contact, VT conducted a surface assay (NIOSH Method 9102) by wiping the surfaces of the products (i.e., plush toy, baby blanket). For liquid products (e.g., the disinfecting spray), VT wiped a surface after cleaning the surface with the liquid product. This process simulated scenarios when a child may touch a surface after cleaning products were used. In the test with the baby blanket and plush toy, VT measured 23 and 13.8 μg/m^2 of total silver, respectively transferred from their surfaces to the wipe. In the test with the disinfecting spray, VT found that the amount of silver transferred to a fabric wipe was 9.03 ± 2.75 μg/m^2.

The potential for exposure to nanosilver in aerosols associated with product use was evaluated using an optical particle counter and a scanning mobility particle sizer system. In scenarios simulating normal, real-world, intended use of the consumer products, ambient aerosol concentrations were not significantly elevated above background levels. VT stated that if these products emit any form of silver-containing aerosols, the emission rates are very low.

4. Conclusion

The objective of these studies was to develop protocols to characterize the potential for children's exposure to nanosilver released from consumer products. VT analyzed 13 products to obtain information on total silver, to determine nanosilver presence, and to quantify levels of leachable silver under simulated use scenarios. The leaching experiments detected measurable silver from some of the tested products. Most of the leached silver was ionic in form, suggesting that dissolution was the main leaching mechanism. The measured ionic silver levels are lower than the EPA recommended concentration of silver in drinking water (0.1 mg/L). This study indicates that exposure to silver leached from a single consumer product (e.g., plush toy) may be low. However, cumulative exposure to silver from multiple consumer products could become a health concern, as more nanosilver-enabled consumer products are introduced into the marketplace. There were three publications generated from this research.[4,5,6]

[4] Quadros M.E., Pierson R. IV, Tulve N.S., Willis R., Rogers K., Thomas T.A., and Marr L.C. (2013) Release of Silver from Nanotechnology-based Consumer Products for Children. *Environ. Sci. Technol.* 47: 8894.
[5] Vance M.E. and Marr L.C. (2015) Exposure to airborne engineered nanoparticles in the indoor environment. *Atmos. Environ.* 106: 503.
[6] Tulve N.S., Stefaniak A.B., Vance M.E., Rogers K., Mwilu S., LeBouf R.F., Schwegler-Berry D., Willis R. Thomas T.A., and Marr L.C. (2015) Characterization of silver nanoparticles in selected consumer products and its relevance for predicting children's potential exposures. *Int. J. Hyg. Environ.* 218: 345.

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 Report

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Disclaimer

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Executive Summary

The U.S. Consumer Product Safety Commission (CPSC) needs methods and data to better understand whether nanosilver is released from children's consumer products under conditions of normal, real-world intended use, making it available for potential exposure to children, since the lack of data on nanosilver release from consumer products has been identified by CPSC as a critical data gap. The objectives of this research project, conducted through an inter-Agency (IA) agreement with CPSC, and carried out by the U.S. Environmental Protection Agency (EPA) and Virginia Tech (VT; contractor to EPA), were to develop tools, approaches, and protocols to categorize and prioritize children's potential exposures to select children's consumer products containing nanosilver and to evaluate selected products in pilot tests.

The research effort comprised the following activities: a literature review was completed to document the state-of-the-science in children's potential exposures to nanosilver from child-specific consumer products; standard operating procedures (SOPs) were developed and evaluated for both chemical and microscopic analyses of various types of consumer products (liquid, fabric, plastic); a systematic approach was developed for experimentally assessing potential exposures to nanosilver; select consumer products were analyzed for total silver and silver nanoparticles as well as the likelihood to leach silver under real-world conditions; and predictions were made on potential pathways of exposure, resulting in a draft exposure framework.

More than 80 consumer products claiming to contain nanosilver and that may be used by or near children were identified and cataloged in the literature survey. From this inventory, 13 products were chosen for intensive evaluation including: one plush toy ([redacted]), three fabric products, one set of breastmilk storage bags, two sippy cups, three cleaning products (spray, surface wipe, and kitchen scrubber), two humidifiers, and one humidifier accessory. A total of 21 samples were generated from these products and evaluated to determine whether nanosilver was present.

A systematic approach was developed to determine the presence of silver, nanosilver, and the potential for silver leaching in various types of consumer products. This approach involved the following steps: measurement of total extractable silver; determination of the presence of nanoscale silver using electron microscopy; and measurement of leachable silver using liquids (synthetic urine, sweat, and saliva; tap water; orange juice; milk formula) relevant to each product's normal, real-world, intended use accompanied by analysis of the forms of silver in the leachate. Analytical standard operating procedures (SOPs) developed to support the systematic approach included: *Preparation of Consumer Product Samples for Silver Analysis by ICP-MS*; *Analytical Methods for Silver Nanoparticle Characterization via Electron Microscopy in Complex Media; Silver Leaching Assays from Solid Materials into Various Liquid Media;* and *Characterization of Aerosols Generated from Nanosilver Consumer Products*. These SOPs are available for use by CPSC as well as other research laboratories. With appropriate laboratory evaluation, it may be possible to adapt these procedures for other nanomaterials and types of products.

All products had at least one component containing silver, except for one humidifier and the humidifier accessory. Scanning and transmission electron microscopy identified silver-containing particles ranging in size from <20 nm to 10 μm in six samples from four products: (1) spray cleaning product, (2) interior foam and exterior fur of [redacted], (3) baby blanket, and (4) rubber ring and stopper from the [redacted] cup (sippy cup #1). Silver particles appeared to be located on the surface of the fabric fibers and embedded in different plastic components of the sippy cup. Nanosilver particles were positively identified in only 3 products ([redacted], baby blanket, spray cleaning product).

Leaching experiments, using liquids (synthetic urine, sweat, and saliva; tap water; orange juice; milk formula) relevant to each product's normal, real-world, intended use, were performed. Synthetic sweat and synthetic urine yielded the largest amount of total silver leached from [redacted] and the fabric products, 6% to 38%, while tap water yielded the lowest amount, 0.5% to 2.6%. Components of the sippy cups were found to leach silver in either milk formula or orange juice. No relationship was found between the pH of the liquid and the level of silver leached from the consumer product. Most, if not all, of the silver leached into the synthetic urine, sweat, or saliva was in ionic form, suggesting dissolution was the main leaching mechanism. In some cases, product aging increased the amount of silver leached from a product, but there was no general trend between aging and leaching. We assessed the transfer of silver to the wipe materials (to estimate dermal exposure) and found that between 0.3 and 23 $\mu g\ m^{-2}$ of silver transferred from products (or surfaces on which products were applied) to the wipes.

The potential for exposure to nanosilver in aerosols associated with humidifier use, spraying a cleaning product, and handling a fabric product and/or plush toy was evaluated. In scenarios simulating normal, real-world, intended use of the consumer products, ambient aerosol concentrations were not significantly elevated above background levels. If these products emit any form of silver-containing aerosols, the emission rates are very low.

Based on the results from this research project and the normal, real-world, intended use for each consumer product evaluated, the plush toy and fabric products are the most likely sources of bioavailable silver. Silver levels to which children may potentially be exposed during normal, real-world, intended use of these consumer products are predicted to be low, and bioavailable silver is expected to be in ionic rather than particulate form. Further research is needed to understand children's exposures, health implications of bioavailable silver, potential health effects from exposure to nanosilver, and mechanisms of toxicity for children's health when using consumer products containing nanosilver.

The results of this work advance our understanding of the potential for exposure to nanosilver in children's consumer products and provide tools and information that CPSC and other researchers can use to investigate the potential for exposure to nanosilver in consumer products. The major outputs from this EPA-CPSC IA are (1) an increased understanding of the potential exposures of children from the use of nanosilver-enabled consumer products, and (2) methods and approaches for evaluating nanosilver in consumer products. Therefore, we recommend that new nanosilver-enhanced products be evaluated using the research approach identified in this report.

Background

Nanosilver is reportedly being used in many different types of consumer products, including products intended for use by children, such as baby bottles, pacifiers, and plush toys. Primary reasons for the sudden emergence of nanosilver in the commercial market include advances in manufacturing as well as its potency as an anti-fungal, anti-bacterial, anti-viral, and anti-microbial agent (Klaine et al., 2008; Morones et al., 2005; Sun et al., 2005; Weir et al., 2008; Yoon et al., 2008).

The potential health impacts of silver nanoparticles in humans are not well understood. Numerous *in vitro* studies have shown that silver nanoparticles are toxic to certain organisms, such as phytoplankton, bacteria, and fish (Luoma, 2008; Navarro et al., 2008), as well as human cells (Hussain et al., 2005; Soto et al., 2005). *In vivo* studies with silver nanoparticles were toxic to rats and the silver nanoparticles translocated between organs (Hyun et al., 2008; Ji et al., 2007; Kim et al., 2008). Luoma (2008) reported 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 use of nanosilver in the commercial market as an anti-fungal, anti-bacterial, anti-viral, and anti-microbial agent has resulted in the need to evaluate children’s potential exposures to silver nanoparticles through multiple routes and pathways (dermal, inhalation, ingestion). Children may be especially affected by the normal use of consumer products designed specifically for them, such as milk bottles, pacifiers, and toys, because of their physiological functions, developmental stage, and activities and behaviors. Few published works address the potential for exposure to silver nanoparticles from consumer products found in the everyday environment (Benn et al., 2010; Hagendorfer et al., 2010; Quadros and Marr, 2010).

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 physicochemical properties. Therefore, methods and data are needed to characterize nanosilver contained in and released from consumer products. Appendix A contains a more detailed literature review.

This report is formatted to align with the research activities identified in the inter-Agency agreement (IA). The activities include:

- Activity #1: Review of Currently Available Information on Children's Potential Exposures to Nanosilver in Children's Consumer Products
- Activity #2: Develop Approaches and Tools for Prioritizing Exposure Potential, Identifying Exposure Scenarios, and Predicting Exposure Pathways
- Activity #3: Select Two or More Children's Consumer Products that Claim to Contain Nanosilver and Determine Whether the Material in the Consumer Product is Nanosilver
- Activity #4: Determine the Form of Silver (Soluble, Metallic) Released from the Product under Conditions of Intended Use
- Activity #5: Identify Pathways of Potential Exposure (Ingestion, Dermal, Inhalation Routes) from the Intended Use of these Products.

Within the body of the report, the methods, results, and discussion are presented by each major research activity. The appendices provide more detailed information for these research activities.

Activity #1: Review of Currently Available Information on Children's Potential Exposures to Nanosilver in Children's Consumer Products

A literature review, entitled "Children's Exposures to Silver Nanoparticles in Consumer Products: Commercially Available Products and Routes of Exposure" was completed (Appendix A). The report discusses the literature search strategy, a list of child-specific products that claim to contain nanosilver, and the potential routes of exposure based on the products identified. The report also acknowledges the challenges with consumer product labeling. For example, products containing nanosilver may not be labeled or advertised as containing nanosilver or products labeled as containing nanosilver may not necessarily contain nano-sized silver. The literature review suggests that most products claiming to contain nanosilver are manufactured in Asian countries. There is no clear indication that these products are readily available in the United States.

Activity #2: Develop Approaches and Tools for Prioritizing Exposure Potential, Identifying Exposure Scenarios, and Predicting Exposure Pathways

Using the literature review and product inventory, a draft exposure framework was developed (Appendix B). The framework lists categories of products claiming to contain nanosilver and potential routes of exposure. Expert judgment suggests that ingestion and dermal are the most likely routes of exposure for children since the products marketed to children are primarily put in the mouth (e.g., bottles, toothbrushes, pacifiers) or worn (e.g., sleep suit, baby blanket, mitts) and laboratory analyses confirmed the presence of nanosilver in many of these products. In addition, children may also be dermally exposed to nanosilver through cleaning products used in the home environment (see Activities 3 and 4 for more information). However, there isn't enough data to predict exposures for different child-specific consumer products and exposure scenarios. Therefore, as more information is learned about the potential for children's consumer products to contain nanosilver, the exposure framework should be updated.

Activity #3: Select Two or More Children's Consumer Products that Claim to Contain Nanosilver and Determine Whether the Material in the Consumer Product is Nanosilver

Activity #3.1: Identification of Potential Children's Consumer Products that Claim to Contain Nanosilver

Activity #3.1.1: Pilot Study Product Identification

A search was conducted using the internet, peer-reviewed literature, direct marketing, and available databases (e.g., the Woodrow Wilson project on emerging nanotechnologies database, http://www.nanotechproject.org/inventories/consumer/) to identify and purchase child-specific consumer products containing nanosilver. This task was difficult primarily because: (1) U.S. product manufacturers do not need to disclose ingredients, (2) products that may have contained nanosilver at one time may no longer contain nanosilver or may no longer be available for purchase, (3) products that truly contain nanosilver may not advertise it, and (4) advertised products from foreign countries have been difficult to obtain. However, we were able to secure a select few items from internet purchases, personal contacts living abroad, CPSC ghost shoppers, personal foreign travel, and personal shopping in an ethnic marketplace. Table 1 lists the products EPA obtained for pilot study evaluation. Figure 1 shows select children's consumer products identified in Table 1.

Table 1. Children's consumer products for pilot study evaluation.

Item Type	Brand Name	Manufacturer	Country of Origin	Website	Nanosilver Claim on Product Packaging
Teddy Bear	[redacted]	[redacted]	US company; made in China	[redacted]	Anti-microbial pure silver (nanotechnology)
Toothbrush	[redacted]	[redacted]	Korea	[redacted]	99.9% edible nanosilver
Toothbrush	[redacted]	[redacted]	Korea		Nanosilver; FDA Reg. No. [redacted]
Child's Sippy Cup (Purple)	[redacted]	[redacted]	UK	[redacted]	No antibacterial or nanosilver claims on packaging
Child's Sippy Cup (Light Blue)	[redacted]	[redacted]	UK	[redacted]	No antibacterial or nanosilver claims on packaging
Mineral Supplement	[redacted]	[redacted]	Westminster, CO, US	[redacted]	Pure silver particles; 20 ppm
Personal Disinfectant Spray	[redacted]	[redacted]	Taiwan		Nanocomposite anti-viral
Toothbrush			Korea		Labeled as nanosilver and anti-bacterial
Scrub Sponges	[redacted]	[redacted]	Korea	[redacted]	Labeled as nanosilver
Bath Towel	[redacted]	[redacted]	Korea	[redacted]	Labeled as nanosilver
Wash Cloth	[redacted]	[redacted]	Korea	[redacted]	Labeled as nanosilver

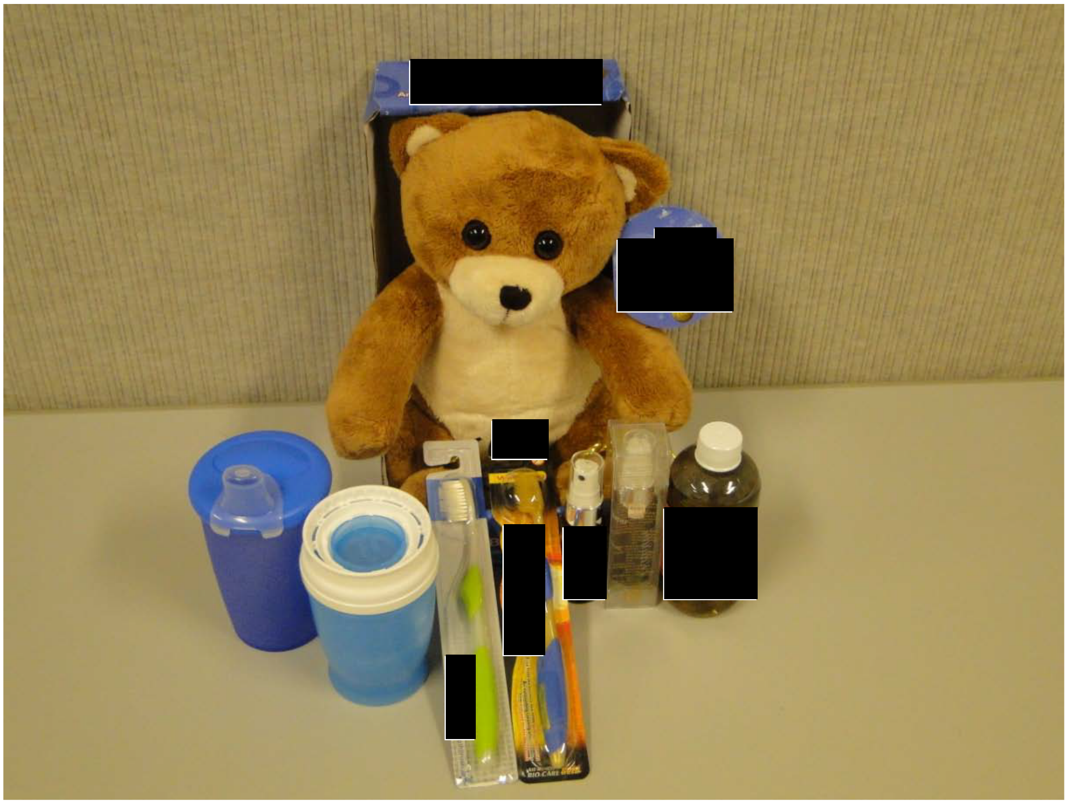

Figure 1. Picture showing select children's consumer products listed in Table 1.

Activity #3.1.2: Main Study Product Identification

Using the internet and available databases (e.g., the Woodrow Wilson project on emerging nanotechnologies database, http://www.nanotechproject.org/inventories/consumer/) VT built an inventory of consumer products claiming to contain nanosilver that may be used by or near children. The product inventory and a discussion of how the inventory was generated are included in Appendix C. More than 80 products were identified as claiming to contain nanosilver and which may be used by or near children. The products were classified by product type (Table 2), with product types ranging from one product (i.e., pacifiers) to 19 products (i.e., dietary supplements [liquids and pills]). In consultation with Dr. Treye Thomas, CPSC, we narrowed the number of products to be tested in the laboratory, with the primary criteria being that the product had to be under the regulatory authority of CPSC. The products that were identified for laboratory analysis are listed in Table 3.

Table 2. Summary of inventory of children's consumer products claiming to contain nanosilver.

Product Type	Number of Products	Item Numbers in Table 3, Appendix C
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

Table 3. Summary of nanosilver products selected for laboratory evaluation in the main study.

	Product	Category	Item Number in Product Inventory (Appendix C)
1	[redacted]	Toys	1
2	Pacifier (not purchased)	Pacifiers	N/A
3	[redacted] Sleepsuit or Babygro	Textiles	14
4	[redacted] Baby Blanket	Textiles	15
5	[redacted] Baby Scratch Mitts	Textiles	16
6	Breastmilk Storage Bags	Food storage	21
7	Sippy Cup #1 ([redacted] Cup), light blue	Food storage	23
8	Sippy Cup #2 [redacted] Cup), purple	Food storage	23
9	[redacted] Antifungal/Antibacterial Disinfecting Spray (four 125 mL bottles)	Surface disinfectants and cleaners	66
10	[redacted] Surface Wipes (one pack of 30)	Surface disinfectants and cleaners	68
11	[redacted] Scrubber	Surface disinfectants and cleaners	69
12	[redacted] Tabletop Humidifier	Humidifiers	72
13	[redacted] Manual Ultrasonic Humidifier	Humidifiers	74
14	[redacted]	Humidifiers	82

Activity #3.2: Determination of Whether the Identified Consumer Products Contain Silver (Chemical Analysis for Total Silver)

Activity #3.2.1: Pilot Study Evaluation for Total Silver

A specific piece or a liquid aliquot of a product was extracted and processed using a MARS XP-1500 microwave extraction instrument (CEM Corporation) (SOP # AirVT-Nanosilver-001: Preparation of Consumer Product Samples for Silver Analysis by ICP-MS [Appendix D]). Originally developed for the extraction of metals from plastics, the extraction method used a ten minute high pressure extraction process in concentrated nitric acid (Millipore, Ultra High Purity). Visual observation of the extracts showed no observable solids. Extracts were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) calibrated using a Perkin-Elmer silver standard solution.

For [redacted], a piece of foam was analyzed; for the toothbrushes, a few bristles were analyzed; for each different sippy cup, a piece of the inner valve material that contacts the liquid was analyzed. Since little or no vendor information was available to identify where the nanosilver (if present) was located in the product, the selection of the part of the solid product to analyze was based on expert judgment. For [redacted], the foam was sampled to include an apparent membrane covering the foam interior. For the sippy cup inner valve material ([redacted] cup), the rubber was sampled from the contact surface. For the two liquid materials, an aliquot was analyzed. The toothbrushes, scrub sponges, bath towel, and wash cloth were purchased on a trip to Korea (ISES-ISEE 2010 Joint International Meeting, August 31, 2010, purchased courtesy of Aleksandr Stefaniak at NIOSH), and these items were considered for inclusion in the main study analyses.

Figure 1 shows the select consumer products that were analyzed in the pilot testing. Table 4 shows the preliminary results for the amount of total silver in the products that were analyzed. Two products, the [redacted] colloidal dietary supplement and the [redacted] commercial particles, were the only products with manufacturer's claims identified on the packaging. For both products, the manufacturer's claim was that the product contained 20 µg/g silver. Analysis of these two products confirmed the manufacturer's claim. In addition, this information also supported the adequacy of the chemical analysis approach used to quantify total silver content. With the exception of the control toothbrush and the [redacted] cup (sippy cup #2), all remaining items contained silver, whereas the manufacturer's claims for these products did not mention the presence of silver. While there is no evidence that the toothbrush purchased at Walgreens should have contained silver, there was evidence that the [redacted] cup should have contained silver. This shows the inherent difficulty in identifying products that may contain silver. It also suggests the heterogeneous distribution of silver in products.

The [redacted] toothbrush bristles had the highest concentration of total silver (858 µg/g), of the products analyzed. The inner valve material of the [redacted] cup (sippy cup #1) contained approximately 200 µg/g silver, and the interior foam from [redacted] contained approximately 100 µg/g silver.

Ideally, if a product contained silver, the next step was to determine via microscopy whether the silver was nanosized. Products containing silver in Table 4 were selected for analysis by Scanning Electron Microscopy (SEM) to look for nanosized particles.

Table 4. Total silver in select children's consumer products analyzed in the pilot study.

Sample	Ag in Extracts Analyzed (EPA Laboratory) (µg/g)	Dilution for ICP-MS Analysis	[Ag] in 25 mL Extracts (µg/g)	Amount of Material Extracted (g)	[Ag] in Material (µg/g)	Manufacturer's Claim (Reported Value) (µg/g)
[redacted] toothbrush bristles	14.0	500	7024	0.2	858	NA
Sippy Cup #1 ([redacted] cup) (light blue) (inner valve material)	7.8	300	2332	0.3	200	NA
Sippy Cup #1 ([redacted] cup) (light blue) (inner valve material)	8.0	300	2388	0.3	199	NA
[redacted] outside foam	6.9	200	1376	0.3	106	NA
[redacted] inside foam	5.8	200	1159	0.3	97	NA
[redacted] outside foam	4.6	200	926	0.3	85	NA
[redacted] silver personal spray	9.0	50	449	0.3	40	NA
[redacted] colloidal dietary supplement	7.7	30	231	0.3	18	20
[redacted] commercial particles	4.3	50	214	0.3	17	20
[redacted] toothbrush (control)	0.1	1	0.1	0.3	Non-Detect	NA
Sippy Cup #2 ([redacted] cup) (purple) (inner valve material)	-0.1	1	-0.1	0.1	Non-Detect	NA

Activity #3.2.2: Main Study Evaluation for Total Silver

Using the product inventory (Appendix C), select children's consumer products were identified for further laboratory evaluation. Products were classified as liquids (Category I), fabrics (Category II), and plastics (Category III), with each product type requiring different sample preparation methods, as shown in Figure 2. VT confirmed that these products contained silver and quantified their total silver content.

For this main study, results of the chemical analysis for total silver determined whether a consumer product was further evaluated using microscopy and leaching analyses. Figure 2 shows the relationship between the three laboratory tasks completed by VT (Task 3, Task 4, and Task 5) and how analyses proceeded depending on the results obtained at each step. For fabrics and plastics, if a product contained a detectable amount of silver, leaching experiments were conducted (Task 5); if silver leached from the product, microscopy analyses were completed (Task 4). If silver did not leach from the product, no further analyses were conducted.

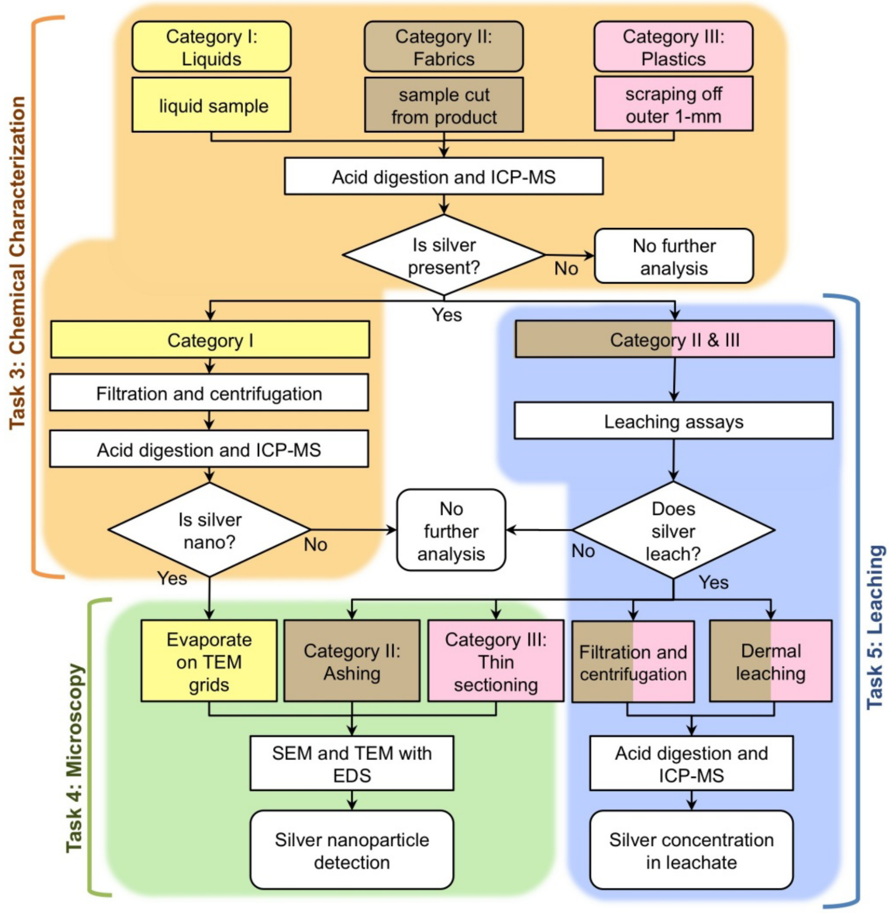

Figure 2. Flowchart summarizing the experimental approach used by VT for the main study.

Table 5 shows the specific leaching media and exposure scenarios evaluated for each consumer product or product component. [redacted] and the baby blanket were the only products evaluated under new and "aged" conditions. In Table 5, sippy cup #1 = [redacted] cup (light blue) and sippy cup #2 = [redacted] cup (purple).

Table 5. Sampling matrix used by VT for nanosilver-containing consumer products analyzed in the main study.

#	Product (component)	Task 3: Total Silver	Task 4: Microscopy – SEM product	Task 4: Microscopy – SEM (ash)	Task 4: Microscopy – TEM product	Task 4: Microscopy – TEM (ash)	Task 5: Leaching – Tap Water	Task 5: Leaching – Orange Juice	Task 5: Leaching – Milk Formula	Task 5: Leaching – Artificial Saliva	Task 5: Leaching – Synthetic Urine	Task 5: Leaching – Synthetic Sweat	Task 5: Dermal	Task 5: Aerosol
1	[redacted] (exterior, new)	X	X	X			X			X	X	X	X	X
2	[redacted] (exterior, aged)						X			X	X	X		
3	[redacted] (interior)	X	X	X		X	X			X	X	X		
4	[redacted] Antibacterial Baby Blanket (new)	X	X	X		X	X			X	X	X	X	
5	[redacted] Antibacterial Baby Blanket (aged)						X			X	X	X		
6	[redacted] 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	[redacted] Antifungal Spray	X			X								X	X
19	[redacted] Surface Wipes	X	X		X								X	
20	[redacted] Scrubber	X											X	
21	[redacted] Table Top Ultrasonic Humidifier						X							
22	[redacted] Ultrasonic Humidifier [redacted]						X							
23	[redacted]						X							X

Methods for Liquid Products

One liquid product ([redacted] antifungal/antibacterial disinfecting spray) was analyzed in the main study. To determine whether the liquid product contained silver in particulate and/or ionic form, an aliquot of liquid product was passed through a series of filters with the following size cutoffs: 1000 nm, 450 nm, 100 nm, and 3KDa (kilodaltons). For the 1000, 450, and 100 nm cutoffs, hydrophilic Teflon filters (Millipore Omnipore) were used. For the 3 KDa cutoff, a centrifugal filtering unit (Millipore) was used because this type of centrifugal filtering unit is recommended for concentrating proteins ~1 nm or larger (Erickson, 2009). Any 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 (HNO_3, 70% ACS certified, Fisher Scientific), diluted 1:100, and analyzed for total silver concentrations using ICP-MS (X-Series, Thermo Electron).

Table 6 shows the concentration of silver obtained by ICP-MS for the liquid product for different particle size cutoffs. This product had an advertised silver concentration of 20 ppm; VT 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% in aggregates larger than 1000 nm, and 14% in ionic form.

To understand whether silver ion would sorb to the filter media, we used 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%, suggesting that the actual proportion of silver in ionic form in the spray product is likely higher than measured.

Table 6. Size-resolved silver concentrations in [redacted] antifungal/antibacterial disinfecting spray (N=3).

Size Cut-Off	**Silver Concentration (ppm) (mean ± std error)**
> 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

<u>Methods for Fabrics and Plastics</u>

Aliquots from the fabric and plastic samples were digested using a combination of nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) (SOP # AirVT-Nanosilver-001: Preparation of Consumer Product Samples for Silver Analysis by ICP-MS [Appendix D]). Benn and Westerhoff (2008, 2010) used 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, at least three aliquots from each product were analyzed. When appropriate, aliquots from different parts of the same product were analyzed. For example, sippy cup #1 ([redacted] cup, light blue) had five different components, whereas sippy cup #2 ([redacted] cup, purple) had three different components that were analyzed (Figure 3).

Figure 3. Close-up of sippy cups #1 ([redacted] cup, light blue) and #2 ([redacted] cup, purple).

The mass of sample aliquot analyzed ranged from less than 100 mg to approximately 2700 mg (Table 7). Small sample sizes resulted when an aliquot of material had to be shaved from the product surface. The surface wipe cleaning products were packed in a solution containing alcohol, resulting in sample masses with large standard deviations due to evaporation of the alcohol. In an attempt to reduce the large standard deviations, one entire wipe was used in the analyses.

Table 7. Products, sample size, mass extracted for acid digestion, and total silver concentration determined by ICP-MS for the children's consumer products evaluated in the laboratory.

Product	Sample Size (N)	Mass Extracted for Acid Digestion (mg) (mean ± std error)	Total Silver Concentration (mg Ag/kg product) (mean ± std error)
1 [redacted]: exterior fur	3	502 ± 3	0.6 ± 0.1
2 [redacted]: interior foam	6	495 ± 3	48.2 ± 5.0
3 Pacifier[1]	-	-	-
4 [redacted] Sleepsuit or Babygro	3	501 ± 1	109.8 ± 4.1
5 [redacted] Baby Blanket	3	501 ± 1	108.3 ± 1.7
6 [redacted] Baby Scratch Mitts	3	501 ± 1	104.2 ± 4.0
7 Breastmilk storage bags	6	506 ± 1	0.9 ± 0.6
8 Sippy Cup #1 ([redacted] cup) (light blue): outside of cup	3	99 ± 1	ND[2]
9 Sippy Cup #1 ([redacted] cup) (light blue): inside of cup	3	99 ± 4	ND
10 Sippy Cup #1 ([redacted] cup) (light blue): rubber ring	3	255 ± 2	24.3 ± 2.9
11 Sippy Cup #1 [redacted] cup) (light blue): white rim	3	201 ± 1	4.9 ± 0.2
12 Sippy Cup #1 ([redacted] cup) (light blue): transparent cap	3	101 ± 4	9.4 ± 1.0
13 Sippy Cup #2 ([redacted] cup) (purple): outside of cup	3	57 ± 10	ND
14 Sippy Cup #2 ([redacted] cup) (purple): inside of cup	3	103 ± 5	ND
15 Sippy Cup #2 ([redacted] cup) (purple): spout	3	100 ± 1	ND
16 Sippy Cup #2 ([redacted] cup) (purple): transparent cap	6	37 ± 13	ND
17 [redacted] Surface Wipes	9	2740 ± 1100	4.5 ± 3.0
18 [redacted] Scrubber	3	505 ± 2	4.6 ± 0.3
19 Non-nano fabric	3	502 ± 2	ND

[1]Not purchased for project.
[2]Not detected. Silver concentrations in the digestion liquid were below 0.5 ppb.

Each aliquot was placed in a 140 mL beaker to which 10 – 20 mL of nitric acid (70% ACS certified, Fisher Scientific) was added. The sample soaked for at least 30 min. Samples were then heated to approximately 90 °C and 1 mL aliquots of nitric acid were added until the sample was completely digested. Since hard plastics do not easily digest in heated nitric acid, samples were heated for at least 2 hr to dissolve the silver into the acid. At the end of this time period, samples were removed from heat, cooled, and 2 mL of hydrogen peroxide (50% ACS certified, Fisher Scientific) was added. Samples were heated until effervescence was minimal and then removed from the hot plate. Samples were cooled until they reached room temperature. After samples reached room temperature, they were diluted to a final volume of 100 mL with ultrapure water and passed through a 450 nm hydrophilic Teflon filter (Millipore Omnipore). Finally, samples were analyzed for total silver concentrations by ICP-MS.

Table 7 shows the total silver content for each fabric or plastic product or product component, reported in mass of silver per mass of product. Samples that had a silver concentration less than 0.5 ppb in the digested liquid were considered below the detection limit (ND). The processes used to incorporate nanosilver into these products are unknown, and it is possible that silver may not be homogeneously distributed throughout the product, potentially resulting in high standard deviations between triplicate samples. When the average result of triplicate samples had a relative standard error > 20%, we collected and analyzed three more samples to bring the total sample size to six.

The interior foam of [redacted] had a silver concentration 8 times higher than the exterior fur. Silver did not seem to be homogeneously distributed throughout the foam, and the relative standard error was approximately 10%. In addition, the pilot study analysis selectively sampled the membrane covering the foam which yielded a higher total silver observed value (Table 4). All [redacted] fabric samples had similar silver concentrations, averaging 107 ± 2 mg Ag/kg product, suggesting that these products were manufactured from the same type of fabric and that the silver application onto the fabric resulted in a relatively homogeneous distribution of silver. Sippy cup #2 ([redacted] cup) had no detectable silver in the product components analyzed, whereas sippy cup #1 ([redacted] cup) had small concentrations in the ring (24 mg Ag/kg product), rim (5 mg Ag/kg product), and transparent plastic (9 mg Ag/kg product). The wide range of silver concentrations measured (ND – 24 mg Ag/kg product) in the components of the sippy cups would suggest that the different materials in the sippy cups have different silver to polymer ratios. The surface cleaning wipe and the kitchen scrubber contained similar silver concentrations (~4 mg Ag/kg product), but the silver was more homogeneously distributed in the scrubber than in the surface wipe.

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 the dynamic light scattering (DLS) results (Figure 4), 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.

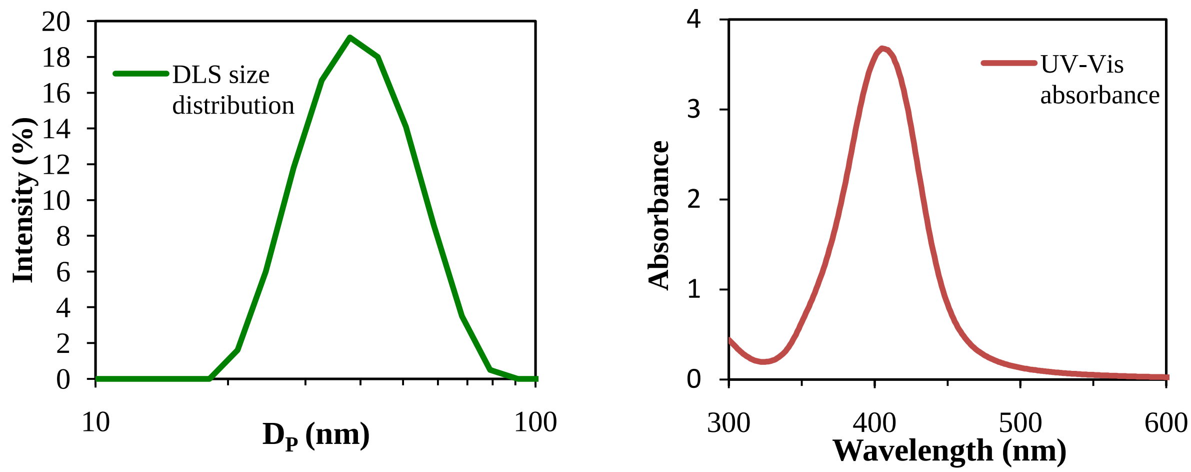

Figure 4. Dynamic light scattering (DLS) size distribution (left) and UV-Vis absorbance spectrum (right) for the standard nanosilver suspension.

Activity #3.3: Determination of Whether Selected Consumer Products Contain Nanosilver (Microscopy)

Activity #3.3.1: Microscopy Results for Select Pilot Study Samples

Scanning Electron Microscopy (SEM) coupled with Energy-Dispersive X-ray Spectroscopy (EDS) was used in an effort to characterize silver-containing particles in a subset of children's consumer products shown in Figure 1. SEM provides information on particle size and morphology, while EDS yields information on particle composition. The EDS detection limit for Ag is estimated to be roughly 0.5 wt %.

In the pilot study, the following products were examined by manual SEM/EDS: (1) [redacted] toothbrush (tapered and untapered bristles); (2) [redacted] cup (sippy cup #1, light blue) (inner valve material and inner wall of cup); and (3) [redacted] cup (sippy cup #2, purple) (inner valve material). For these three products, little or no information was available from the vendor indicating where the nanosilver (if present) was located. As a result, the selection of parts to analyze was based on expert judgment. For the two sippy cups, small (few mm) sections of the inner valve material and inner cup wall [redacted] cup (sippy cup #1) only) were scraped from the unused cups using a scalpel, attached to 12 mm aluminum specimen mounts using carbon sticky tabs, and coated with approximately 300 Å of carbon to minimize charge build-up on the sample. For the toothbrush, individual tapered and untapered bristles were mounted on specimen mounts and carbon-coated.

Preliminary sample analyses of the three consumer products were conducted at the U.S. EPA's National Exposure Research Laboratory (NERL) Electron Microscopy Laboratory (Research Triangle Park, NC) and at the Advanced Measurement Laboratory at the National Institute of Standards and Technology (NIST) (Gaithersburg, MD). The analyses in NERL and NIST were limited to EDS analyses to confirm the presence of silver, and SEM/EDS searches for Ag-rich particles larger than about 20 nm (NIST) and 100 nm (NERL), which are the effective imaging resolutions of the instruments available at each institution. Figures 5a and 5b are low magnification images of the two types of bristles in the [redacted] toothbrush acquired at the NERL facility. SEM/EDS failed to detect silver particles in either bristle.

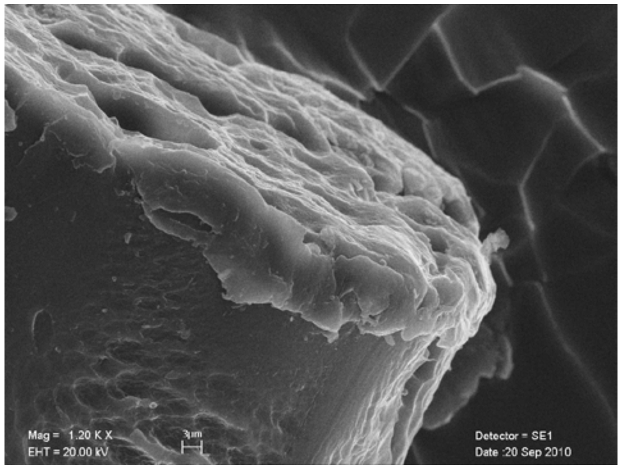

Figure 5a. End of untapered bristle.

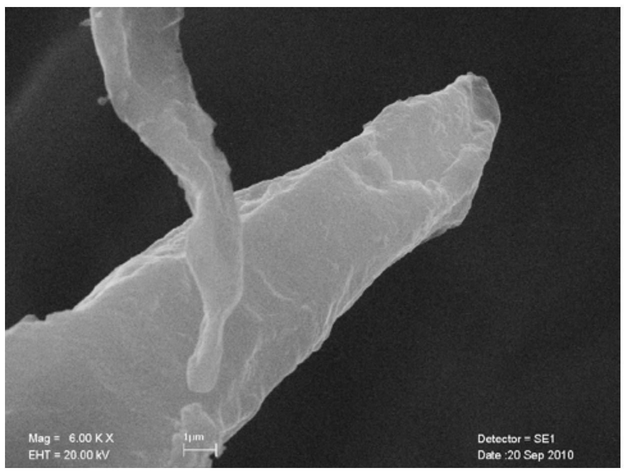

Figure 5b. End of tapered bristle.

Rare isolated silver particles in the size range of one to several microns were detected by SEM/EDS in the valve material of the two sippy cups. It is possible that these particles were accidentally introduced into the product during manufacturing and are unrelated to any intentional nanosilver-doping of these products. Figures 6a and 6b show low-resolution images and EDS spectra for two particles detected in the valve of the [redacted] cup (sippy cup #1). The images were collected in the backscatter detection mode in which a particle's brightness is related to its average atomic number. Silver particles thus appear very bright against a carbonaceous matrix, making them easy to identify down to approximately 100 nm in size for the NERL SEM. The upper left image in Figure 6a is a low-magnification image of the sample. The area within the enclosed box was magnified in the upper right image. The silver particle is the bright particle in the zoomed field. The scale bar at the base of this image indicates that the particle is approximately 1 µm. The EDS spectrum acquired from this particle shown below the field images confirmed that the composition was silver. The small carbon and oxygen peaks are probably associated with the carbonaceous composition of the valve material.

The bright silver particle in Figure 6b is almost 10 µm in length. The EDS spectra from both of these particles suggested pure elemental silver composition, unassociated with other elements. There was no distinctive morphology to the silver particles.

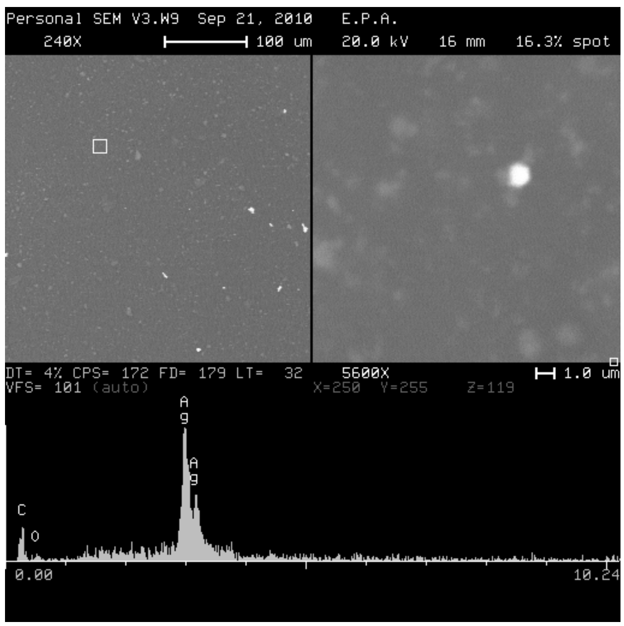

Figure 6a. Silver particle #1 in [redacted] cup (sippy cup #1) inner valve material.

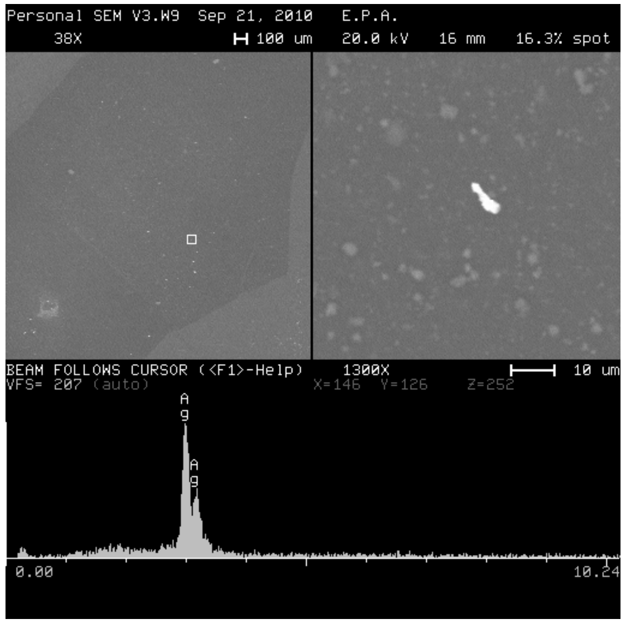

Figure 6b. Silver particle #2 in [redacted] cup (sippy cup #1) inner valve material.

Occasional silver particles were also found in the inner wall of the [redacted] cup (sippy cup #1). Figure 7 shows a high resolution image of the inner wall of the [redacted] cup (sippy cup #1) acquired at NIST using a high resolution Field Emission SEM. The 500 nm scale bar shows that detection of silver nanoparticles down to approximately 20 nm is possible with this instrument. However, particles this small in size were not detected.

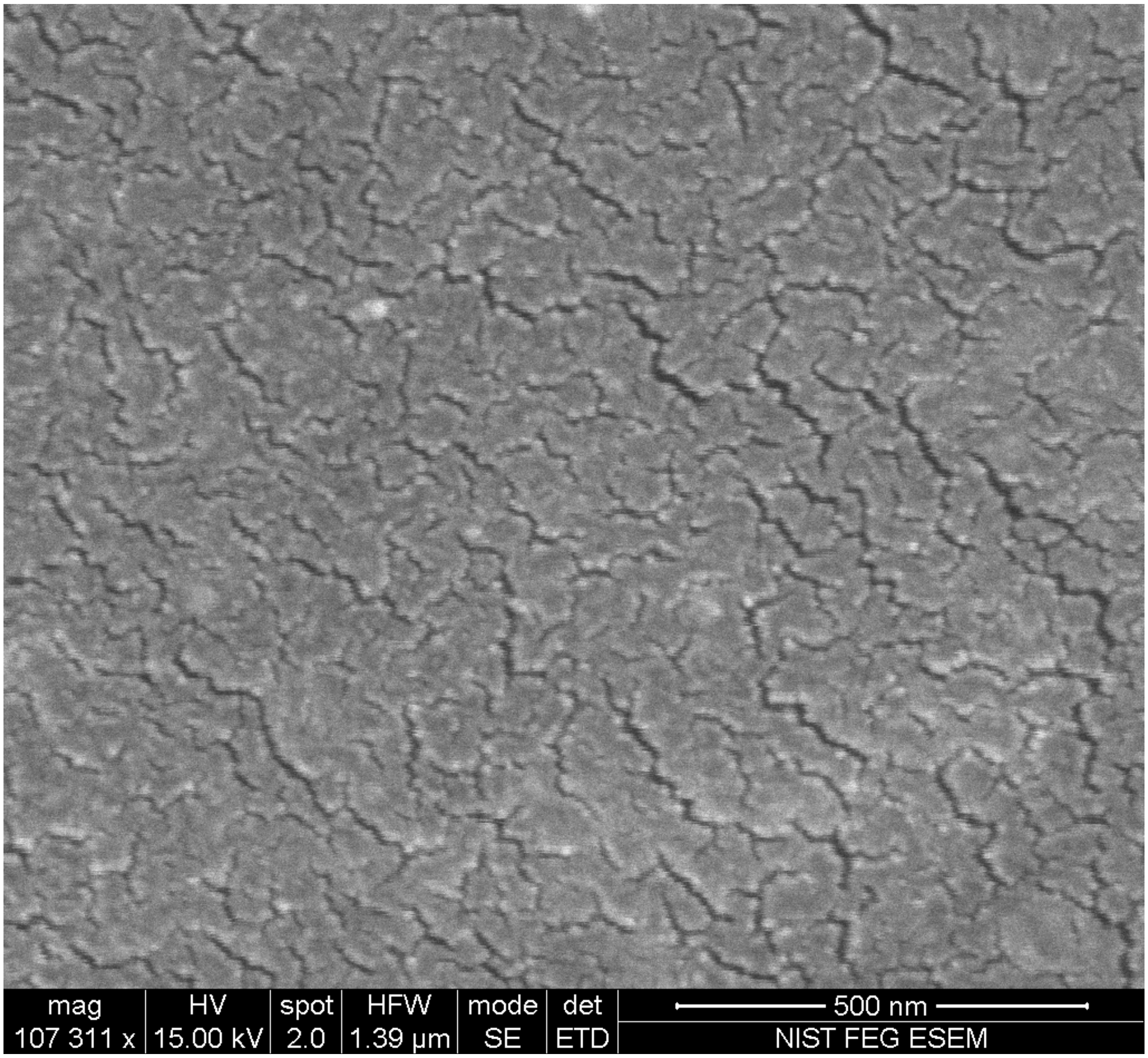

Figure 7. High resolution image of inner wall of the [redacted] cup (sippy cup #1) acquired with high resolution Field Emission SEM at NIST (Courtesy: J. Conny, NIST).

Figure 8 shows a 2 µm silver particle from the inner wall of the [redacted] cup (sippy cup #1).

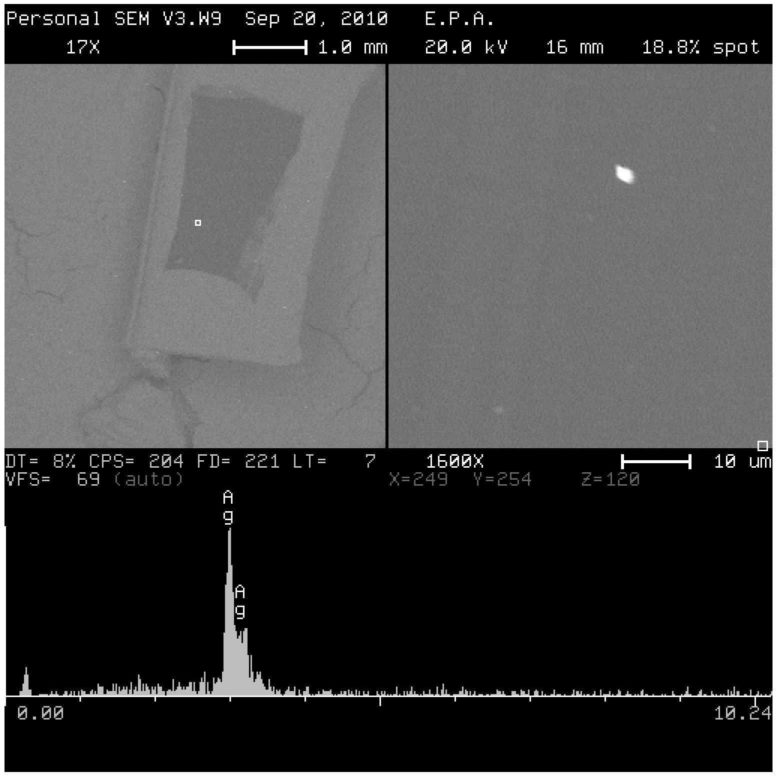

Figure 8. Two µm silver particle from the inner wall of the [redacted] cup (sippy cup #1).

Figure 9 shows an isolated silver particle from the [redacted] cup (sippy cup #2) inner valve material.

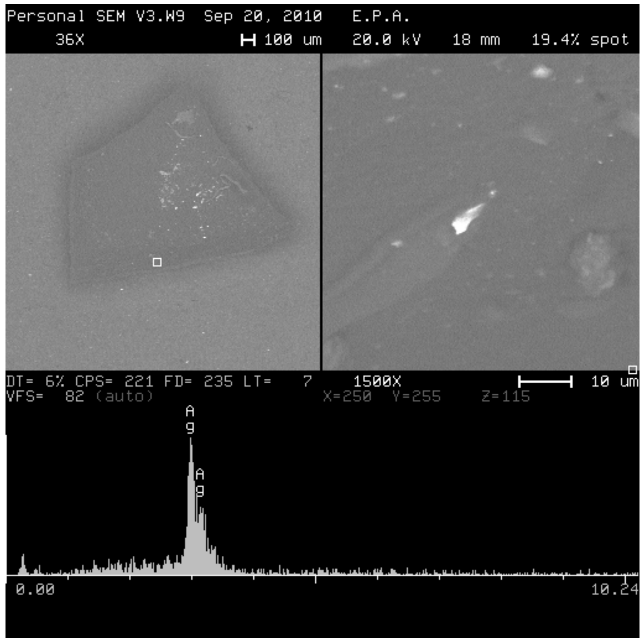

Figure 9. Silver particle in [redacted] cup (sippy cup #2) inner valve material.

In summary, no silver was detected on the [redacted] toothbrush bristles in these preliminary SEM/EDS analyses. Silver was detected by EDS in the valve materials of the sippy cups and in the inner wall of the [redacted] cup (sippy cup #1), and isolated micron-sized silver particles were found by SEM/EDS in these samples, but no nanosized silver particles were detected. One cannot conclude, however, that nanosilver is absent in these products. If nanosilver particles were present, however, these results suggest that they were smaller than the limiting resolution (20 nm) of the instruments available to EPA.

Activity #3.3.2: Microscopy Results for Select Main Study Samples

After laboratory analysis confirmed the presence of silver in select children's consumer products, the mass ratio was quantified (micrograms of silver per gram of product) using ICP-MS, and microscopy was used to determine if the silver was nanosized. Microscopy methods and sample preparation 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 D). Microscopes used by VT for the main study are listed in Table 8.

Table 8. Microscopes at Virginia Tech used in the main study.

Name / Description of Method	Instrument	Description / Rationale for Selection	Resolution or Limit of Detection
Environmental SEM with 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.

Microscopy Sample Preparation and Analysis

Liquid products containing 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 contained and leached silver, pieces of each product were adhered to SEM stubs using carbon tape, sputter-coated with gold to make the samples conductive, and analyzed by SEM/EDS.

Ashing and TEM

For the baby blanket (new product component) and [redacted] (new product component), we adapted a method applied by Benn and Westerhoff (2008) which consisted of ashing the material and dusting the residue onto carbon tape for SEM analysis. Samples were partially ashed in a muffle furnace at 450 – 500 °C, suspended in water, sonicated, evaporated onto carbon-coated TEM grids, and analyzed using the Titan high-resolution TEM/EDS (FEI Company, Hillsboro, OR).

To determine whether the ashing method altered the silver particles in the samples, a control sample using a silver nanoparticle suspension applied to a non-nano fabric ([redacted] 100% cotton t-shirt) was prepared and evaluated. The nanosilver suspension was dispersed over the cotton fabric at the same silver concentration as observed in the nanosilver fabric samples and then subjected to the same ashing and microscopy methods that were applied to the nanosilver fabric and [redacted] samples.

A method described by Heard et al. (1983) was used to synthesize EDTA-stabilized silver nanoparticles. The resulting particles were characterized by DLS 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, then 10 μL droplets were placed onto copper TEM grids coated with a holey carbon film (SPI), and viewed using the Philips EM 420 TEM at 120 keV. These samples were also diluted 1:100, acidified with 5% nitric acid, and analyzed by ICP-MS to determine the total silver concentration.

Microscopy Analysis of [redacted] Antifungal/Antibacterial Disinfecting Spray

Figure 10 shows a TEM micrograph of the [redacted] antifungal/antibacterial disinfecting spray. The product contained aggregates of widely distributed sizes and primary particles of narrowly distributed sizes. Figure 11 shows this product’s primary particle size distribution, estimated using ImageJ software (N=253 particles).

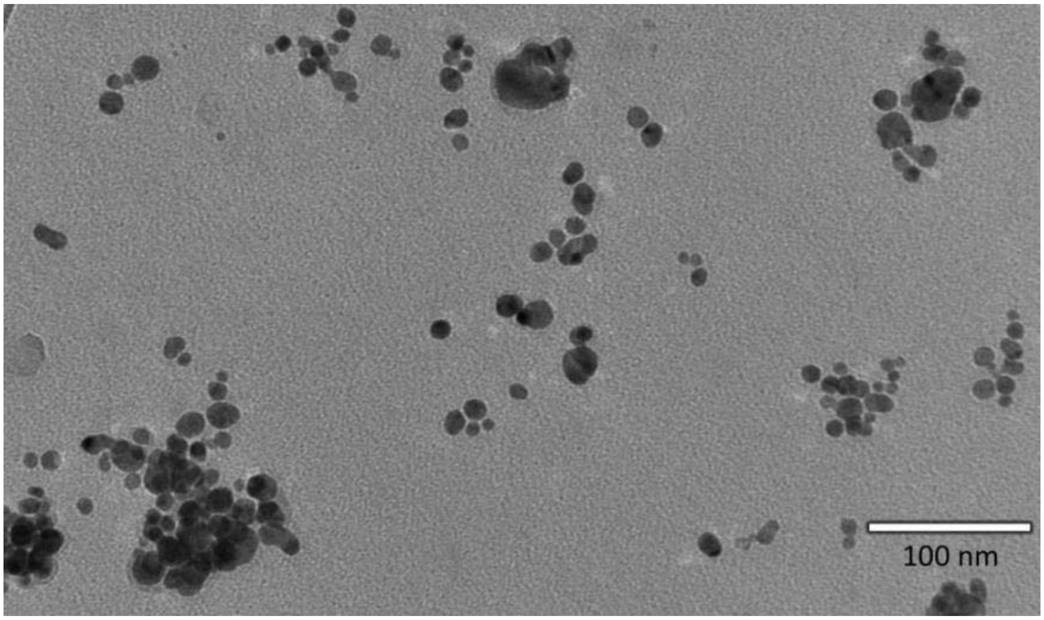

Figure 10. TEM micrograph of the [redacted] antifungal/antibacterial disinfecting spray.

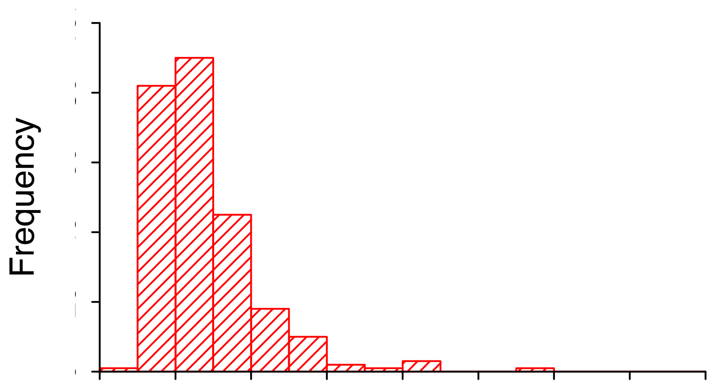

Figure 11. Primary particle size distribution of the [redacted] antifungal/antibacterial disinfecting spray (not counting aggregates).

Even though the disinfecting spray contained 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 10) and the size distribution obtained by DLS (Figure 12). The DLS size distribution has three peaks at 0.8 nm, 3.2 nm, and 255 nm. DLS results are more accurate when size distributions are unimodal, so the intensities shown in Figure 12 do not directly represent the amount of particles present in each size range. The UV-Vis absorbance spectrum peaks at 396 nm (Figure 12), close to the peak for colloidal silver, which occurs at ~400 nm.

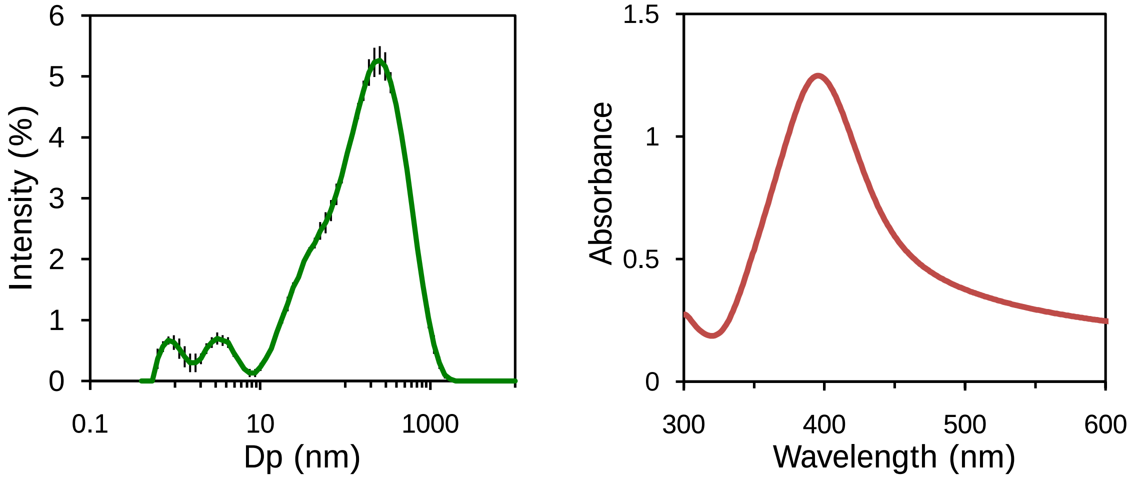

Figure 12. DLS size distribution (left) and UV-Vis absorbance spectrum (right) for the [redacted] antifungal/antibacterial disinfecting spray.

<u>Microscopy Analysis of Synthesized Silver Nanoparticles</u>

Figure 13 shows a TEM micrograph of the silver nanoparticles synthesized in VT's laboratory. The average particle diameter, as measured by DLS, was 36.2 ± 0.2 nm, whereas the average particle diameter estimated by ImageJ software was 25.1 ± 0.6 nm (Figure 14). Particle diameters measured by DLS are usually larger than those measured from TEM micrographs, probably because water molecules surround each particle affecting the particles' scattering properties.

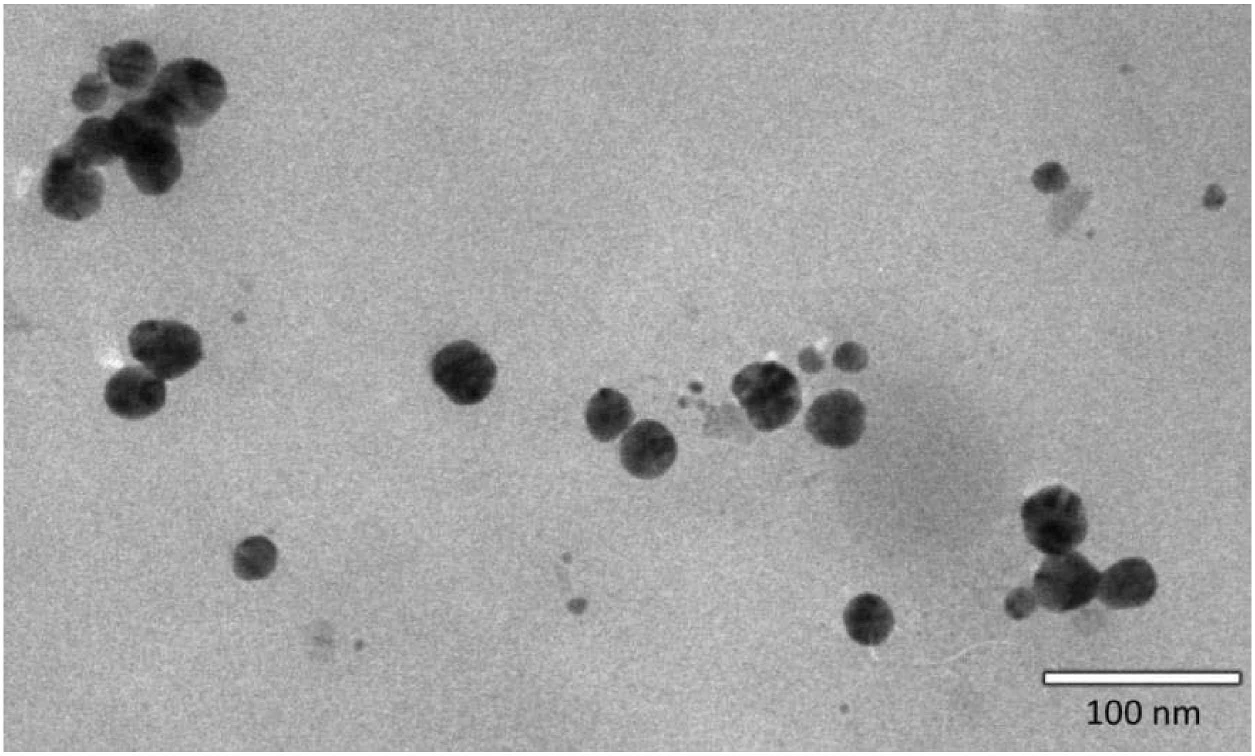

Figure 13. TEM micrograph of nanosilver particles synthesized in the VT laboratory.

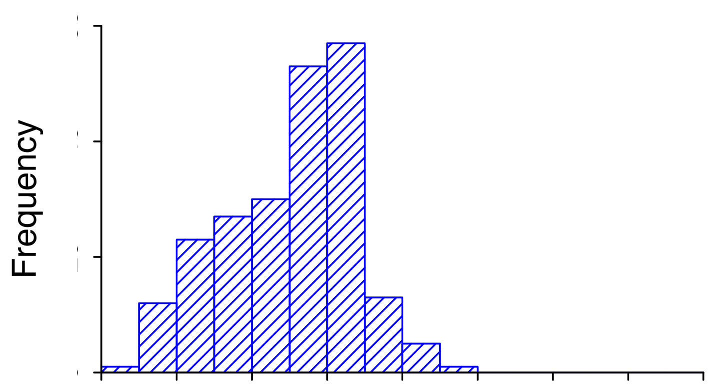

Figure 14. Particle size distribution of nanosilver particles created in the laboratory, estimated using ImageJ software (N=222 particles).

<u>Microscopy Analysis of the Non-Nano Control Fabric</u>

Figure 15 shows an SEM image of the non-nano control fabric ([redacted] 100% cotton t-shirt) treated with a standard nanosilver suspension. This image was obtained using the electron detector in backscatter detection mode, through which heavy elements shine more brightly. The bright spots observed on the fiber are silver nanoparticles and nanoparticle aggregates. EDS spectra for this sample can be found in Appendix E.

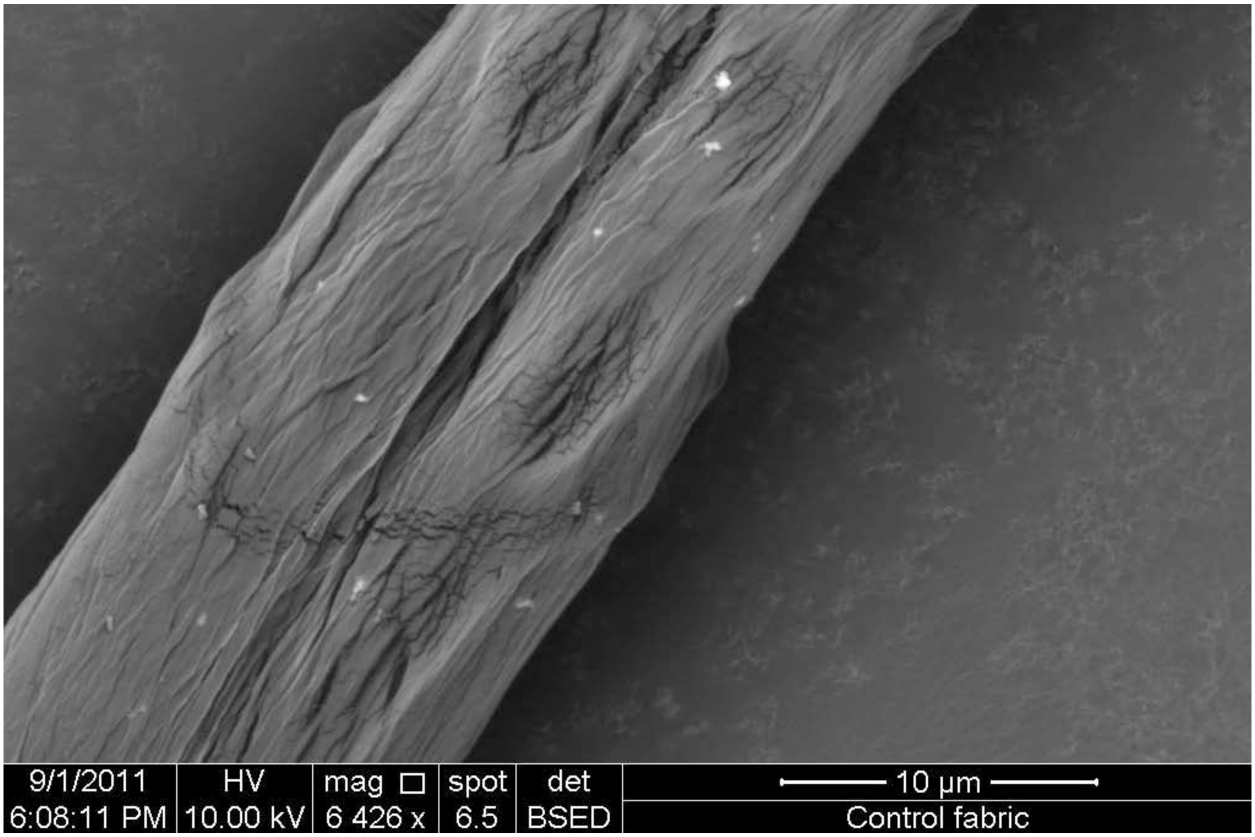

Figure 15. SEM micrograph of the control fabric treated with a standard nanosilver suspension (obtained in backscatter detection mode).

Figure 16 shows a high-resolution TEM (HR-TEM) image of an ashed sample of 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 E). Comparing this image with the micrograph of the nanosilver suspension (Figure 13), 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.

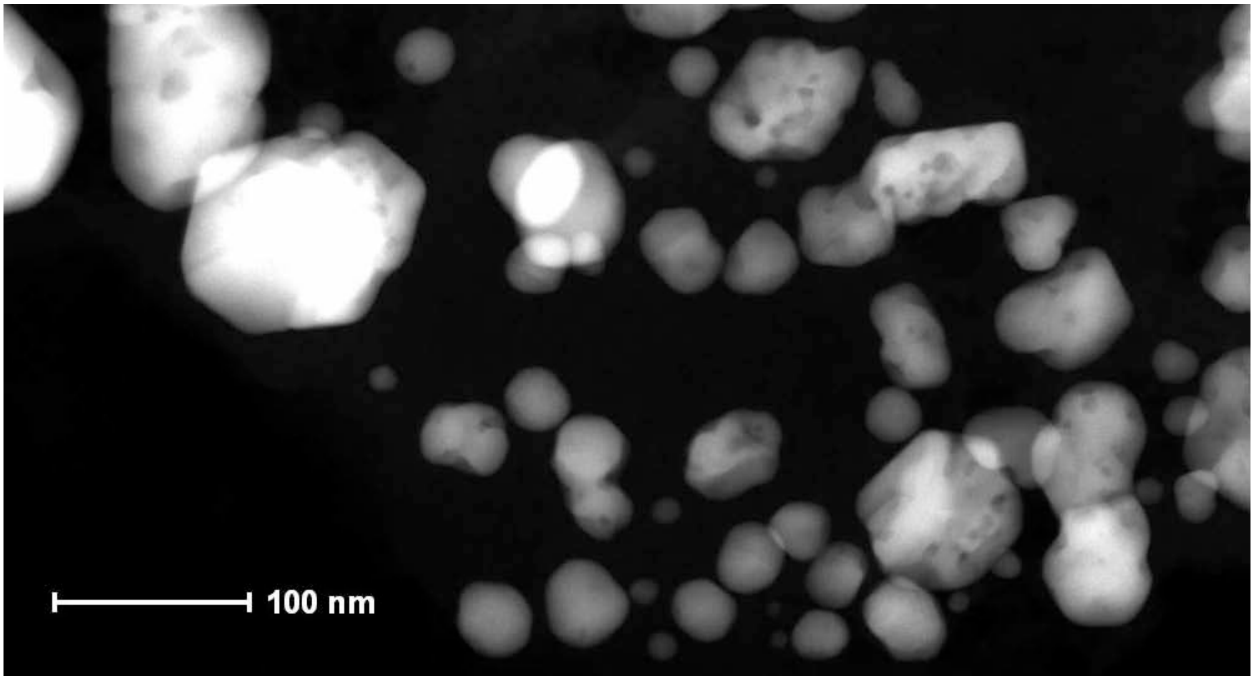

Figure 16. High angle annular dark field (HAADF) micrograph of ashed sample of the non-nano control fabric ([redacted] 100% cotton t-shirt) treated with a standard nanosilver suspension.

Microscopy Analysis of Fabrics and Plastics

Figure 17 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 E). 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 the EDS, which has an effective excitation spot size of approximately 1 µm × 1 µm.

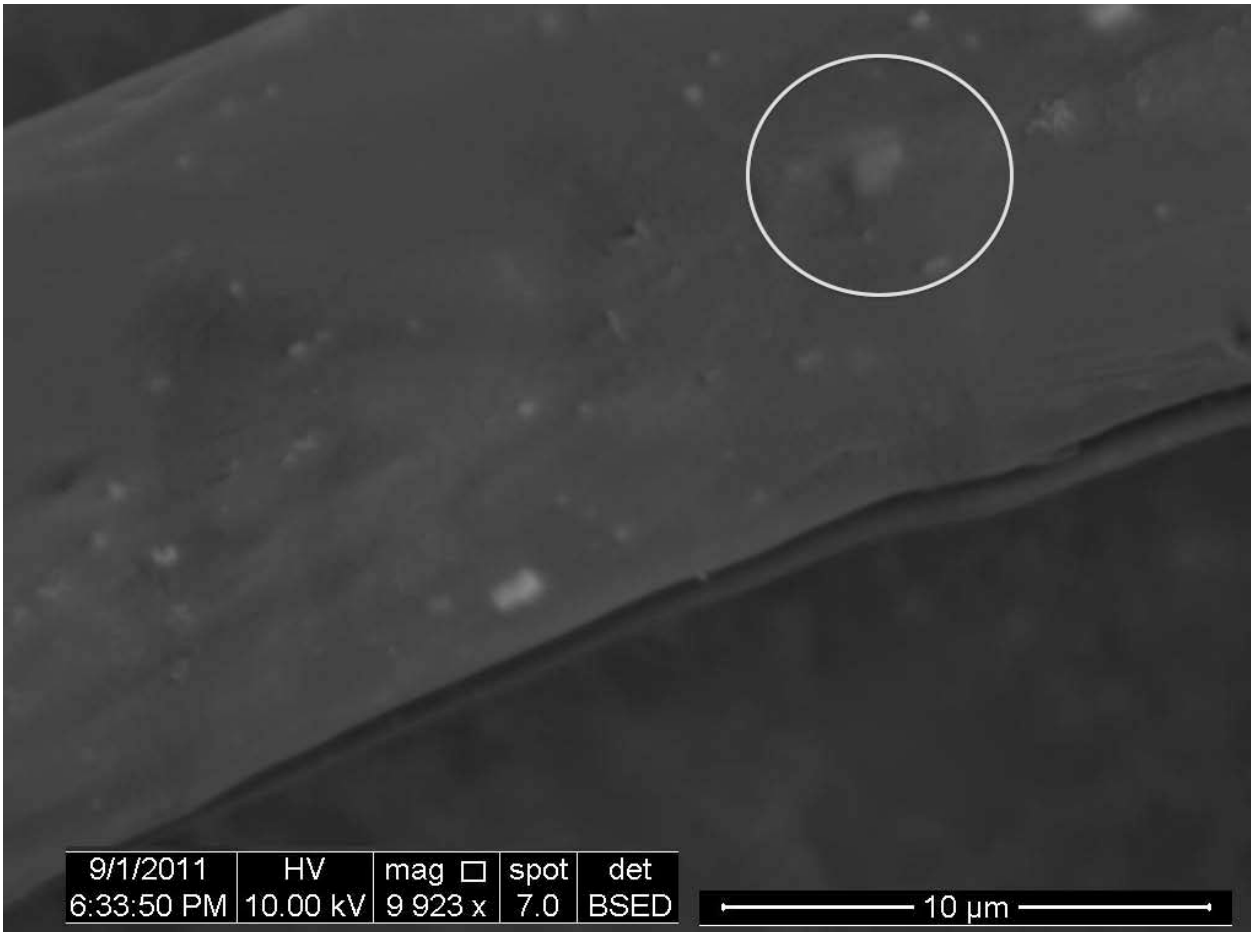

Figure 17. SEM micrograph of the baby blanket (obtained in backscatter detection mode).

Figure 18 shows a HR-TEM micrograph of an ashed sample from the baby blanket. The image was acquired in the high angle annular dark field (HAADF) mode in which the particle brightness increased roughly as Z^2, where Z is the particle's atomic number. 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 19 shows a detailed micrograph from the same sample. 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 E. We hypothesize that the differences in sintering between the control fabric and the baby blanket may be due to how the nanosilver is incorporated into the various fabrics. However, further research is needed.

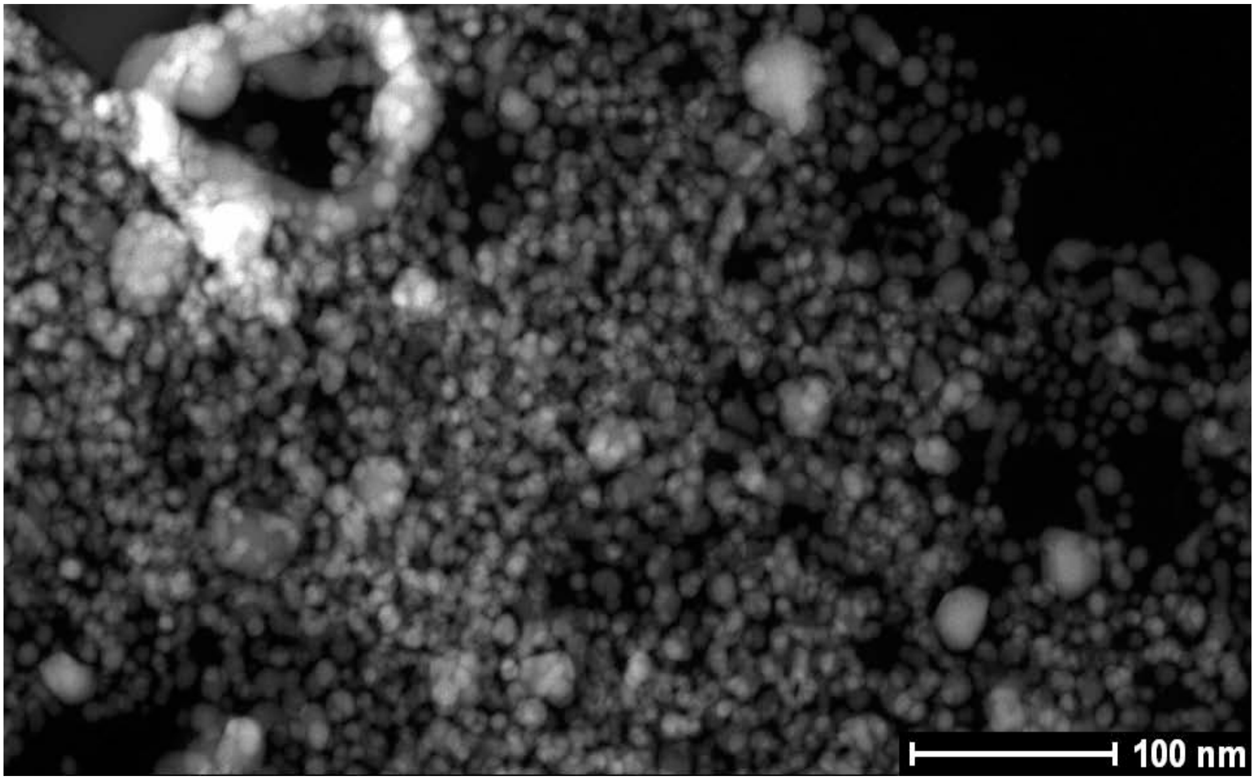

Figure 18. HAADF micrograph of ashed sample from the baby blanket.

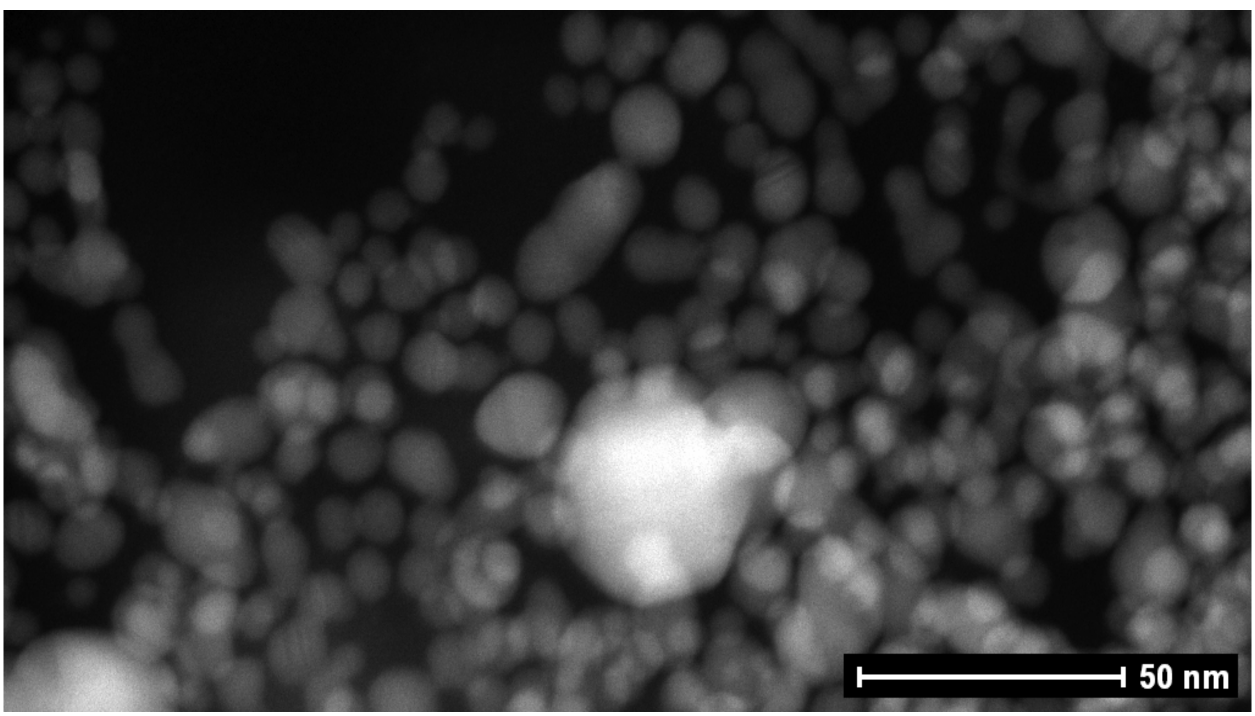

Figure 19. HAADF micrograph of ashed sample from the baby blanket (detail showing one silver nanoparticle).

Microscopy Analysis of [redacted] Samples

ICP-MS analysis of [redacted] exterior fur showed 0.6 $\pm$ 0.1 mg Ag/kg product (Table 7). In addition, we were able to locate a few silver-containing particles in the fibers of [redacted] fur using SEM, indicated by the circles in Figure 20.

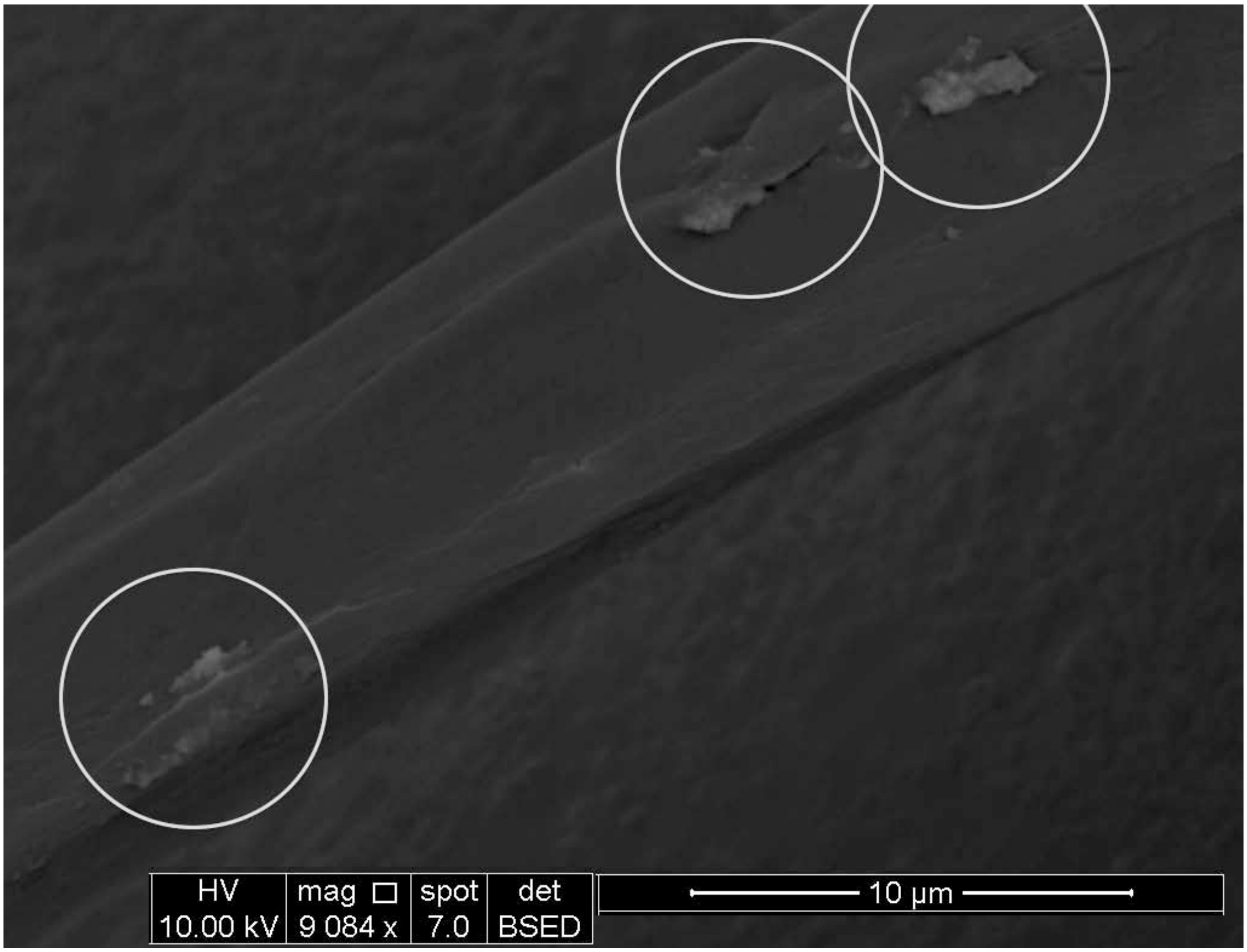

Figure 20. SEM micrograph of a fiber from [redacted] exterior fur (obtained in backscatter detection mode).

Using SEM, we were able to find a wide range of silver-containing particles, from >2000 nm aggregates to ~100 nm particles/aggregates in [redacted] interior foam. These appear as bright spots in Figure 21. EDS confirmed the composition of the silver particles.

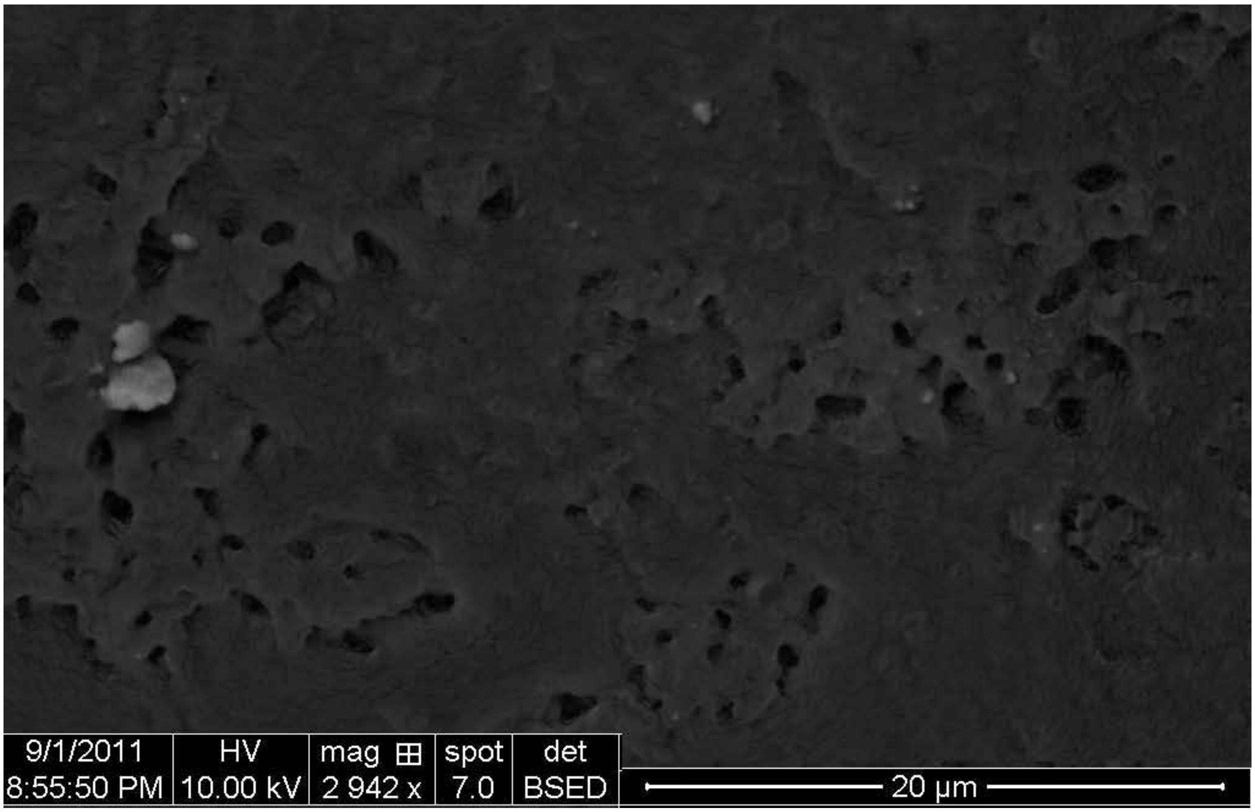

Figure 21. SEM micrograph of [redacted] interior foam (obtained in backscatter detection mode).

In ashed samples of [redacted] interior foam, silver nanoparticles were not as abundant as in the other ashed samples. Figure 22 shows a silver nanoparticle aggregate consisting of primary particles that are 15 – 21 nm in diameter.

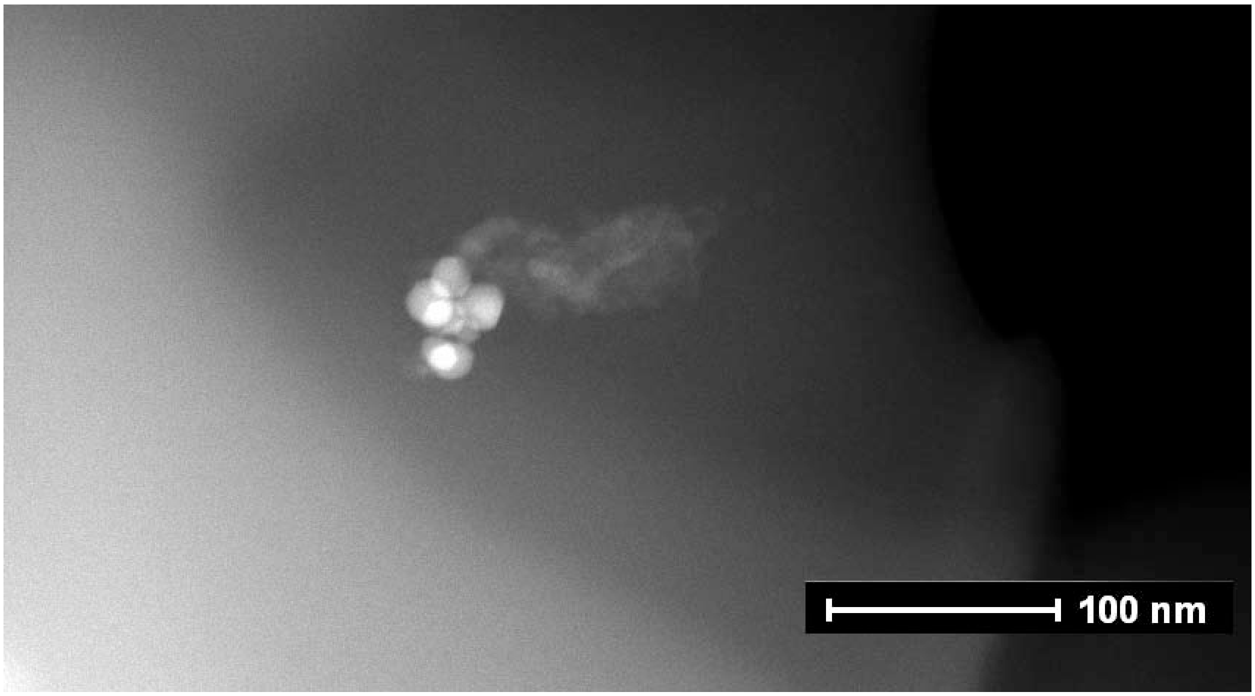

Figure 22. HAADF micrograph of ashed sample from the interior foam of [redacted].

Microscopy Analysis of Sippy Cups and Surface Wipe Samples

For the sippy cup samples, we were able to identify silver-containing particles using SEM/EDS on sippy cup #1 ([redacted] cup). Figure 23 shows one silver-containing particle (~2 µm in size) from the rubber ring from sippy cup #1 ([redacted] cup). Figure 24 is an SEM micrograph of the transparent stopper from sippy cup #1 ([redacted] cup), showing silver particles ranging in size from ~600 nm – 10,000 nm in diameter.

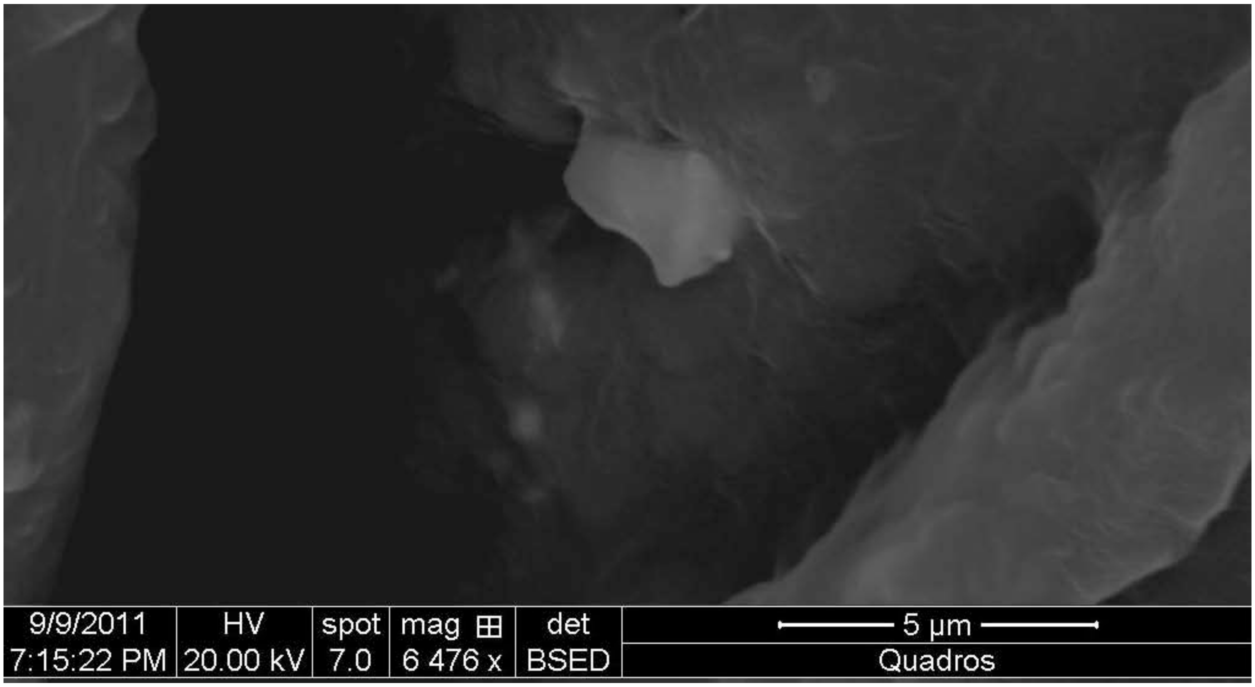

Figure 23. SEM micrograph of the rubber ring from sippy cup #1 ([redacted] cup) (obtained in backscatter detection mode).

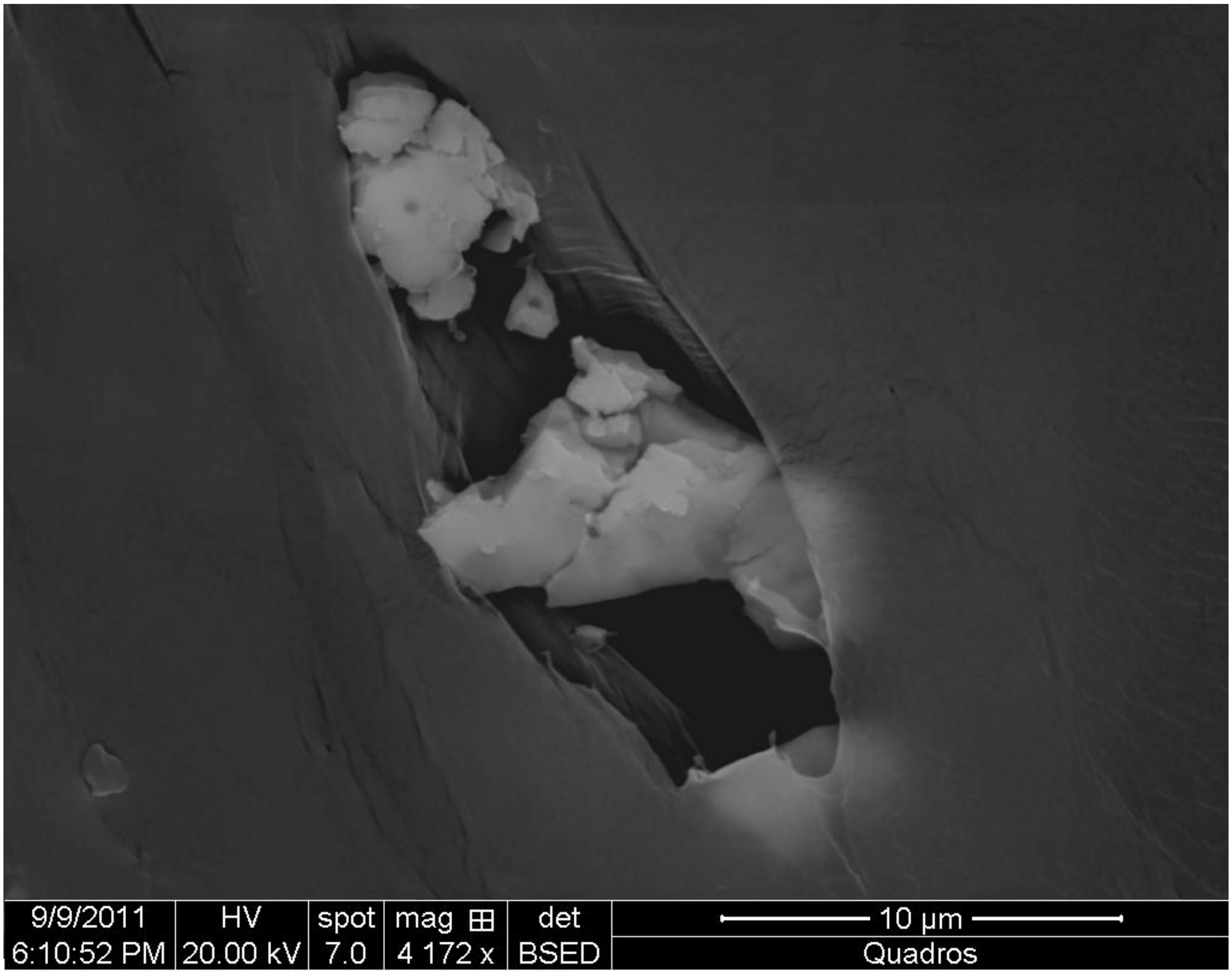

Figure 24. SEM micrograph of the transparent stopper from sippy cup #1([redacted] cup) (obtained in backscatter detection mode).

We were not able to observe silver-containing particles in the surface wipe or the transparent cap from sippy cup #2 [redacted] cup) using SEM or TEM.

Results of the microscopy analyses are summarized in Table 14. While silver particles were observed in many of the products, silver nanoparticles were positively identified only in [redacted] interior foam, baby blanket, and the [redacted] antifungal/antibacterial disinfecting spray.

Activity #4: Determine the Form of Silver (Soluble, Metallic) Released from the Product under Conditions of Intended Use (Main Study)

Products and/or product components containing silver were selected for leaching experiments using application-specific liquids, transfer experiments using wipe materials, or aerosol emissions experiments (Table 9). To determine whether silver was released from the products into different liquids, protocols described in published studies (Benn et al., 2010; Benn and Westerhoff, 2008) were adapted and used. Detailed methods for the leaching assays are presented in SOP # AirVT-Nanosilver-003: Silver Leaching Assays from Solid Materials into Various Liquid Media (Appendix D).

The leaching media included tap water from the city of Blacksburg, VA, synthetic saliva (Gal et al., 2001), synthetic sweat (Kulthong et al., 2010), and synthetic urine (Mayrovitz and Sims, 2001), orange juice ([redacted] orange juice from concentrate, no pulp, 0.5 gal), and milk formula ([redacted] Infant Formula, 360 g). Leaching media were prepared and stored in a refrigerator at 5 °C. A detailed description of each leaching media is listed in Appendix F.

An experiment comparing the leaching media used by VT and CPSC was also performed. Leaching media used in the past by CPSC have included: (1) a saline solution (as a surrogate for saliva) and (2) a solution of hydrochloric acid (HCl, 0.07 M) to simulate ingestion (ASTM, 2008). The baby blanket was used for the leaching experiments because there was a lot of blanket available and results from the chemical analysis for total silver reported consistent concentrations of silver that appeared to be homogeneously distributed.

To simulate product aging, one piece of the baby blanket and one leg of [redacted] were placed under a UV germicidal fluorescent lamp (GE G875, 8W, 254 nm) for approximately 2 weeks, then hung on an outdoor clothesline for 9 days (Figure 25). At the end of that time period, each product was rubbed against a concrete block for 1 min and subjected to the same leaching experiments as the new, un-aged products (tap water, synthetic saliva, synthetic sweat, and synthetic urine).

Figure 25. [redacted] leg and a piece of the baby blanket ([redacted] fabric) exposed to weathering (hanging on an outdoor clothesline).

For leaching experiments using the synthetic sweat and synthetic urine, three aliquots of product (0.5 g each) were placed into three beakers containing 25 mL of synthetic media and the beakers were soaked in a water bath at ~38 °C for 2 hr. As recommended by ASTM (2008), a factor of 50 was used for the ratio between leaching media volume and product mass. Samples were then acidified with 20% nitric acid and analyzed by ICP-MS.

For leaching experiments using synthetic saliva, small aliquots of product (0.16 ± 0.01 g) were placed into 2-mL bead-beating vials. Approximately 0.3 g of 1-mm glass beads were added along with 1.5 mL of synthetic saliva (at a temperature of 37 – 38 °C), and beat for 30 s in a bead beater at 2500 rpm. The amount of product and synthetic saliva added to each beaker resulted in the same product-mass-to-leaching-volume ratio as with the consumer products analyzed using synthetic sweat and urine. Samples were acidified with 20% nitric acid and analyzed by ICP-MS.

For the leaching assays using milk formula in the breastmilk storage bags, milk formula was prepared according to the manufacturer's instructions, heated to ~38 °C, and poured into three bags (100 mL per bag). For leaching assays using milk formula and sippy cup components, 0.5 g aliquots were shaved from the product components and placed into three beakers containing 25 mL of milk formula at a temperature of approximately 38 °C. The same process was used with the orange juice, except the temperature was approximately 5 °C. The beakers were covered with plastic film and placed in a refrigerator (5 °C) for 6 days (milk bags were placed lying on their sides).

To determine whether the silver present in the leachate was in ionic or particulate form, select samples that were shown to contain silver were filtered using a 3 KDa centrifugal filtering unit (Millipore) and analyzed again by ICP-MS.

Table 9. Activities performed on select consumer products or product components.

	Product	Activity
1	[redacted]: exterior fur[1] (new)	Leaching: tap water, saliva, sweat, urine Surface assay to estimate dermal exposure Aerosol emissions
2	[redacted]: exterior fur[1] (aged)	Leaching: tap water, saliva, sweat, urine
3	[redacted]: interior foam[1]	Leaching: tap water, saliva, sweat, urine
4	[redacted] Fabric[2] (new)	Leaching: tap water, saliva, sweat, urine Surface assay to estimate dermal exposure Aerosol emissions
5	[redacted] Fabric[2] (aged)	Leaching: tap water, saliva, sweat, urine
6	Breastmilk Storage Bags	Leaching: milk formula
7	Sippy Cup #1 ([redacted] cup) light blue: rubber ring	Leaching: milk formula, orange juice[3]
8	Sippy Cup #1 ([redacted] cup) light blue: transparent cap	Leaching: milk formula, orange juice
9	Sippy Cup #2 ([redacted] cup) (purple): cap	Leaching: milk formula, orange juice
10	[redacted] Antifungal/Antibacterial Disinfecting Spray	Surface assay to estimate dermal exposure Aerosol emissions
11	[redacted] Surface Wipes	Surface assay to estimate dermal exposure
12	[redacted] Scrubber	Surface assay to estimate dermal exposure
13	[redacted] Humidifier (non-nanosilver)	Leaching: tap water (blank control) Aerosol emissions (blank control)
14	[redacted] Tabletop Humidifier	Leaching: tap water
15	[redacted] Manual Ultrasonic Humidifier	Leaching: tap water
16	[redacted] (placed into a [redacted] humidifier)	Leaching: tap water Aerosol emissions

[1]Interior and exterior pieces of [redacted] were tested for leaching separately.
[2]Since all three [redacted] products had approximately the same silver concentration, the leaching, dermal, and aerosol experiments were performed on only one product.
[3]The sippy cup leaching experiments were restricted to two leaching media because of the small sample mass available for use.

Leaching from Humidifiers

The water reservoir from each humidifier was filled with tap water from the town of Blacksburg, VA (2.92 mg l^{-1} total chlorine, pH of 7.6) and allowed to sit at room temperature for 5 – 6 days. At the end of this time period, aliquots were collected, acidified with 10% nitric acid, and analyzed by ICP-MS. Additional aliquots were collected after 17 days of soaking.

In addition, the silver concentration in the vapor produced by each humidifier was measured. The humidifier reservoirs were filled with tap water from the town of Blacksburg, VA. The port of each humidifier was plugged with a PVC reducing pipe and tubing connected to a vacuum system. The vapor passed through a sealed beaker that was submerged in ice to promote condensation inside the beaker (Figure 26). These liquid samples were then acidified with 10% nitric acid and analyzed by ICP-MS.

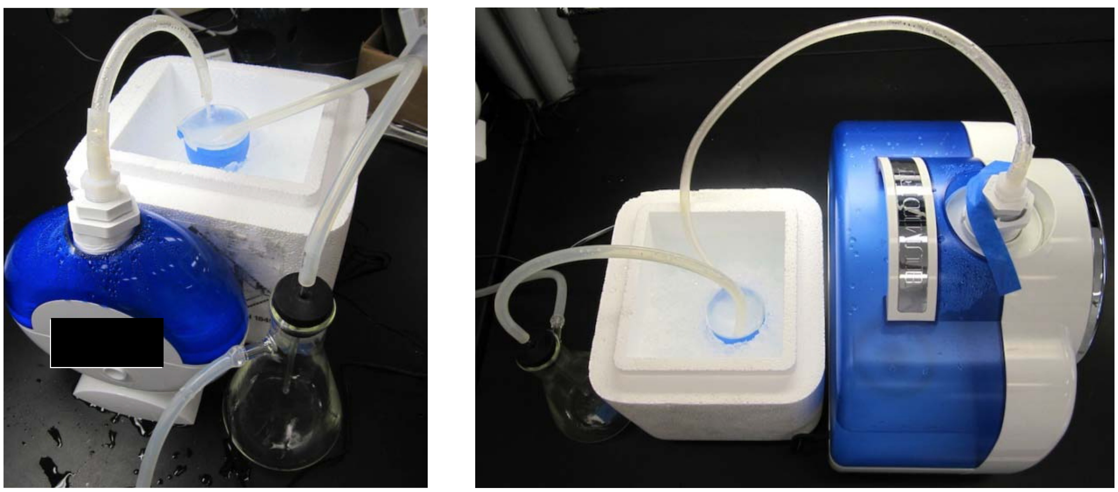

Figure 26. Setup for sampling the vapor from the humidifiers while in use (shown here with the [redacted] (left) and [redacted] (right)).

Aerosol Experiments

For products shown to contain silver and with the potential to produce aerosolized nanosilver (sprays, powders, and plush toys), aerosol emissions were characterized using methods developed at Virginia Tech (Quadros and Marr, 2010a, b), as described in SOP # AirVT-Nanosilver-004: Characterization of Aerosols Generated from Nanosilver Consumer Products (Appendix D).

Aerosol emissions testing was performed using a protocol designed to mimic real-world scenarios in which select products might be used. For these experiments, a test room with the following characteristics was used: floor area of 13.6 m^2; volume of 36 m^3; carpeted floors; one door; one window; 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 ^{o}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.

The following products were tested for aerosol emissions:

- An ultrasonic humidifier ([redacted]), which contained the [redacted] in the water reservoir. For comparison, experiments were repeated using an identical humidifier without the silver cube which served as a blank control (non-nano).
- The [redacted] antifungal/antibacterial disinfecting spray. In addition, the experiment was repeated using a bottle filled with ultrapure water and with the disinfecting spray's pump which served as a blank control.
- A nanosilver fabric ([redacted] sleepsuit). This experiment was repeated with a [redacted] 100% cotton t-shirt as a non-nano control.
- [redacted].

Experimental methods were tailored to mimic each product's normal, real-world, intended use. The humidifiers were placed in a corner of the test room and ran, individually, for 90 min, during which time approximately 300 mL of water from each reservoir was vaporized. The disinfecting spray was sprayed 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. The nanosilver fabric was handled in a repetitive fashion for 30 min: pick up, shake, fold, and set back down on a surface located 0.3 m horizontally from the sampling inlets. For [redacted], a procedure similar to the fabric was used: pick up, shake, and beat on surface.

The following parameters were monitored 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.

Surface Assays to Estimate Dermal Exposures

For products containing silver and meant to be used in close contact with skin (e.g., fabrics, cups, pacifiers), their potential to release silver upon contact with skin was evaluated using surface sampling wipes. Surface assays to estimate dermal exposures were performed on the following products: [redacted] (exterior fur); [redacted] baby blanket; [redacted] antifungal/antibacterial disinfecting spray; [redacted] surface wipes; [redacted] scrubber. We followed NIOSH Method 9102: Elements on Wipes (NIOSH, 2003) using benzalkonium chloride moist towelettes to estimate dermal exposure to the products.

For the disinfecting products ([redacted] antifungal/antibacterial disinfecting spray and Silver Shield surface wipes), in the laboratory we mimicked a real-life situation in which a child may touch a surface after it has been cleaned using one of these products. The disinfecting spray and wipes were applied to three individual 1 ft × 1 ft commercial vinyl floor tiles ([redacted], [redacted] then the tiles were wiped with the dermal wipes using horizontal and vertical strokes.

For other products ([redacted], baby blanket, [redacted] scrubber), areas on the product's surface were isolated and wiped. Three different areas on the baby blanket and scrubber were sampled. However, since the amount of [redacted] was limited, repeated wiping was conducted on one section as shown in Figure 27. Wipes were digested in nitric acid and hydrogen peroxide according to SOP # AirVT-Nanosilver-001 (Appendix D).

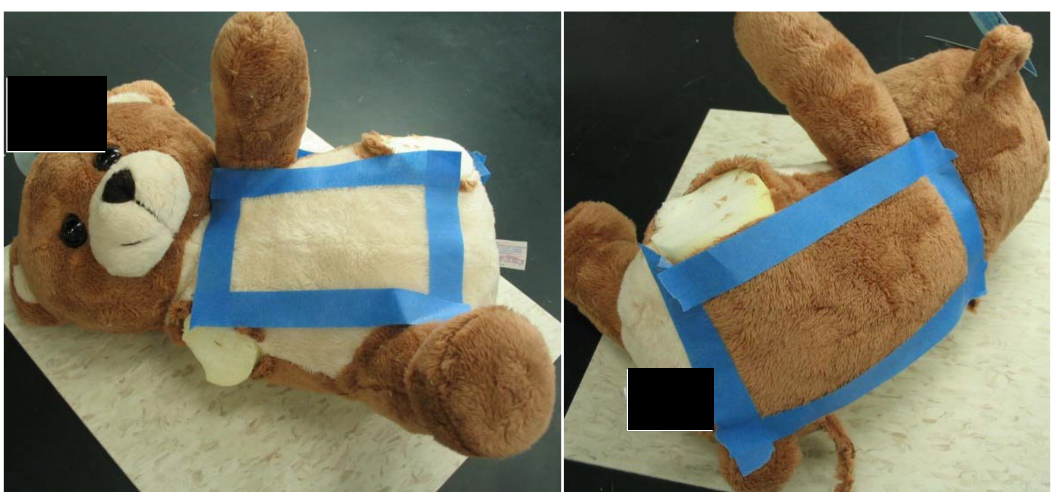

Figure 27. Areas selected for the surface assays to estimate dermal exposure from [redacted] are shown in the pictures (outlined in blue tape).

Leaching Experiments

Table 10 shows the amount of silver that leached from each product into application-specific liquids. Synthetic sweat and synthetic urine yielded the largest amount of silver (between 6% and 38%), while tap water yielded the lowest amount (0.5% to 2.6%). No relationship was observed between the liquid media's pH and the level of silver leaching, which indicates that ionic strength of the media may also play a role in silver leaching.

Even though the baby blanket had a higher silver concentration than [redacted], the blanket's silver-leaching yield was lower than the [redacted] (Figure 28). On average, the blanket leached 2.9% of its silver concentration, while [redacted] interior foam leached 9.5% of its silver. On the other hand, [redacted] exterior fur only leached 0.1% of its silver (on average). Silver leaching was generally low and variable for the components of the two different sippy cups, suggesting a heterogeneous distribution of silver throughout the different plastic components.

Table 10. Amount of silver leached into relevant liquid media, mass of silver per mass of product, and percent of silver leached from product (N=3).

Product	**Liquid Media**	**Amount of Silver Leached** mg Ag/kg Product (mean ± std error)	**%**
[redacted]: exterior fur	Tap water	ND[1]	-
	Saliva	0.03 ± 0.001	5.6
	Sweat	0.14 ± 0.002	24
	Urine	ND	-
[redacted]: interior foam	Tap water	0.24 ± 0.02	0.5
	Saliva	1.77 ± 0.03	3.7
	Sweat	18.5 ± 1.1	38.3
	Urine	17.4 ± 0.8	36.1
[redacted] Baby Blanket	Tap water	1.6 ± 0.3	1.5
	Saliva	1.2 ± 0.1	1.1
	Sweat	4.8 ± 0.3	4.8
	Urine	3.7 ± 0.3	3.4
Breastmilk Storage Bags	Milk formula	ND	-
Sippy Cup #1 ([redacted] cup): rubber ring	Milk formula	ND	-
	Orange juice	0.41 ± 0.01	1.7
Sippy Cup #1 ([redacted] cup): transparent cap	Milk formula	0.020 ± 0.001	0.2
	Orange juice	0.07 ± 0.01	0.7
Sippy Cup #2 ([redacted] cup): transparent cap	Milk formula	0.93 ± 0.02	23.5
	Orange juice	ND	-

[1]Not detected. Silver concentrations in the liquid sample were below 0.5 ppb.

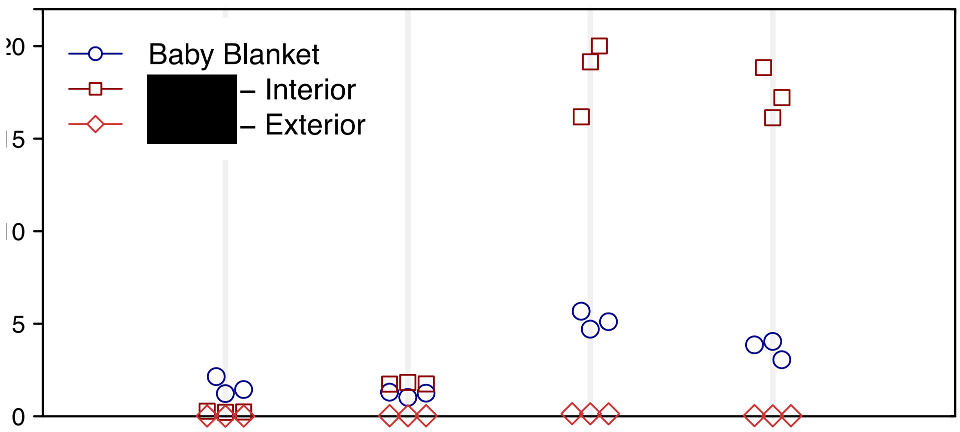

Figure 28. Amount of silver leached into different leaching media (all data points shown). The three data points for each replicate are slightly offset horizontally to improve legibility.

For the leachate from [redacted] and the baby blanket, a 3 KDa cutoff centrifugal filtering unit was used to separate ionic and particulate silver. Figure 29 shows the ionic silver fraction in different leaching media for both products. Since the ionic fraction is high for all products and leaching media, and considering that the centrifugal filtering units are known for absorbing some silver ions, we can infer that most, if not all, of the silver that leaches from these products is in dissolved ionic form.

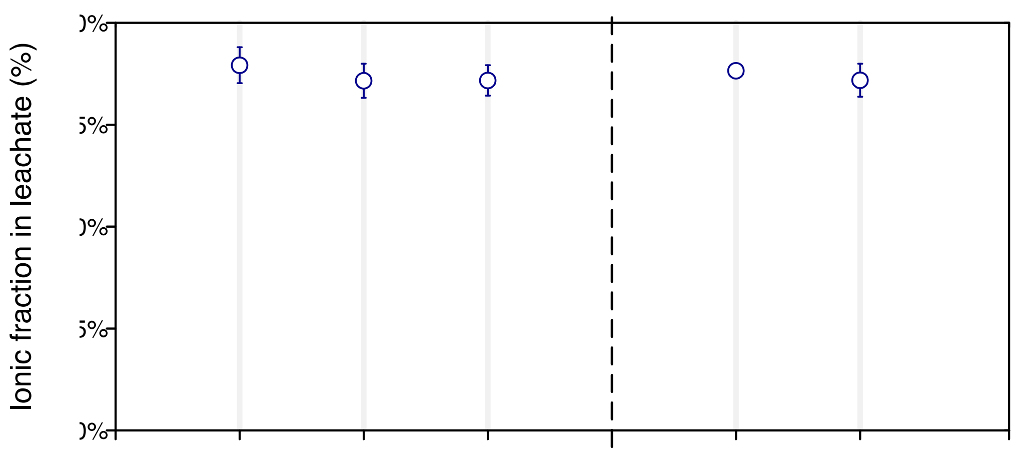

Figure 29. Ionic silver fraction in leachate for baby blanket and [redacted] grouped by leaching media (left) and product (right).

Figure 30 shows a comparison between the new products and those subjected to simulated aging in regards to silver leaching. While no general trend is apparent, the aged samples soaked in sweat leached more silver than did new samples from the same products.

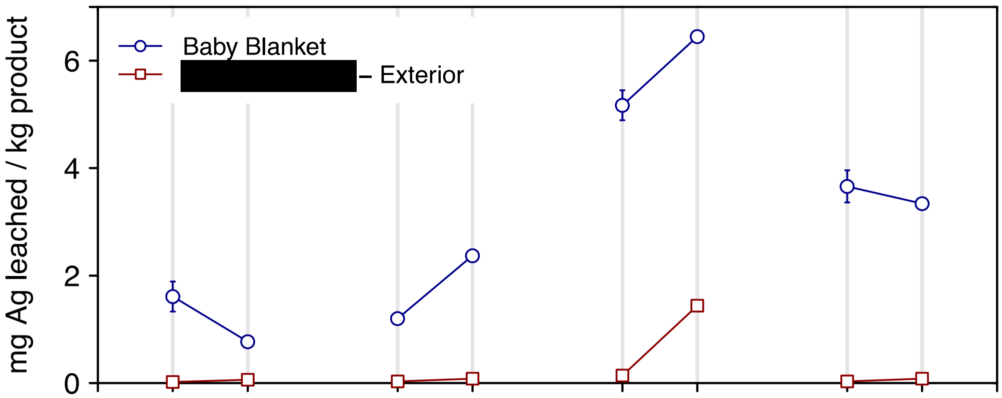

Figure 30. Comparison between new and aged products in regards to silver leaching.

To compare the protocols used by VT and CPSC, the baby blanket was also subjected to leaching in two media used by CPSC (HCl and saline). Figure 31 shows the amount of silver leached from the baby blanket into six different leaching media (tap water; synthetic sweat, urine, and saliva; HCl; saline). The amount of silver leached from the baby blanket was highest with the synthetic sweat, followed by the HCl and saline.

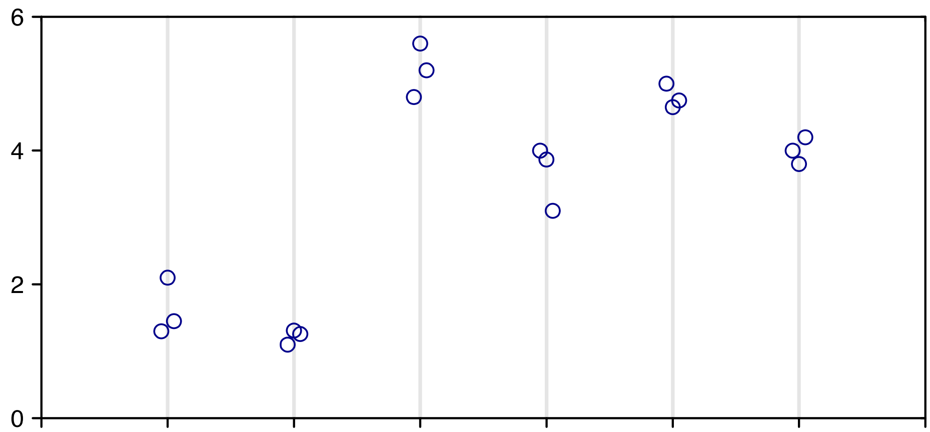

Figure 31. Comparison between the amount of silver leached from the baby blanket into the leaching media used for this study and CPSC (all data points shown).

Table 11 shows the results from the surface assays to estimate dermal exposures for the [redacted] antibacterial/antifungal disinfecting spray, fabric, and plastic products. For a surface assay to estimate dermal exposure from the [redacted] antifungal/antibacterial disinfecting spray, the amount of silver transferred per surface area was 9.03 ± 2.75 $\mu g/m^2$. Based on the concentration of silver in the product and the volume of product sprayed onto the surface, we estimated that ~6% of the silver that was applied to the surface transferred to the surface wipes. The baby blanket, which is the product with the highest silver concentration, displayed the highest amount of transfer to the wipe materials.

Table 11. Amount of silver transferred from surfaces onto surface wipes, in terms of silver mass per surface area.

Product	**Amount of Silver Transferred ($\mu g/m^2$)**
[redacted] Baby Blanket	23.0 ± 1.4
[redacted]: exterior fur	13.8 ± 8.4
[redacted] Antifungal/Antibacterial Disinfecting Spray	9.0 ± 2.8
[redacted] Surface Wipes	2.3 ± 0.2
[redacted] Scrubber	0.3 ± 0.1

We performed an aerosol experiment using the [redacted] antifungal/antibacterial disinfecting spray. It was sprayed onto a surface once per minute for 30 min. This period is represented by the yellow shaded area in Figure 32, 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 the disinfecting spray's pump as a control. Figure 33 shows the size distributions of aerosols in the room before and during spraying.

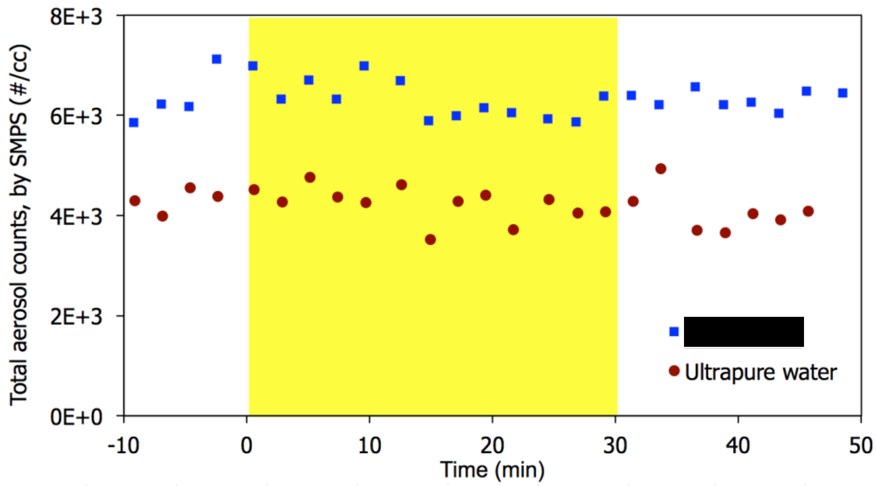

Figure 32. Time series of concentrations of aerosols between 14 – 750 nm, measured by a scanning mobility particle sizer (SMPS) before, during, and after spraying of the [redacted] antifungal/antibacterial disinfecting spray and control (ultrapure water). The yellow shaded area indicates the period when the product and control were sprayed.

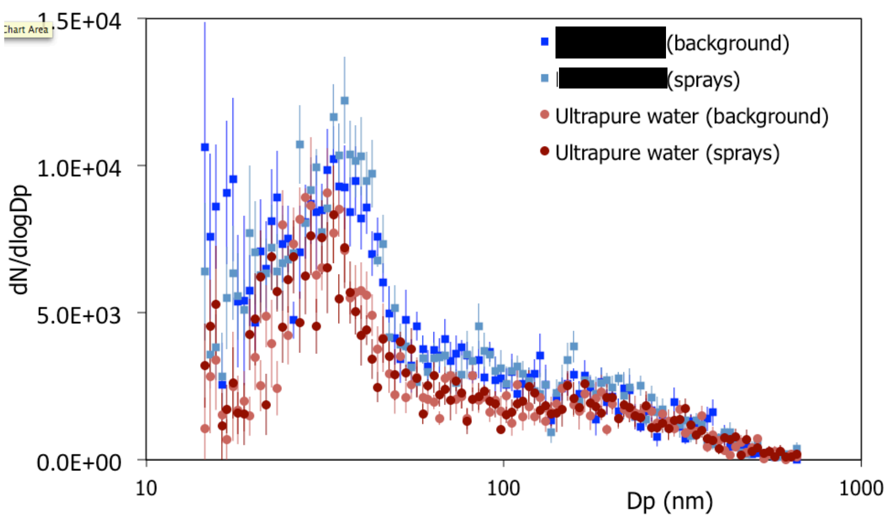

Figure 33. Size distributions of aerosols before and during spraying, as measured by a scanning mobility particle sizer (SMPS).

Figures 32 and 33 show that aerosol concentrations in the 14 – 750 nm range in the test room were not significantly different between the [redacted] antifungal/antibacterial disinfecting spray and control sample. Figure 34 shows the total aerosol concentrations, for particles 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 the [redacted] antifungal/antibacterial disinfecting spray, but these counts continued to increase even after spraying stopped, suggesting that the increase was caused by fluctuations in the background aerosol levels in the room.

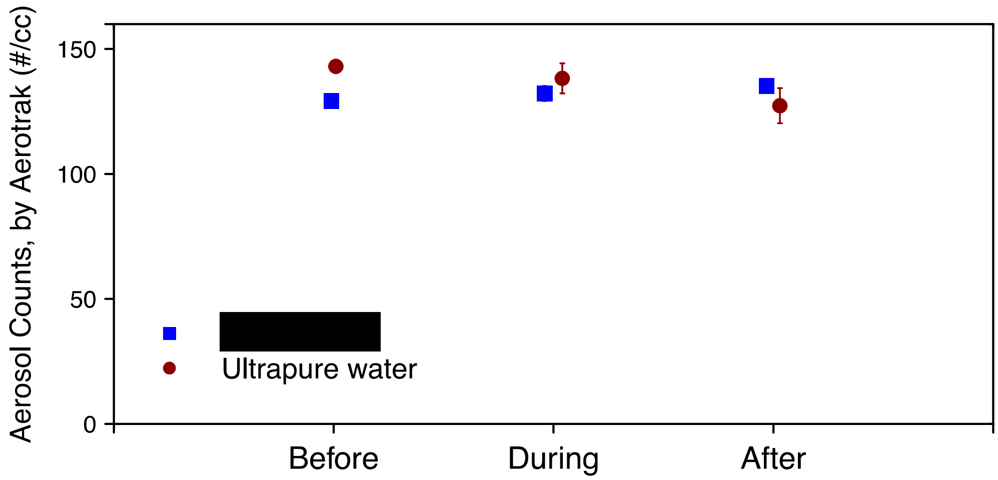

Figure 34. Total concentrations of aerosols between 300 nm and 10 µm in diameter, before, during, and after 30 min of spraying, as measured by the optical particle counter.

We also measured the potential for aerosol exposure associated with handling of the nanosilver fabric ([redacted] Sleepsuit), [redacted], and a [redacted] 100% cotton t-shirt (non-nano control). Figure 35 shows a time series of total concentrations of aerosols between 14 – 750 nm in diameter, where the yellow shaded area represents the 30 min period when materials were shaken.

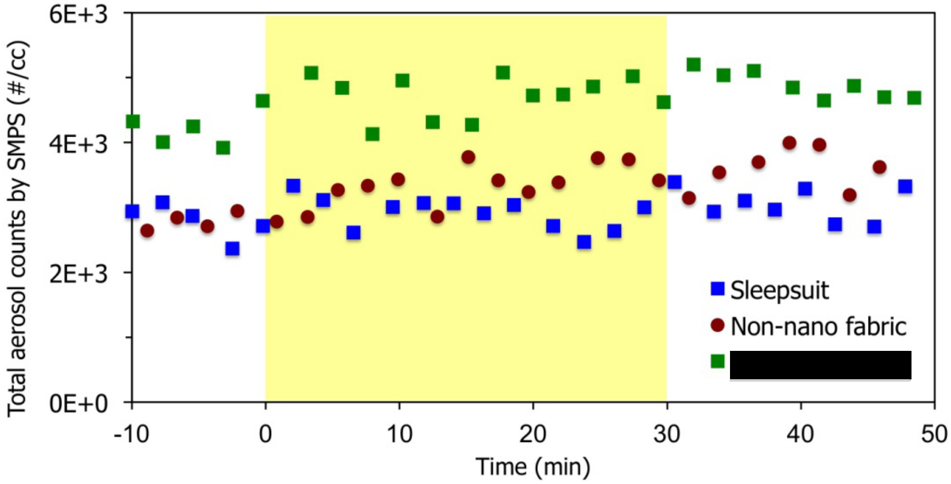

Figure 35. Time series of aerosol concentrations between 14 – 750 nm before, during, and after handling of plush toy and fabrics. The yellow shaded area indicates the period when the products were handled.

Figures 36 and 37 show the size distributions of aerosols in the room before and during the shaking procedure.

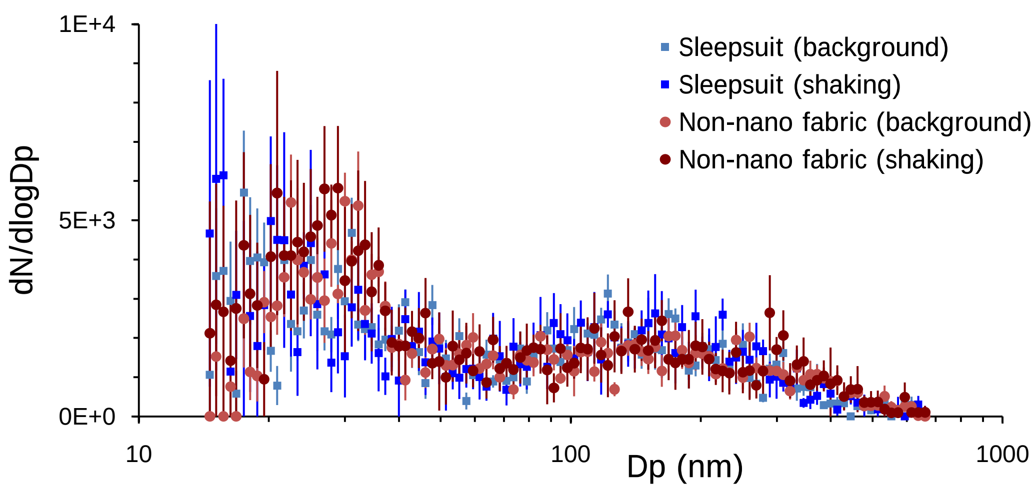

Figure 36. Size distributions of aerosols before (background) and during shaking for fabrics.

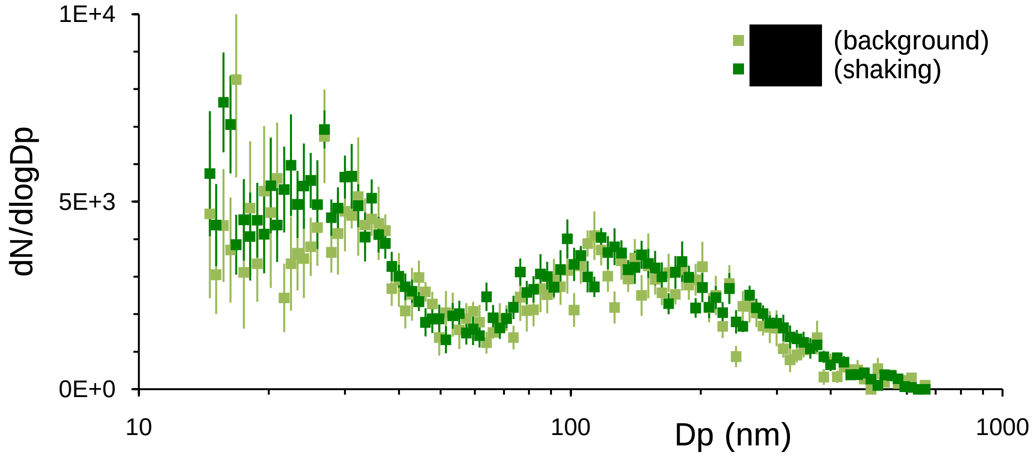

Figure 37. Size distributions of aerosols before (background) and during shaking for [redacted].

As with the spray product, handling of the nanosilver fabric ([redacted] Sleepsuit) and the plush toy ([redacted]) did not result in elevated aerosol concentrations in the 14 – 750 nm size range.

Figure 38 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 [redacted] and the nanosilver sleepsuit. Aerosol concentrations increased during shaking of the [redacted] 100% cotton t-shirt (non-nano control) and then decreased. Since this behavior was not observed during the shaking of the nanosilver material, we believe it may be attributed to the suspension of particles from the fabric or fluctuations in the background aerosol concentrations.

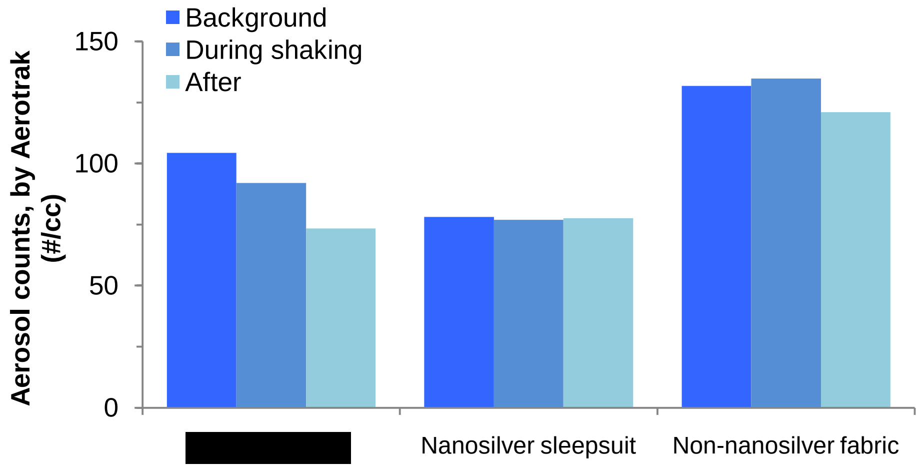

Figure 38. Total concentrations of aerosols between 300 nm and 10 μm in diameter, before, during, and after 30 min of handling.

The aerosol emissions 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 very low.

Humidifiers

Table 12 shows silver concentrations in water stored in the reservoirs of four different humidifiers. In all cases, silver concentrations were below the detection limit of the ICP-MS.

Table 12. Silver concentrations in humidifier reservoir water after different periods of soaking.

Product	Soaking Time (days)	Silver Concentration (ppb)
[redacted] Tabletop Humidifier	6	ND[1]
	18	ND
[redacted] Manual Ultrasonic Humidifier	6	ND
	18	ND
[redacted] (into a non-nano humidifier)	5	ND
	17	ND
Non-nano humidifier (blank)	5	ND

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

Table 13 shows silver concentrations in the vapor collected from the humidifiers' output. Only the [redacted] tabletop humidifier emitted silver-containing vapors.

Table 13. Silver concentrations in humidifier vapor during use.

Product	Silver Concentration (ppb)
[redacted] Tabletop Humidifier	2.3 ± 0.7
[redacted] Manual Ultrasonic Humidifier	ND[1]
[redacted] (into a non-nano humidifier)	ND
Non-nano humidifier (blank)	ND

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

The aerosol experiments were performed using two [redacted] humidifiers, one of which contained the [redacted] in the water reservoir. Figure 39 shows a time series of the concentration of aerosols between 14 – 750 nm in diameter. Background measurements began ~15 min before the humidifiers were turned on. The humidifiers ran for 90 min.

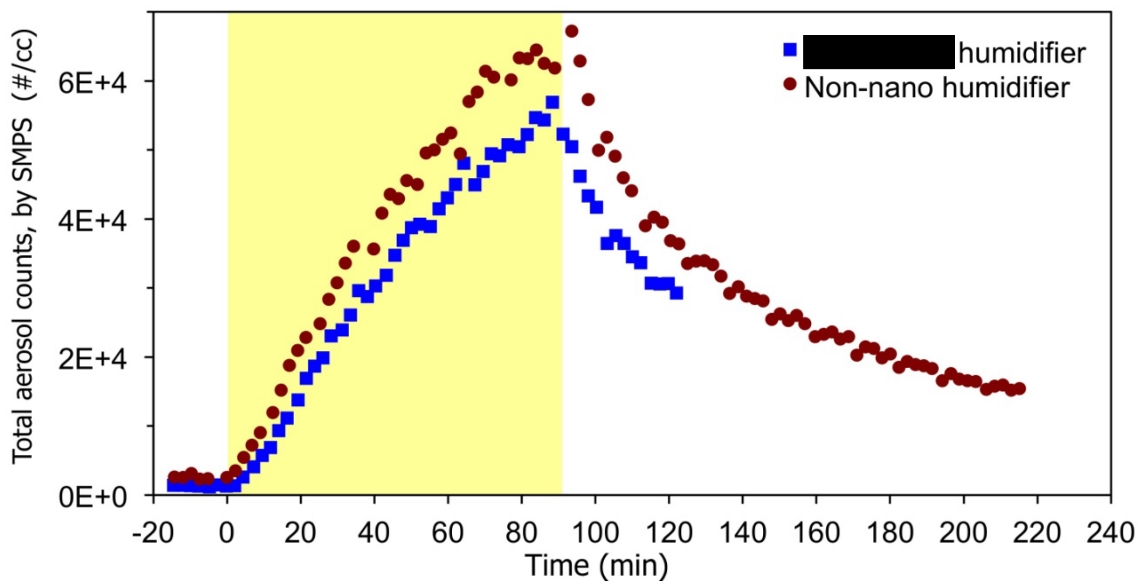

Figure 39. Time series of concentrations of aerosols between 14 – 750 nm before, during, and after humidifier operation. The yellow shaded area indicates the period when the humidifiers were running.

Ambient aerosol concentrations (14 – 750 nm) were higher during use of the non-nano humidifier than during use of the silver cube humidifier. 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 [redacted] and the one without it, respectively.

Figure 40 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 [redacted], as compared to the one without it.

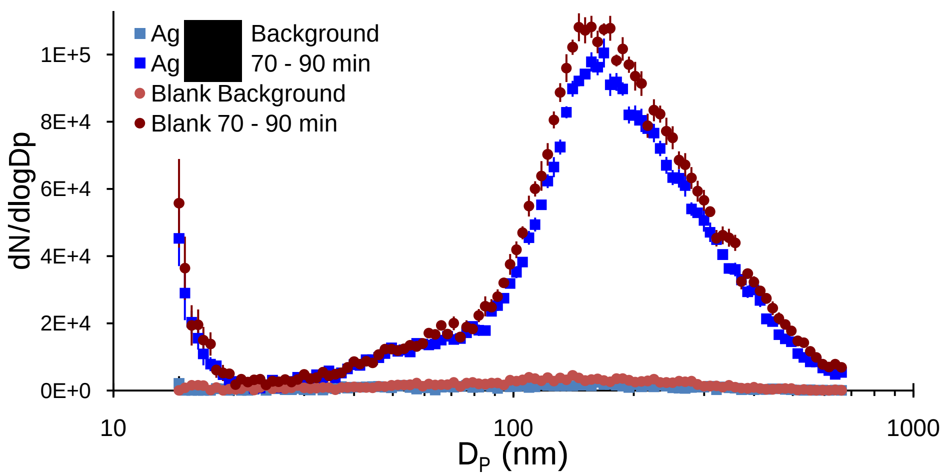

Figure 40. Aerosol size distributions during operation of the humidifiers. The blank humidifier is the one without the [redacted].

When compared to the humidifier without the [redacted], these results suggest that, under these experimental conditions, use of the [redacted] was not associated with greater potential for exposure to aerosols.

Bioavailability of Nanosilver

Bioavailability of nanomaterials in environmental studies is a concept that does not yet have standard testing methods (Ehlers and Luthy, 2003; Semple et al., 2004; SERDP and ESTCP, 2008). For this project, silver bioavailability was assessed by determining the following:

- Total concentration of silver in each product and whether the location of silver within the product allowed for contact during normal use (i.e., surface versus bulk).
- Average size or size distribution of silver nanoparticles (if silver is indeed nanosized).
- Whether silver leaches from the products into relevant liquid media and the type and extent of contact that liquid media has with the human body (e.g., ingestion, dermal contact).

Of the 21 samples evaluated, 12 contained silver at levels above the experimental detection limit. All products had at least one component containing silver. Particulate silver was identified in six samples from four products: (1) spray cleaning product, (2) interior foam and exterior fur of [redacted], (3) baby blanket, and (4) rubber ring and stopper from the [redacted] sippy cup (sippy cup #1). Table 14 summarizes all products evaluated for this project and whether biologically accessible nanosilver was found consistently above background levels. Silver particles ranged in size from <20 nm to 10 µm and appeared to be located on the surface of the fabric fibers and embedded in the components of the sippy cup.

Table 14. Summary of products evaluated, whether silver was detected, whether nanosilver particles were present, as determined by microscopy, and whether biologically accessible silver was present, as determined through leaching, transfer to wipe materials, and aerosol exposure experiments.

Product	Detection of Silver in Product	Nanosilver Observed	Presence of Biologically Accessible Silver
[redacted]: exterior fur	Yes (<1 ppm)	No[1]	Yes
[redacted]: interior foam	Yes	Yes	Yes
[redacted] Baby Blanket	Yes	Yes	Yes
[redacted] Sleepsuit or Babygro	Yes	Yes[2]	Yes[3]
[redacted] Baby Scratch Mitts	Yes	Yes[2]	Yes[3]
[redacted] Antifungal/Antibacterial Disinfecting Spray	Yes	Yes	–[4]
[redacted] Surface Wipes	Yes	No	Yes
Sippy Cup #1 ([redacted] cup): outside of cup	No	NA[5]	NA
Sippy Cup #1 ([redacted] cup): inside of cup	No	NA	NA
Sippy Cup #1 ([redacted] cup): rubber ring	Yes	No[1]	Yes
Sippy Cup #1 ([redacted] cup): white rim	Yes (<5 ppm)	NA	NA
Sippy Cup #1 ([redacted] cup): transparent cap	Yes (<10 ppm)	No[1]	Yes
Sippy Cup #2 ([redacted] cup): outside of cup	No	NA	NA
Sippy Cup #2 ([redacted] cup): inside of cup	No	NA	NA
Sippy Cup #2 ([redacted] cup): spout	No	NA	NA
Sippy Cup #2 ([redacted] cup): transparent cap	No	No	Yes[6]
[redacted]	Yes (<5 ppm)	NA	Yes
Breastmilk Storage Bags	Yes (<1 ppm)	NA	No
[redacted] Tabletop Humidifier	No	NA	No
[redacted] Manual Ultrasonic Humidifier	No	NA	No
[redacted]	No	NA	No

[1]Silver particles > 100 nm were identified.

[2]Product not analyzed by microscopy; nanosilver is expected to be present as observed in the baby blanket; products seemed to be made of the same material.

[3]Product not tested for leaching; leaching is expected to be the same as the baby blanket; products seemed to be made of the same material.

[4]Leaching scenarios not applicable.

[5]NA = not analyzed.

[6]Silver was not detected in piece extracted by acid, but was detected in leachate from a different piece.

Synthetic sweat and synthetic urine yielded the largest amount of silver (6% to 38%), while tap water yielded the lowest amount (0.5% to 2.6%). No relationship was observed between the liquid media's pH and the level of silver leaching. Most, if not all, of the silver leached into the synthetic saliva, sweat, or urine was in ionic form, suggesting that dissolution was the main leaching mechanism. Bioavailable silver is most likely in ionic form.

In some cases, product aging increased the amount of silver leached from a product, but there was no general trend between aging and leaching. Between 0.3 and 23 $\mu g\ m^{-2}$ of silver transferred from select consumer products or surfaces on which products were applied, to surface wipes, suggesting that dermal is a potential route of exposure. Based on these observations, sweat was deemed most likely to impact the potential route of exposure to silver from these consumer products.

Particle size distributions can be used to assess bioavailability of inhaled aerosols from consumer products, since deposition in the human respiratory tract is size dependent (Quadros and Marr, 2010; Oberdoster et al., 1995). In scenarios simulating real-world use of these products, 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 very low.

Based on all results from this project and the intended use for each product, product categories were ranked from most likely to least likely to be a potential source of bioavailable silver:

- Plush toy and fabric products (e.g., [redacted], clothing, blanket);
- Cleaning products (e.g., disinfecting spray and surface wipes);
- Sippy cups;
- Humidifiers;
- Breastmilk storage bag and kitchen scrubber.

The levels of silver to which children may be exposed during normal use of these consumer products is likely to be low, and bioavailable silver is expected to be in ionic rather than particulate form. Further research is needed to understand the health implications of bioavailable silver, potential health effects from exposure to nanosilver, and mechanisms of toxicity for children's health when using consumer products containing nanosilver.

Activity #5: Identify Pathways of Potential Exposure (Ingestion, Dermal, Inhalation Routes) from the Intended Use of these Products

Using the literature review and product inventory, a draft exposure framework was developed (Appendix B). The framework lists categories of products claiming to contain nanosilver and potential routes of exposure. Expert judgment suggests that ingestion and dermal are the most likely routes of exposure for children since the products marketed to children are primarily put in the mouth (e.g., bottles, toothbrushes, pacifiers) or worn (e.g., sleep suit, baby blanket, mitts) and laboratory analyses confirmed the presence of nanosilver in many of these products. In addition, children may also be dermally exposed to nanosilver through cleaning products used in the home environment (see Activities 3 and 4 for more information). As more information is learned about the potential for children's consumer products to contain nanosilver, the exposure framework should be updated.

Summary

Recent advances in technology have allowed the production and incorporation of nanosilver particles into a range of consumer products, including those intended for use by or near children. The objective of this research project was to develop tools, approaches, and protocols to categorize and prioritize children's potential exposures to select children's consumer products containing nanosilver and to evaluate selected products in pilot tests.

To meet this objective, a research approach was developed that involved (1) identifying and selecting consumer products claiming to contain nanosilver that may be used by or near children and (2) testing selected products using the SOPs that were developed. The first step involved selecting consumer products claiming to contain nanosilver that may be used by or near children and pose a potential exposure risk. The second step involved testing selected products to quantify the presence, release, and speciation of silver (i.e., nanoscale or ionic) in select items from three product classes: liquids, fabrics, and plastics.

From a market survey of products claiming to contain nanosilver that may be used by or near children, 80 products were identified. Of these, 13 were selected for further analyses. The experimental approach used to understand whether nanosilver may be present involved the following steps: measurement of total extractable silver; determination of the presence of nanosilver using electron microscopy; and measurement of leachable silver using liquids (synthetic urine, sweat, and saliva; tap water; orange juice; milk formula) relevant to each product's normal, real-world, intended use accompanied by analysis of the forms of silver in the leachate. This experimental approach required the development and evaluation of SOPs for chemical and microscopic analyses, leaching experiments, and characterization of aerosols.

All products had at least one component containing silver, except for one humidifier ([redacted] Manual Ultrasonic Humidifier) and the humidifier accessory [redacted]). Neither humidifier showed measurable release of silver into the water reservoir. However, silver was detected at low concentration in the water vapor from the [redacted] Tabletop Humidifier. Electron microscopy confirmed the presence of nanosilver (< 100 nm) in 3 products ([redacted], baby blanket, [redacted] antifungal/antibacterial disinfecting spray). The transfer of silver to the wipe materials (to estimate dermal exposure) was assessed and found that between 0.3 and 23 $\mu g\ m^{-2}$ of silver transferred from products (or surfaces on which products were applied) to the wipes. Leaching experiments showed measurable ionic silver from [redacted], fabric, and sippy cups under conditions simulating normal, real-world, intended use of these products. The amounts of silver released from [redacted] and fabric were highest with the synthetic sweat and urine compared to tap water or saliva, and components of the sippy cups were found to release silver in either milk formula or orange juice.

Based on these results and the normal, real-world, intended use for each consumer product evaluated, predictions were made on potential pathways of exposure. Of the products tested in this project, [redacted] and the fabric products are predicted to be the most likely sources of bioavailable silver. Overall, silver levels to which children may potentially be exposed during normal, real-world, intended use of the consumer products tested in this study are low.

The maximum amount of silver ion that may possibly be ingested (tap water, saliva, milk formula or juice) during a single encounter with the tested products (i.e., assuming a 100 g saturated aliquot from [redacted] or fabric or 1 g aliquot from a sippy cup exposed surface leached with 50 mL liquid) would range from 0.00093 mg (sippy cup) to 0.16 mg (fabric) under the test conditions used in this study. To place these values into context, the EPA recommends that the concentration of silver in drinking water not exceed 0.1 mg/L (ATSDR ToxFAQs). Although results of this study suggest that a single exposure to silver leached from a single product (e.g., fabric) may be low, as more nanosilver-enhanced products are introduced into the marketplace (e.g., products intended to reduce the adverse effects of microbes such as communicable disease, odor, and material degradation), aggregate and cumulative exposures to silver could become a concern.

Conclusions and Recommendations

This research project provides CPSC and other researchers with tools, approaches, and protocols for categorizing, prioritizing, and assessing children's potential exposures to nanosilver in children's consumer products. The information gained by applying these tools will support and advance CPSC's mission of regulating nanosilver in the consumer product marketplace. The systematic approach developed for nanosilver can potentially be adapted to investigate exposure potential to other nanomaterials in consumer products.

A wide range of variables are involved in the manufacture of nanoscale silver-enhanced commercial products, including the synthesis, stabilization, and immobilization of these materials into consumer products. In addition, this technology may be applied to a wide range of products (both known and to be manufactured) leading to a variety of potential exposure scenarios involving the release of ionic or nanosilver particles. Consequently, further research is needed to:

- Evaluate new products using the research approach identified in this report (measurement of total extractable silver, determination of the presence of nanoscale silver using electron microscopy, and measurement of leachable silver under simulated real-world conditions);
- Understand children's exposures, health implications of bioavailable silver, potential health effects from exposure to nanosilver, and mechanisms of toxicity for children's health when using consumer products containing nanosilver; and
- Understand the potential for cumulative exposures to multiple nanosilver-enhanced products, especially the exposure and health impacts for children.

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Appendix A: Literature Review

Children's Exposures to Silver Nanoparticles in Consumer Products:

Commercially Available Products and Routes of Exposure

Final Report

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1.0 Introduction

Nanoparticles (NPs), which are defined by having at least one dimension < 100 nm (Oberdorster et al., 2005), often possess different physical and chemical properties relative to their bulk cousins. Increased reactivity, strength, and conductivity in the nano-phase are the key features which are leading to a boom in NP production. Sometimes, these differences can be attributed to increased surface area. However, in many cases, even the surface-area normalized reactivity of the nanoparticle is greater than its bulk counterpart. In these instances, the increased activity may be due to surface defects or the fact that the particle size is approaching quantum regime (Hood, 2004).

Engineered NPs consist of many different materials. The most common commercial NPs include zinc, titanium, and cerium oxides, carbon nanotubes, quantum dots, and silver. A study by Lux Research estimates that, by 2014, $2.6 trillion of NPs will be incorporated into manufactured goods (Lux, 2006). The prominence of NPs is also illustrated by an online database supported by the Woodrow Wilson Nanotechnology Project (WWNTP). As current regulations do not require specific product labeling for NPs, the information contained in this database may be somewhat misleading. Therefore, the WWNTP database is not necessarily a definitive source. However, the WWNTP database does give a clear indication of the upcoming prominence of NPs in our everyday life.

The data available in the WWNTP database and other sources (Zweck et al., 2008) reveals that carbon nanotubes and nanosilver (nAg) have the widest range of commercial applications. The primary reason for the sudden emergence of nAg in the commercial market is its potency as an anti-fungal, anti-bacterial, anti-viral, and anti-microbial agent (Morones et al., 2005; Sun et al., 2005; Klaine et al., 2008; Weir et al., 2008; Yoon et al., 2008). In fact, one source claims that nAg is up to 45% more effective at killing bacteria than ionic silver (Ag^+) (US EPA). The commercial sector is certainly taking advantage of these unique properties. According to the WWNTP database, there are ~1,015 commercial products which contain NPs as of May 2010. Within this list, about 250 (25%) of these products contain nAg. According to the WWNTP and the International Center for Technology Assessment (ICTA) databases, potential uses of nAg include impregnation into fabrics and ceramics (Impellitteri et al., 2009; Brown and Sobsey, 2010; Duran et al., 2010), air freshening systems, water disinfectant systems (Li et al., 2008), food storage containers, catheters, wound dressings, vacuum cleaners, escalator rails, doorknobs, refrigerators, personal care products, bedding, several children's products, and a wide range of other applications (ICTA; WWNTP).

While the use of nAg surges ahead, research into the human health effects of nAg lags severely behind. Although the unique properties of nAg are desirable (or, at least, saleable) in many applications, it is unclear how increased exposure of nAg will affect human health. The primary focus of this report will begin the process of learning the effects of nAg on human health. Specifically, this report will target commercially available products containing nAg marketed for and used by children. Principle routes of exposure will be detailed for each product as well as a short description regarding the known toxicological effects of nAg. In Section 3, a list of nAg-containing products marketed towards children is presented. In addition, the likely routes of exposure are detailed for each individual product. Section 4 also details routes of exposure, but focuses on known toxicity data for each exposure pathway. Finally, Section 5 addresses the challenges ahead, while Section 6 provides a brief summary.

2.0 Approach

In this Report, a mixed search strategy was employed. Scientific literature was targeted for information regarding nAg toxicity, chemical transformations of nanoparticles, and nAg exposure routes. The primary tool used to identify children's products containing nAg was the WWNTP database. Other methods were also used, as described below.

2.1 Scientific Literature Search:

The literature search was accomplished using the Web of Knowledge (WoK). The WoK utilizes data from thousands of peer-reviewed journals, including the top environmental, health, and nanoscience journals. The search phrases and relevancy of the search phrases used in the WoK are shown below:

Terms used in Web of Knowledge Literature Search (# hits)

silver nanoparticles (> 10,000)
nanoparticles and children (3)
silver nanoparticle health effects (25)
silver nanoparticle health effects humans (0)
consumer silver nanoparticles (18)
nanoparticles in children toys (0)
silver in toys (7)
nanoparticles and pacifiers (0)
silver and breastmilk (0)
silver nanoparticles in consumer products (14)
silver nanoparticles children products (0)
silver nanoparticle health effects mammals (0)
silver and children (408)
silver in children toys (2)
nanoparticles in toys (1)
silver and pacifiers (0)
nanoparticles and breastmilk (0)

2.2 Consumer Product Search

The primary mechanism to identify nAg-containing products to which children may be exposed was the WWNTP database. This database, which was founded in 2005, details consumer products which contain NPs. As of May 2010, over 1000 items were listed. These lists include the product manufacturer and, if known, the type of nanoparticle used in the product. While this database is not necessarily comprehensive, it is a primary resource for identifying nanoparticle-containing products around the world. In this database, products relevant to this report were identified using two methods:

1) Browsing the Inventory focusing on "Goods for Children." (http://www.nanotechproject.org/inventories/consumer/browse/categories/goods_for_children/)

   Entries were individually examined and goods containing nAg were entered into Table 1.

2) Browsing the Inventory focusing on "Silver Nanoparticles." (http://www.nanotechproject.org/process/assets/files/7039/silver_database_fauss_sept2_final.pdf)

   Entries were individually examined and goods specific for children were entered into Table 1.

Another online database used in this Report is provided by the International Center for Technology Assessment (ICTA). Much of the information in this database is repeated from the WWNTP database. However, this database provides information on some unique products and is organized in a more readable format than the WWNPT database.

Sub-mechanisms used to identify nAg-containing products include commercial websites such as [redacted]. For example, at [redacted], the search phrase "nano silver" was entered and 50 consumer products were listed. Of these, 2-3 are used as children's products and are listed in Table 1.

Whenever possible, the information gathered from the above sources was independently verified. To verify the claims, the product manufacturer's website was examined. If the manufacturer claims that nAg is present, then the product is considered to contain nAg. When conflicting information was detected between the WWNTP database and the manufacturer's website, it is noted below Table 1. It is noted, however, that product labels are often misleading and that some of the manufacturer claims may be false. The only way to prove nAg is present is to directly test the product in a laboratory.

Again, it is stressed that only products specific to children are listed in this Report. Broad-spectrum products containing nAg (such as humidifiers, water treatment systems, air fresheners, and wound dressings) would likely expose children to nAg, but are not included here.

3.0 Current nAg Products Marketed to Children

Table 1

Product	Company(s)	Exposure Route	Country of Origin	Reference
Silver nano Toothbrush	[redacted]	Oral	Korea	(Trading) (Nanogist) (WWNTP) (Lucky) (WWNTP)
Silver nano Toothpaste	[redacted].	Oral	Korea	(Nanogist) (WWNTP)
Plush Toys* ([redacted] and [redacted])	[redacted]	Dermal / Oral	USA	(WWNTP)
Baby Wet Wipes	[redacted]	Dermal	Korea	(Nanogist)
Baby Bottle Brush	[redacted]	Oral	Korea	(SangShin)
Pacifier	[redacted]	Oral	Taiwan	(WWNTP)
[redacted] Baby Changing Mat	[redacted]	Dermal	Australia	(EMP)
Baby Mug	[redacted]	Oral / Dermal	Korea	(Babydream)
Teeth Developer	[redacted]	Oral	Korea	(Babydream)
Baby Milk Bottle	[redacted]	Oral / Dermal	Korea	(Babydream)
Stroller	[redacted]	Dermal / Oral	Korea	(Silverfox)

* On the Pure Plushy website, there is no evidence that nAg is currently being used on these toys. Presently, the manufacturer has modified production and the Plush Toys are made free of silver nanoparticles ([redacted]; US EPA).

4.0 Routes and Pathways of Exposure

Exposure to nAg can occur via at least three different routes: i) direct contact with the skin (i.e., dermal), ii) inhalation, and iii) via the gastrointestinal (GI) tract (i.e., ingestion). The exposure pathway will likely play a large role in the extent and mechanism of toxicity (if any). In this section, the most probable pathways of exposure are discussed for each product listed in Table 1. In addition, the known toxicological mechanism for each exposure route is presented.

4.1 Direct Skin Contact

Of the products listed in Table 1, the wet wipes, baby changing mat, and stroller are most likely to directly interact with skin. A few researchers have identified that nAg leaches out of textile products in aqueous conditions (as both nAg and Ag^+) (Benn and Westerhoff, 2008; Geranio et al., 2009; Impellitteri et al., 2009). In addition, one study found that sweat can lead to release of nAg from fabrics (Kulthong et al., 2010). Therefore, it is likely that some amount of nAg will leach out of these products as a result of sweating and other mechanical stressors and have direct contact with skin.

If the toxicity of nAg is to be understood, it is important to first understand the chemical speciation of the Ag when in contact with the skin. Laboratory studies have shown that under high chloride conditions (such as sweat from humans), nAg is oxidized and converted to silver chloride (AgCl) (Scheckel et al., 2010). This is significant because AgCl is far less toxic than both Ag^+ and nAg (Scheckel et al., 2010). Interactions between the highly active nAg colloids and the lower layers of the skin may have significant implications on the overall health effects of nAg (Chen and Schluesener, 2008).

Previous researchers have found that intact skin is pervious to nanoparticles such as quantum dots and fullerenes (Ryman-Rasmussen et al., 2006; Rouse et al., 2007). If one extrapolates from these studies, it is likely that unreacted nAg is also able to penetrate through intact skin. At present, there is one study which details nAg interactions with the skin (Samberg et al., 2010). In this study, a dose-dependent response was observed only for unwashed nAg. Carbon-coated nAg and washed nAg showed no harmful effects to the skin. In the case where the skin is wounded, nAg would have a direct line into the bloodstream. Here, it is probable that nAg build-up in the liver would result (Trop et al., 2006; Vlachou et al., 2007).

4.2 Inhalation

With the possible exception of the plush toys, the nAg in the products listed in Table 1 is unlikely to be aerosolized. Therefore, inhalation is not expected to be a major pathway of exposure to children and only a very brief description of the topic is presented here. It should be noted, however, that inhalation may be an important route of exposure to nAg for broad-spectrum applications such as air fresheners and humidifiers.

Because nAg has an inherently small diameter, there is a high probability that it will proceed to the alveolar lining in the lungs (Choi and Kim, 2007). Currently, it is not clear what speciation changes may take place there. The nAg will either 1) become oxidized by alveolar macrophages to silver ions or 2) be directly transported into the bloodstream. More research is needed to determine the fate of nAg in this media. However, for a more detailed discussion of metal transport and particle removal within the lungs specific to nAg, the reader is referred to outside references (Takenaka et al., 2001; Ji et al., 2007; Sung et al., 2008, 2009; Wijnhoven et al., 2009) and the references therein.

4.3 GI Tract

Table 1 reveals that most of the products marketed towards children are meant to be used orally (toothbrushes, toothpaste, milk bottles, pacifiers, and baby teethers). In addition, infants have a strong tendency to place toys and blankets in their mouths. Continued mouthing of toys or textiles may cause nAg to dislodge and become mobile. Therefore, the plush toys, some of which have silver interspersed into the inside foam, may also be an important route of exposure if the toy is broken apart.

Once nAg enters the GI tract, there are many potential interactions likely to occur before it is released. Chang et al. reports the presence of blue-gray pigmentation on the skin (argyria) after ingestion of so-called "immunity boosters" containing nAg (Chang et al., 2006). This finding implies that the particles themselves may be absorbed through the GI tract.

Another interaction will be with the hydrochloric acid in the stomach. This acidic and chloride-rich environment provides ample opportunity for nAg speciation changes. Liu and Hurt observed that, under acidic conditions, (pH = 4), the dissolution rate of nAg (to Ag^+) happens quite fast (assuming that stomach acid is oxygenated) (Liu and Hurt, 2010). As mentioned in Section 4.1, Scheckel (2010) found that in aqueous solutions rich in chloride, the nAg will generate a coating of silver chloride (AgCl). These observations imply that the relatively inert AgCl will be produced from the nAg not absorbed by the body before contact with stomach acid. However, because laboratory experiments directly involving acidic and high-chloride conditions have not been performed, the nature of how nAg will act in a high-chloride and acidic environment (such as stomach acid) remains unresolved.

When taking into account the types of products available and the habits of most infants, it is clear that the most likely route of exposure for children is through ingestion. Presently, there is little-to-no research which has attempted to elucidate the mechanisms and speciation changes once nAg enters the body in this manner. Given the high probability of nAg leaching into a child's mouth, one of the most active avenues of research should be to observe the behavior of nAg in situations that closely resemble the conditions of the mammalian GI tract.

4.4 A Note Regarding nAg Toxicity

The exposure of nAg by the above routes is only as important as the relative toxicity of nAg. In one review article, the authors state that understanding the effect of nAg toxicity "is in its beginning stages (Park et al., 2010)." Even in these early stages of research, however, preliminary data from several investigators show clear toxic effects of nAg to aquatic life (Naddy et al., 2007; Asharani et al., 2008), plants (Kumari et al., 2009), and other organisms (Navarro et al., 2008; Fabrega et al., 2009). In fact, nAg has been shown to be far more toxic to aquatic organisms than Ag^+ ions (Griffitt et al., 2008; Chae et al., 2009). Data is sparser for mammalian cells, although some does exist. For example, cytotoxic effects have been observed in mammalian stem cells (Braydich-Stolle et al., 2005). Also, nAg may oxidize within the body and cause damage to the liver and the brain (Hussain et al., 2005, 2006). If the nAg becomes blood-borne, then it may accumulate in the liver, kidney, and spleen (Takenaka et al., 2001; Panyala et al., 2008).

The toxicity of any nanomaterial will be influenced by several factors like oxidation state, surface charge, solubility, morphology, chemical speciation, and binding specificity to a biological site. Depending on the application, nAg may be synthesized via dozens of routes with various capping agents and coatings. Thus, as reviewed in Stone et al. (2010), there are thousands of possible combinations for the synthesis of nAg and the ability to predict toxicity of one product based on data from another product becomes extremely challenging (Stone et al. 2010). If the toxicity of nAg is to be better understood, then experiments which differ in capping agents and nAg synthesis routes must be performed. In addition, consistency among experiments is mandatory if inter-study results are to be interpreted with success and meaning.

5.0 Limitations and Challenges

The toxicity of nAg broadly depends on two parameters: 1) the primary route(s) of exposure (inhalation, ingestion, or dermal) and 2) the mechanism of toxicity for each route of exposure. As presented in Table 1, it is relatively easy to predict the routes of exposure. Those assigned in Table 1 are based on two criteria: 1) what is the designed use of the product and 2) would child behavior (such as hugging or mouthing) alter or cause any additional exposure routes. The greatest challenge in determining the absolute toxicity of nAg is the current lack of understanding of how nAg behaves in the human body. For example, even though ingestion appears to be the common route of exposure for children, it may be insignificant if the toxicity resulting from ingestion is very low. Therefore, in order to adequately prioritize the exposure routes, data regarding the toxicity of each exposure route must be generated.

While the WWNTP database is an invaluable database for determining which consumer products contain NPs, it is not a definitive source. Since there are currently no regulations regarding the labeling on a particular product, it is unclear which products actually contain nAg. This confusion could result in both false positives and false negatives. First, products may claim to use nAg as a selling point, but not actually contain nAg. Conversely, there is evidence that some products which do not mention nAg on the labels actually do contain nAg.

6.0 Summary

In this report, commercially available products which are thought to contain nAg and are marketed towards children are detailed. Given the nature of the products, ingestion exposure appears to the primary pathway of exposure for children, followed by dermal exposure, and then inhalation.

The literature regarding the toxicity of nAg on various ecosystems and environments is extensive. However, there is only a small amount of known data regarding the toxicity of nAg on humans and other mammals. This small amount of data does show, however, clear indications that human exposure to nAg results in some degree of toxicity. However, the toxicological pathways are not yet developed. Before moving ahead with any regulations, it is imperative that data are developed regarding the potential adverse health effects of nAg on humans.

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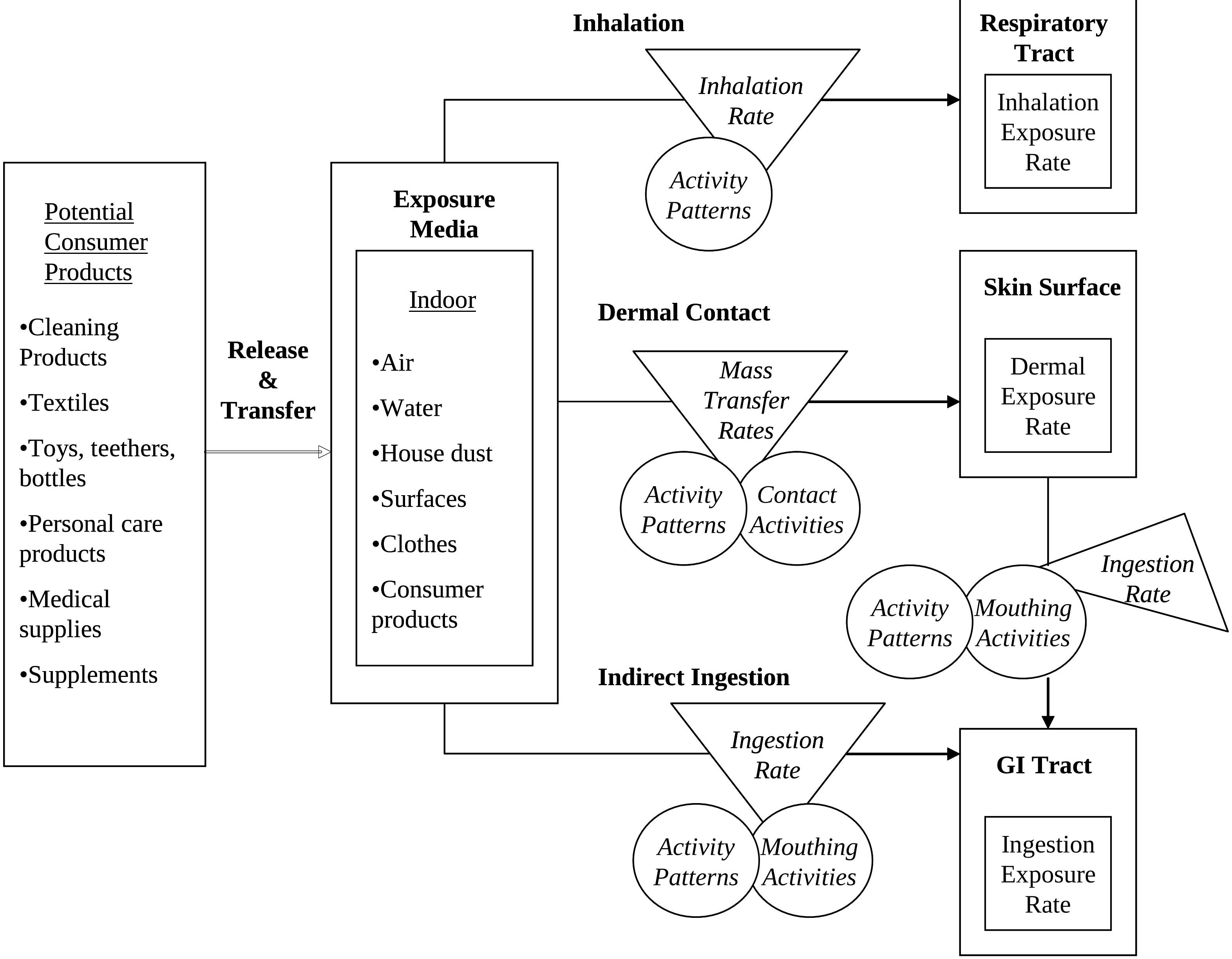
Appendix B: Exposure Framework

Children's residential exposures to nanosilver - proposed



(Modified from Cohen Hubal *et al*., 2000)

Appendix C: Product Inventory

PRODUCT INVENTORY

Exposure Assessment of Silver Nanoparticles in Select Children's Consumer Products

(RFQ-RT-10-00249)

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5 April 2011

1. METHODS

Our objective is 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 consumer products inventory were added to our inventory when described as being safe for consumption by children and currently available for purchase by end-consumers in the US market [1,2]. We have 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 will also visit large department and international stores in the local area. Our search comprised mainly the stores listed in Table 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 have also searched for products with descriptions that are suggestive of the use of nanosilver, even if it is not explicitly advertised (e.g., Table 3, item 5: nano toothbrush). Products previously purchased by EPA researchers have also been added to this inventory.

Table 1. Example listing of nanosilver products considered for testing.

Name of store	Location (link if online store, city if physical)
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	Blacksburg, VA
[redacted]	Blacksburg, VA
[redacted]	Blacksburg, VA
[redacted]	Blacksburg, VA
[redacted]	Christiansburg, VA
[redacted]	Christiansburg, VA
[redacted]	Christiansburg, VA
[redacted]	Christiansburg, VA
[redacted]t	Christiansburg, VA

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

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

- Milk bottles and sippy cups
- Plates, bowls, food containers, and utensils
- Pacifiers
- Toothbrushes
- Throat or nasal sprays
- Dietary supplements / homeopathic supplements

We have 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 2 presents a summary of products listed in the inventory, the details of which are shown in Table 3.

Table 2. Summary of inventory of nanosilver children's products.

Product Type	Number of Products	Item Numbers in Table 3
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

In consultation with EPA, we will select 10-20 products from this inventory to be tested, including up to four items of each type from different manufacturers. We will purchase products available and legally permitted to be distributed in the US. 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 added to illustrate examples of products that might enter the US market through distributors (e.g., Table 3, item 3: pacifiers and Table 3, item 12: cloth diapers).

Table 3. Nanosilver products to be considered for testing.

(1)	**Product Name:** [redacted] Antimicrobial Toy	**Product Type (likely exposure route):** Toy (ingestion, dermal)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
Advertised form of silver: “Patented animal toy blends memory foam with silver nanotechnology”		
Comments: $27.99. Product sold out, according to [redacted] We have one bear that was purchased by EPA. “[redacted]” was also advertised to contain silver nanotechnology.		

(2)	**Product Name:** [redacted]	**Product Type (likely exposure route):** Toy (dermal)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
Advertised form of silver: “silver nano”		
Comments: $7.99 for a 6-pc set.		

(3)	**Product Name:** [redacted]	**Product Type (likely exposure route):** Pacifier (ingestion)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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:** [redacted]	**Product Type (likely exposure route):** Toothbrush (ingestion)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
Advertised form of silver: "Nano-silver bristles."; "(…) proprietary nano-silver technology (…)"		
Comments: $4.99 each, available in pink and yellow.		

(5)	**Product Name:** [redacted]	**Product Type (likely exposure route):** Toothbrush (ingestion)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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:** [redacted]	**Product Type (likely exposure route):** Toothbrush (ingestion)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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:** [redacted]	**Product Type (likely exposure route):** Toothbrush (ingestion)
Manufacturer (Country): [redacted]		**Retailer:** Unknown
Retailer website: Not Applicable		
Advertised form of silver: "silver particle(s)"		
Comments: Product purchased in Seoul by EPA staff.		

(8)	**Product Name:** [redacted]	**Product Type (likely exposure route):** Toothbrush (ingestion)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer [redacted] [redacted]		
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: [redacted]	Product Type (likely exposure route): Toothpaste (ingestion)
Manufacturer (Country): [redacted]		Retailer: [redacted]
Retailer website: [redacted]		
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: [redacted]	Product Type (likely exposure route): Toothpaste (ingestion)
Manufacturer (Country): [redacted]		Retailer: [redacted]
Retailer website: [redacted]		
Advertised form of silver: "Nano Silver"		
Comments: $4.85 for a 120-g tube. Directions for children were not mentioned.		

(12)	Product Name: [redacted]	Product Type (likely exposure route): Cloth diapers (dermal)
Manufacturer (Country): [redacted]		Retailer: [redacted]
Retailer website: [redacted]		
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:** [redacted]	**Product Type (likely exposure route):** Fabric (dermal)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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 [redacted] online inventory.		

(14)	**Product Name:** [redacted]	**Product Type (likely exposure route):** Clothing (dermal)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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 [redacted] online inventory.		

(15)	**Product Name:** [redacted]	**Product Type (likely exposure route):** Blanket (dermal)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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 [redacted] online inventory.		

(16)	**Product Name:** [redacted]	**Product Type (likely exposure route):** Clothing (dermal)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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 [redacted] online inventory.		

(17)	**Product Name:** [redacted]	**Product Type (likely exposure route):** Clothing (dermal)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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 [redacted] online inventory.		

(18)	**Product Name:** [redacted]	**Product Type (likely exposure route):** Clothing (dermal)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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:** [redacted]	**Product Type (likely exposure route):** Textile (ingestion)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer [redacted]		
Advertised form of silver: "The [redacted] antimicrobial-protected lining helps reduce odor-causing bacteria"		
Comments: Price: $7.99.		

(20)	**Product Name:** [redacted]	**Product Type (likely exposure route):** Bath towel (dermal, ingestion)
Manufacturer (Country): [redacted]		**Retailer:** Unknown
Retailer website: [redacted]		
Advertised form of silver: Unknown		
Comments: Product purchased in Seoul by EPA staff.		

(21)	**Product Name:** [redacted]	**Product Type (likely exposure route):** Food storage (ingestion)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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:** [redacted]	**Product Type (likely exposure route):** Sippy cups (ingestion)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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:** [redacted]	**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:** [redacted]	**Product Type (likely exposure route):** Food storage (ingestion)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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:** [redacted]	**Product Type (likely exposure route):** Food storage (ingestion)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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:** [redacted]	**Product Type (likely exposure route):** Food storage (ingestion)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer[redacted]		
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:** [redacted]	**Product Type (likely exposure route):** Nail polish (ingestion, dermal)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
Advertised form of silver: “Nano Silver”		
Comments: $7.50 for a 0.5-oz bottle.		

(28)	**Product Name:** [redacted]	**Product Type (likely exposure route):** Skin care (dermal)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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:** [redacted]	**Product Type (likely exposure route):** Skin care (dermal)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
Advertised form of silver: "Colloidal Silver Topical Gel with Aloe, Copper, Zinc and Essential Oils"; "Provides you with 240 ppm of pure collodial 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:** [redacted]	**Product Type (likely exposure route):** Skin care (dermal)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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: [redacted]	Product Type (likely exposure route): Skin care (dermal)
Manufacturer (Country): [redacted]		Retailer: [redacted]
Retailer website: [redacted]		
Advertised form of silver: "24 ppm product [sic]"		
Comments: $14.59 for a 4-oz bottle.		

(32)	Product Name: [redacted]	Product Type (likely exposure route): Skin care (dermal)
Manufacturer (Country): [redacted]		Retailer: [redacted]
Retailer website: [redacted]		
Advertised form of silver: "Uses only the finest particle-size colloids to ensure maximum bioavalability and efficiency" "safe for children."		
Comments: $17.70 for a 3-oz tube.		

(33)	Product Name: [redacted]	Product Type (likely exposure route): Skin care (dermal)
Manufacturer (Country): [redacted]		Retailer: [redacted]
Retailer website: [redacted]		
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:** [redacted]	**Product Type (likely exposure route):** Skin care (dermal)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
Advertised form of silver: "Colloidal Silver"		
Comments: $25.40 for a 100-g tub.		

(35)	**Product Name:** [redacted]	**Product Type (likely exposure route):** Skin care (dermal)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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:** [redacted]	**Product Type (likely exposure route):** Skin care (dermal)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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: [redacted]	Product Type (likely exposure route): Skin care (dermal)
Manufacturer (Country): [redacted]		Retailer: [redacted]
Retailer website: [redacted]		
Advertised form of silver: "Colloidal Silver (250 ppm)";		
Comments: $ 9.59 for a 1-oz tub.		

(38)	Product Name: [redacted]	Product Type (likely exposure route): Shampoo (dermal, ingestion)
Manufacturer (Country): [redacted]		Retailer: [redacted]
Retailer website: [redacted]		
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: [redacted]	Product Type (likely exposure route): Shampoo (dermal, ingestion)
Manufacturer (Country): [redacted]		Retailer: [redacted]
Retailer website: [redacted]		
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:** [redacted]	**Product Type (likely exposure route):** Shampoo (dermal, ingestion)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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:** [redacted]	**Product Type (likely exposure route):** Conditioner (dermal, ingestion)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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:** [redacted]	**Product Type (likely exposure route):** Throat spray (ingestion and inhalation)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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:** [redacted]	**Product Type (likely exposure route):** Throat spray (ingestion, inhalation)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
Advertised form of silver: "contains Argentum metallicum (silver)" "Spray for sore throat"		
Comments: $9.53 for a 2-oz bottle.		

(44)	**Product Name:** [redacted]	**Product Type (likely exposure route):** Throat/nasal spray (ingestion, inhalation)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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:** [redacted]	**Product Type (likely exposure route):** Nasal spray (ingestion, inhalation)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
Advertised form of silver: "colloidal silver nasal spray"		
Comments: $6.85 per spray bottle.		

(46)	Product Name: [redacted]	Product Type (likely exposure route): Nasal spray (ingestion, inhalation)
Manufacturer (Country): [redacted]		Retailer: [redacted]
Retailer website: [redacted]		
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: [redacted]	Product Type (likely exposure route): Dietary supplement (ingestion)
Manufacturer (Country): [redacted]		Retailer: [redacted]
Retailer website: [redacted]		
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: [redacted]	Product Type (likely exposure route): Dietary supplement (ingestion)
Manufacturer (Country): [redacted]		Retailer: [redacted]
Retailer website: [redacted]		
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:** [redacted]	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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:** [redacted]	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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:** “[redacted]	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
Advertised form of silver: “"[redacted]" 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: [redacted]	Product Type (likely exposure route): Dietary supplement (ingestion)
Manufacturer (Country): [redacted]		Retailer: [redacted]
Retailer website: [redacted]		
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: [redacted]	Product Type (likely exposure route): Dietary supplement (ingestion)
Manufacturer (Country): [redacted]		Retailer: [redacted]
Retailer website: [redacted]		
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: [redacted]	Product Type (likely exposure route): Dietary supplement (ingestion)
Manufacturer (Country): [redacted]		Retailer: [redacted]
Retailer website: [redacted]		
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: [redacted]	Product Type (likely exposure route): Dietary supplement (ingestion)
Manufacturer (Country): [redacted]	**Retailer:** [redacted]
Retailer website: [redacted]	
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: [redacted]	Product Type (likely exposure route): Dietary supplement (ingestion)
Manufacturer (Country): [redacted]	**Retailer:** [redacted]
Retailer website: [redacted]	
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: [redacted]	Product Type (likely exposure route): Dietary supplement (ingestion)
Manufacturer (Country): [redacted]	**Retailer:** [redacted]
Retailer website: [redacted]	
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.	

<table>
<tr><td>(58)</td><td>Product Name:
[redacted]</td><td>Product Type (likely exposure route):
Dietary supplement (ingestion)</td></tr>
<tr><td colspan="2">Manufacturer (Country):
[redacted]</td><td>Retailer:
[redacted]</td></tr>
<tr><td colspan="3">Retailer website:
[redacted]</td></tr>
<tr><td colspan="3">Advertised form of silver:
“Ionic Colloidal Silver at 6ppm”</td></tr>
<tr><td colspan="3">Comments:
$21.45 for a 200-mL bottle.
This NZ-based retailer ships to the US and advertises prices in US dollars.</td></tr>
</table>

<table>
<tr><td>(59)</td><td>Product Name:
[redacted]</td><td>Product Type (likely exposure route):
Dietary supplement (ingestion)</td></tr>
<tr><td colspan="2">Manufacturer (Country):
[redacted]</td><td>Retailer:
[redacted]</td></tr>
<tr><td colspan="3">Retailer website:
[redacted]</td></tr>
<tr><td colspan="3">Advertised form of silver:
“Uses only the finest particle-size colloids to ensure maximum bioavalability and efficiency”
“Take one teaspoon with a meal three times daily”
“18 ppm”</td></tr>
<tr><td colspan="3">Comments:
$32.95 for a 4-oz bottles.
Directions for children were not given.</td></tr>
</table>

<table>
<tr><td>(60)</td><td>Product Name:
[redacted]</td><td>Product Type (likely exposure route):
Dietary supplement (ingestion)</td></tr>
<tr><td colspan="2">Manufacturer (Country):
[redacted]</td><td>Retailer:
[redacted]</td></tr>
<tr><td colspan="3">Retailer website:
[redacted]</td></tr>
<tr><td colspan="3">Advertised form of silver:
“Colloidal Silver”
“World's Smallest Particle Size, less than 1 nm on average”</td></tr>
<tr><td colspan="3">Comments:
$29.97 for an 8.45-oz bottle.</td></tr>
</table>

(61) **Product Name:** [redacted]	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): [redacted]	**Retailer:** [redacted]
Retailer website: [redacted]	
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:** [redacted]	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): [redacted]	**Retailer:** [redacted]
Retailer website: [redacted]	
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:** [redacted]	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): [redacted]	**Retailer:** [redacted]
Retailer website: [redacted]	
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:** [redacted]	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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:** [redacted]	**Product Type (likely exposure route):** Dietary supplement (ingestion)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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:** [redacted]	**Product Type (likely exposure route):** Surface disinfectant (dermal, ingestion)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
Advertised form of silver / Directions: "Colloidal Silver" "World's Smallest Particle Size, less than 1 nm on average" "Use in the kitchen, bathroom and childrens room (…) [sic]"		
Comments: $27.99 for a 125-mL bottle.		

(67)	**Product Name:** [redacted]	**Product Type (likely exposure route):** Surface disinfectant (dermal, ingestion)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
[redacted]		
Advertised form of silver: "Uses only the finest particle-size colloids to ensure maximum bioavalability 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:** [redacted]	**Product Type (likely exposure route):** Surface disinfectant (dermal, ingestion)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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:** [redacted]	**Product Type (likely exposure route):** Surface cleaner (dermal, ingestion)
Manufacturer (Country): [redacted]		**Retailer:** Unkown
Manufacturer website: [redacted]		
Advertised form of silver: "Nano Silver"		
Comments: Product purchased in Seoul by EPA staff (set of 2).		

(70)	**Product Name:** [redacted]	**Product Type (likely exposure route):** Surface cleaner (dermal, ingestion)
Manufacturer (Country): [redacted]		**Retailer:** Unkown
Manufacturer website: [redacted]		
Advertised form of silver: "Nano Silver"		
Comments: Product purchased in Seoul by EPA staff (set of 4).		

(71)	**Product Name:** [redacted]	**Product Type (likely exposure route):** Humidifier (inhalation)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
Advertised form of silver: "Includes demineralization cartridge and ionic silver stick"		
Comments: Cost: $169.99		

(72)	**Product Name:** [redacted]	**Product Type (likely exposure route):** Humidifier (inhalation)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
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: [redacted]	Product Type (likely exposure route): Humidifier (inhalation)
Manufacturer (Country): [redacted]		Retailer: [redacted]
Retailer website: [redacted]		
Advertised form of silver: "Silver clean technology fights mold and bacteria growth in water tank"		
Comments: Cost: $122.37		

(74)	Product Name: [redacted]	Product Type (likely exposure route): Humidifier (inhalation)
Manufacturer (Country): [redacted]		Retailer: [redacted]
Retailer website: [redacted]		
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: [redacted]	Product Type (likely exposure route): Humidifier (inhalation)
Manufacturer (Country): [redacted]		Retailer: [redacted]
Retailer website: [redacted]		
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: [redacted]	Product Type (likely exposure route): Humidifier (inhalation)
Manufacturer (Country): [redacted]		Retailer: [redacted]
Retailer website: [redacted]		
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: [redacted]	Product Type (likely exposure route): Humidifier (inhalation)
Manufacturer (Country): [redacted]		Retailer: [redacted]
Retailer website: [redacted]		
Advertised form of silver: "[redacted] inhibits growth of mold bacteria"		
Comments: Cost: $119.99		

(78)	Product Name: [redacted]	Product Type (likely exposure route): Humidifier (inhalation)
Manufacturer (Country): [redacted]		Retailer: [redacted]
Retailer website: [redacted]		
Advertised form of silver: "patented [redacted] [redacted] inhibiting the growth of mold bacteria"		
Comments: Cost: $79.99		

(79)	**Product Name:** [redacted]	**Product Type (likely exposure route):** Humidifier (inhalation)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
Advertised form of silver: "patented [redacted] inhibiting the growth of mold bacteria"		
Comments: Cost: $99.99		

(80)	**Product Name:** [redacted]	**Product Type (likely exposure route):** Humidifier (inhalation)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
Advertised form of silver: "[redacted]		
Comments: Cost: $139.99		

(81)	**Product Name:** [redacted]	**Product Type (likely exposure route):** Humidifier (inhalation)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
Advertised form of silver: "The patented [redacted] works nicely with William, inhibiting the growth of mold bacteria."		
Comments: Cost: $299.99		

(82)	**Product Name:** [redacted]	**Product Type (likely exposure route):** Humidifier (inhalation)
Manufacturer (Country): [redacted]		**Retailer:** [redacted]
Retailer website: [redacted]		
Advertised form of silver / directions: "Releases silver ions into the water" "For use with most humidifiers on the market"		
Comments: Cost: not advertised		

REFERENCES

1. The Project on Emerging Technologies, A database of silver nanotechnology in Commercial Products. 2010.
2. Fauss, E., *The silver nanotechnology commercial inventory*. 2008, Project on Emerging Technologies: Charlottesville.

Appendix D: Standard Operating Procedures (SOPs)

SOP # AirVT-Nanosilver-001

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

May 16, 2011

By

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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.

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.

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.

MATERIALS AND REAGENTS

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)

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

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%)

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.

Filters

a. 100, 450, and1000-nm PTFE (Teflon) filter membrane or syringe filters
b. 3 or 10 KDa cut-off (such as Millipore Amicon or similar)

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).

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.

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 extraction 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.

4. Microwave-Assisted Extraction Method

Silver nanoparticles from liquid, plastic, foam and fabrics are extracted using microwave extraction and reagent-grade nitric acid (HNO_3).

a. The maximum mass of sample that can be extracted with the Mars CEM Microwave Extraction Unit is 0.5 g. Each sample regardless of media is weighed out to roughly 0.3 g using pipette, spatula, or forceps to handle the respective samples. After weighing out, each sample is placed into a Mars CEM XP-1500 Plus Extraction Tube.
b. To each sample, 10 mL of reagent-grade HNO_3 is added. Foam and fabric samples may require additional nitric acid to cover the sample in the extraction tube.
c. Each sample is extracted using a Mars CEM Microwave Extraction Unit. Samples are heated and pressurized to 800 psi and 200 ºC and held at this temperature and pressure for 10 minutes.
d. Samples must be allowed to cool before the extraction tubes are removed from the microwave unit. When the pressure inside the tubes has reached ambient levels the extraction tubes can be removed from the microwave unit and opened in a fume hood.
e. Upon opening, each sample is allowed to vent in a fume hood for at least 30 minutes. After 30 minutes, if upon swirling gently the sample releases fumes into the hood or is not yet a clear white color, it is allowed to sit for an additional 30 minutes.
f. When samples no longer release fumes upon swirling and become clear and colorless they are transferred to 25-mL volumetric flasks, and diluted to volume using ultrapure water.
g. Transfer to vial for AA or ICP analysis.

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.

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.

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.

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

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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.

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.

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.

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 550 ºC.

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.

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.

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.

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.

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.

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

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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.

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.

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 1 shows a flow chart depicting this method in detail.

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).

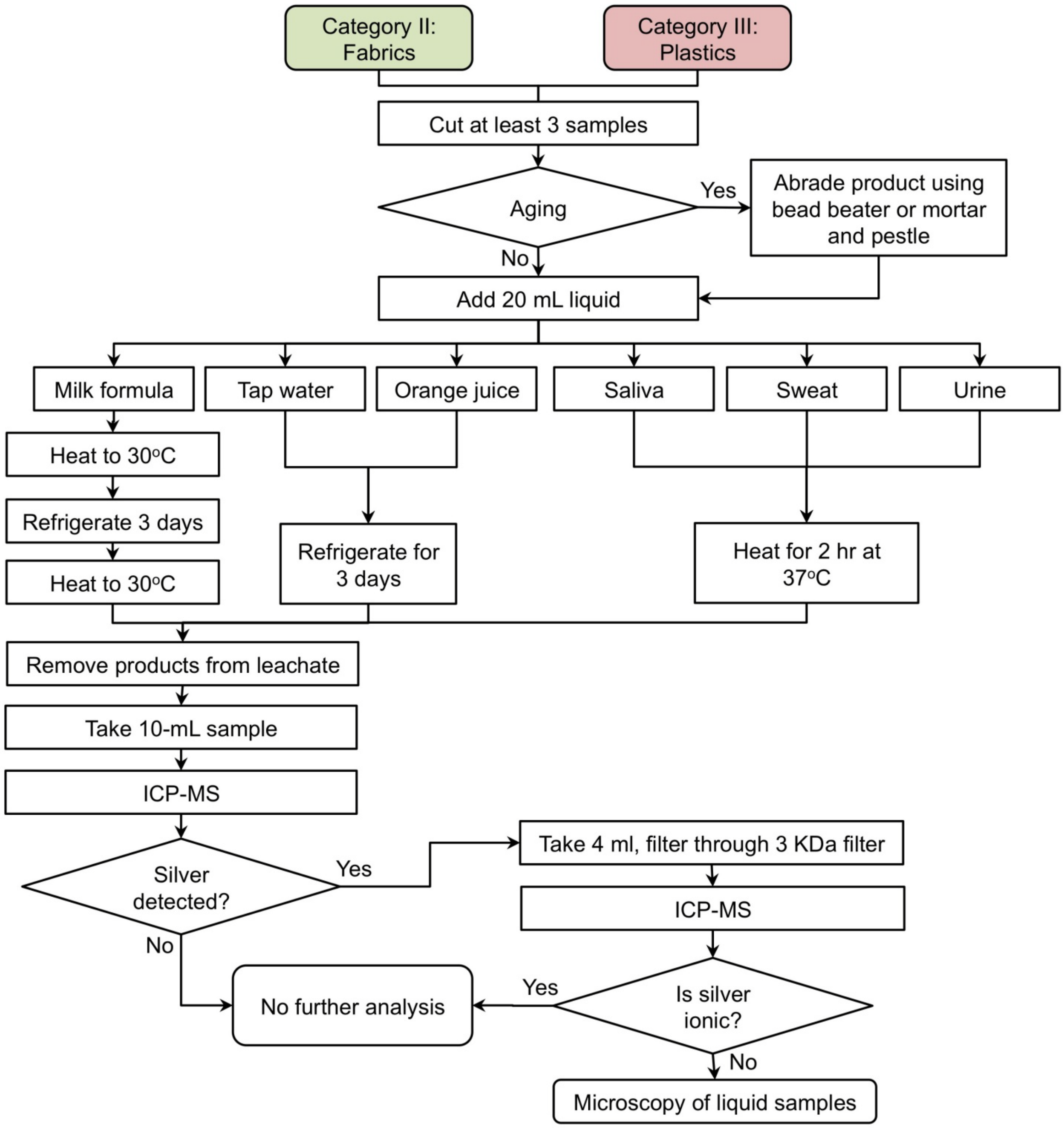

Figure 1. Flowchart summarizing the analytical methods for leaching assays of nanosilver consumer products.

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).

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.

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.

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.

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.

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SOP # AirVT-Nanosilver-004

CHARACTERIZATION OF AEROSOLS GENERATED FROM NANOSILVER CONSUMER PRODUCTS

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By

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Virginia Tech Principal Investigator — Date

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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.

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.

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.

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)

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.

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 μm).
   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.

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.

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.

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.

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.

American Society of Heating, Refrigerating, and Air Conditioning Engineers (ASHRAE), Thermal Environmental Conditions for Human Occupancy, Standard 55-2010.

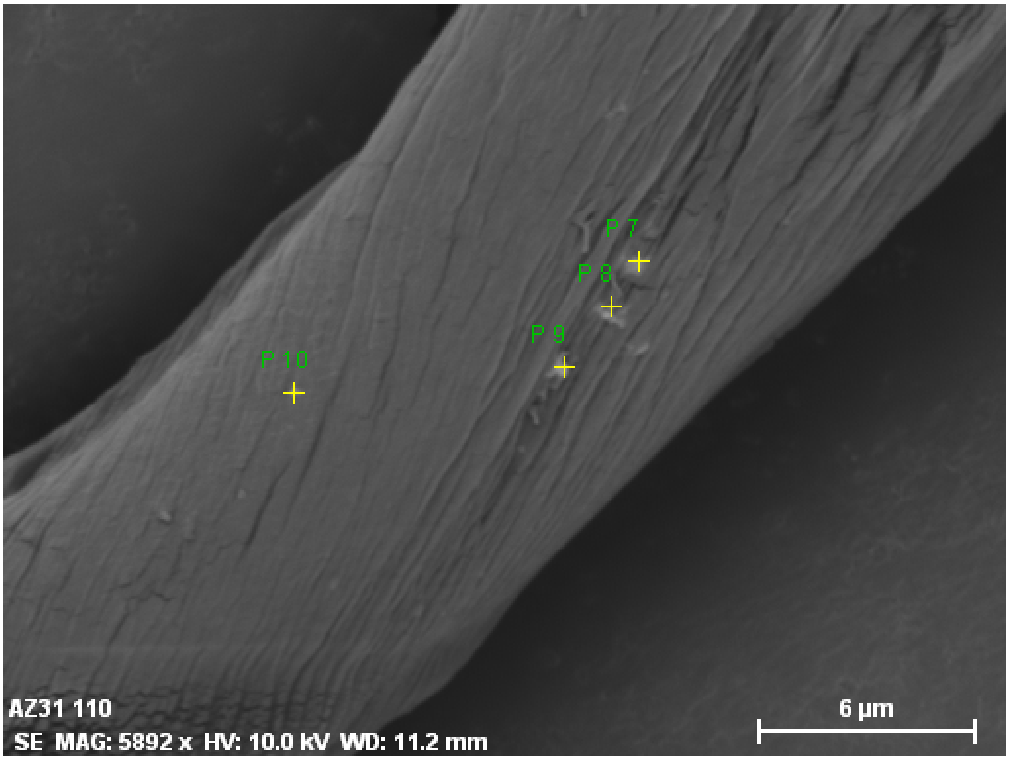
Environmental Protection Agency (EPA), A Brief Guide to Mold, Moisture, and Your Home, 2010. EPA 402-K-02-003, Office of Air and Radiation, Indoor Environments Division, Washington, DC.

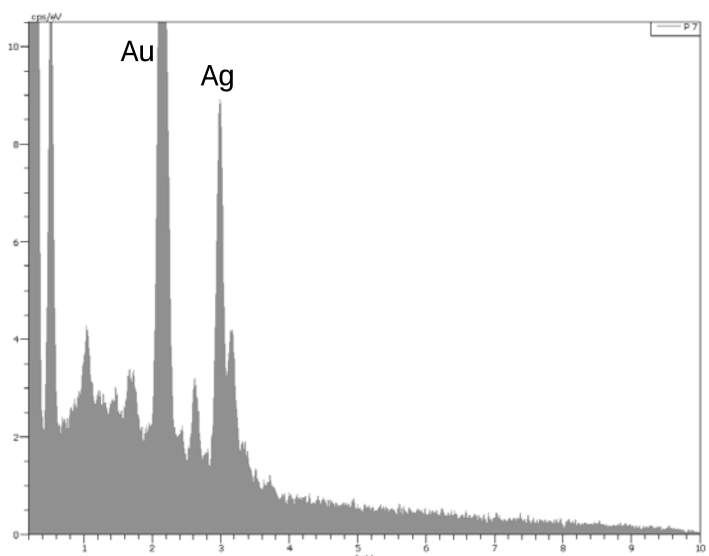
Quadros, M.E., C.T. Faria, and L.C. Marr, *A thermophoretic sampler for collecting airborne nanoparticles (Poster)*, in *1st International Conference on the Environmental Implications of Nanotechnology* 2009, 1st ICEIN: Washington, DC.

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

Appendix E: Microscopy

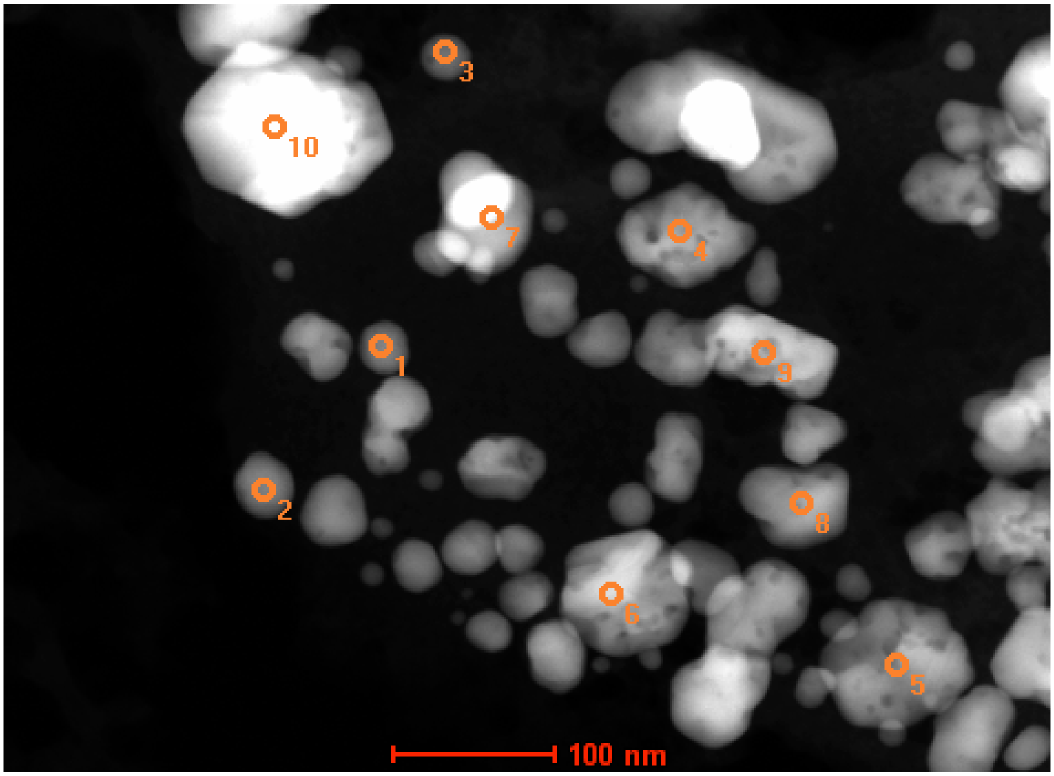
Nanosilver control sample – SEM/EDS



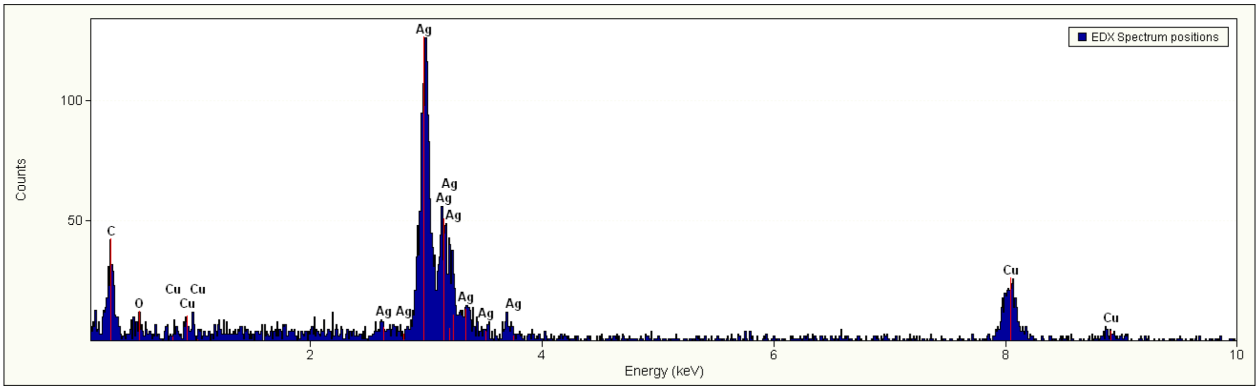


Spectrum representative of positions P7, P8, P9, P10, which yielded identical spectra (gold is from sputter coating).

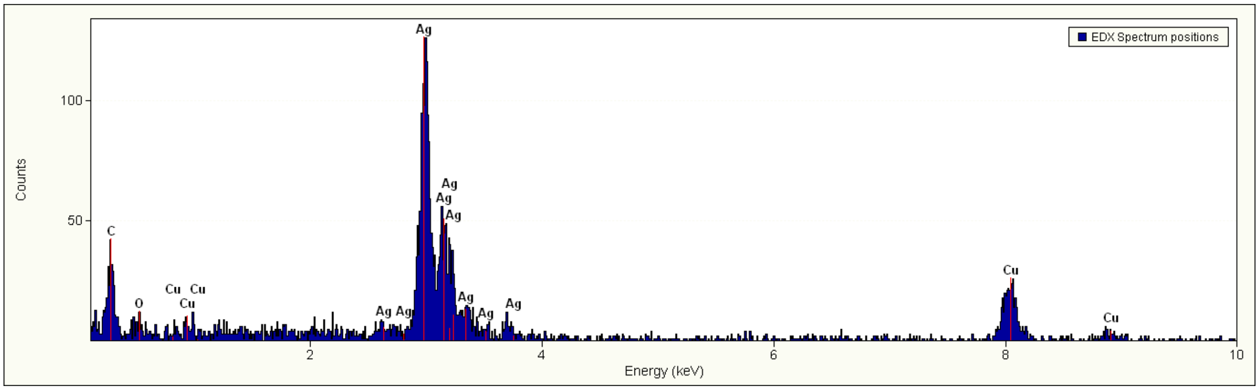
Nanosilver ash control



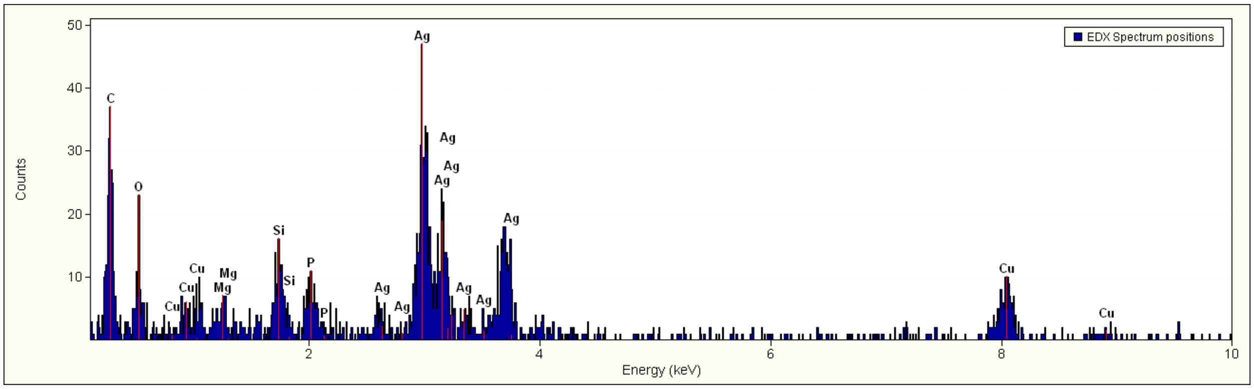
Location of EDS spectra.



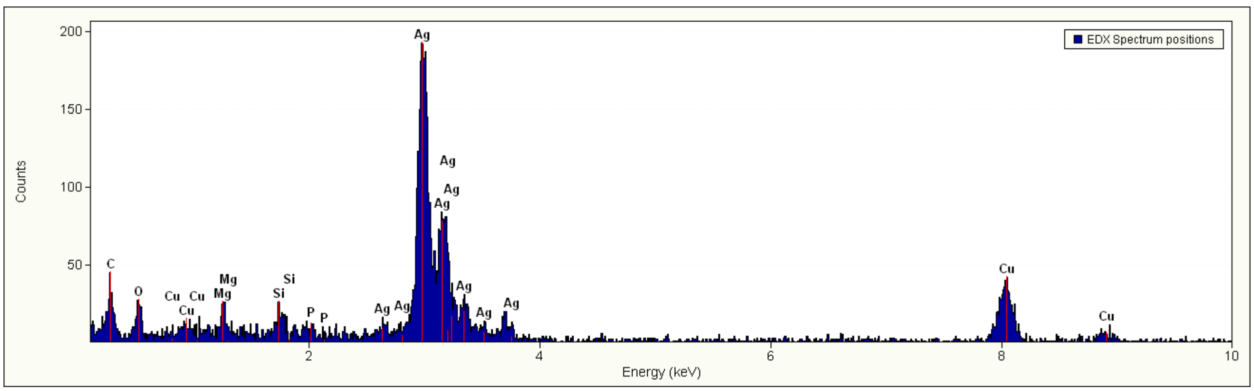
Spectrum Position O_1.



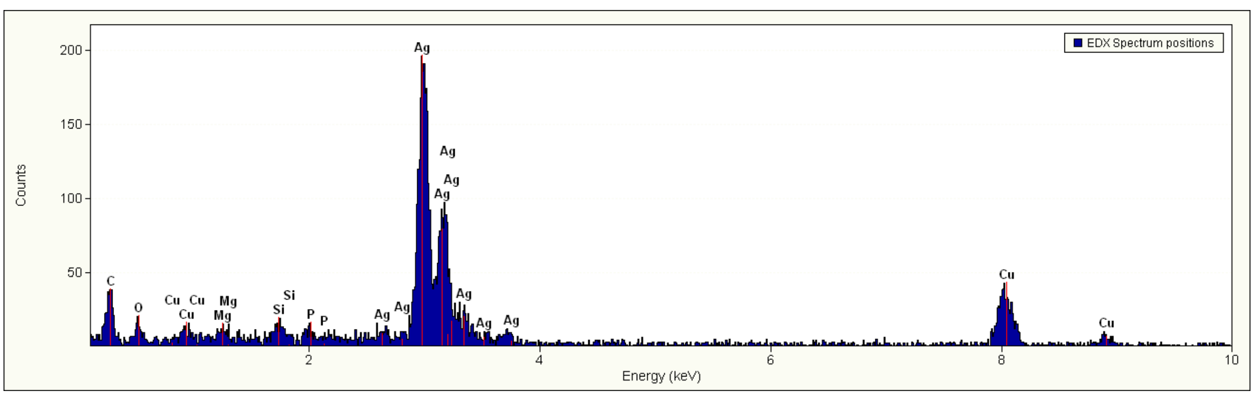
Spectrum Position O_2.



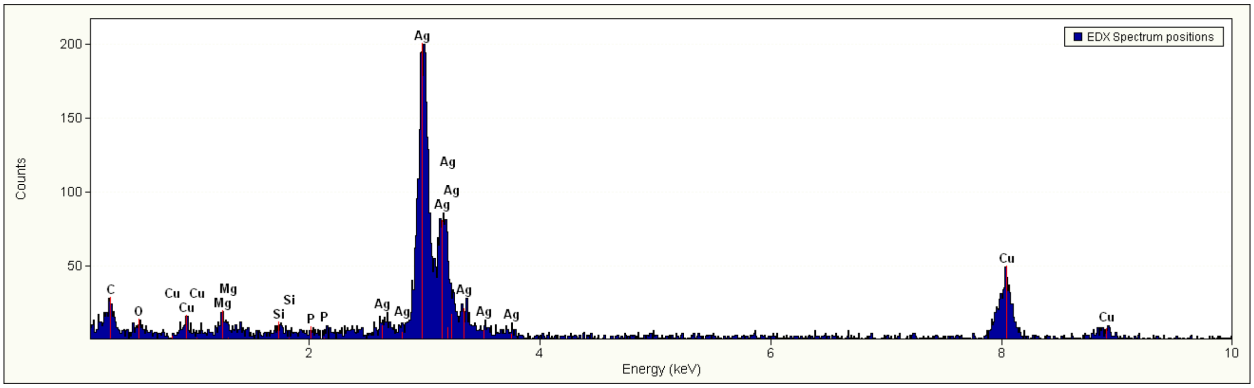
Spectrum Position O_3.



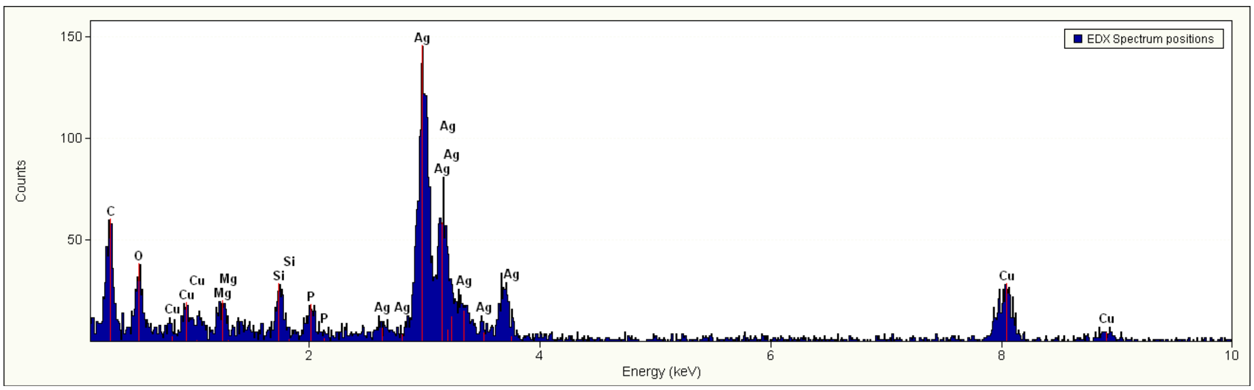
Spectrum Position O_4.



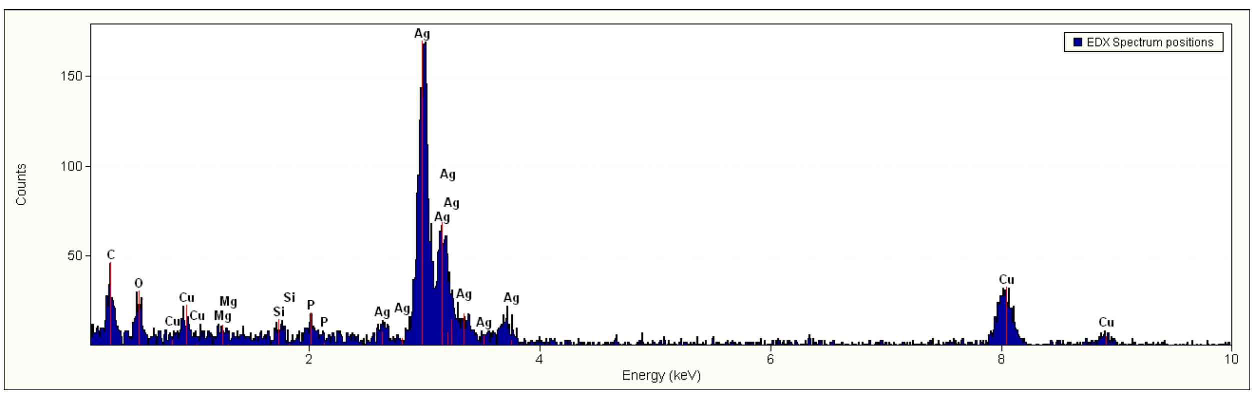
Spectrum Position O_5.



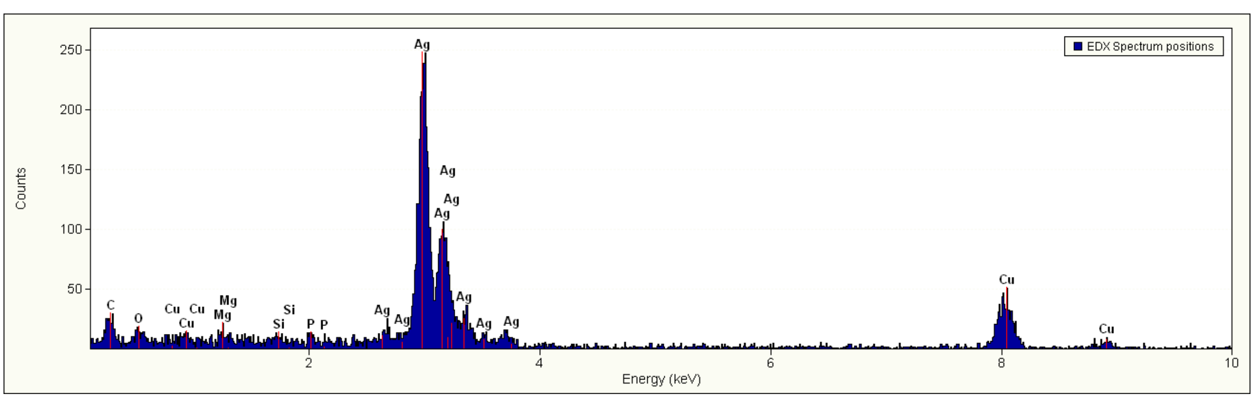
Spectrum Position O_6.



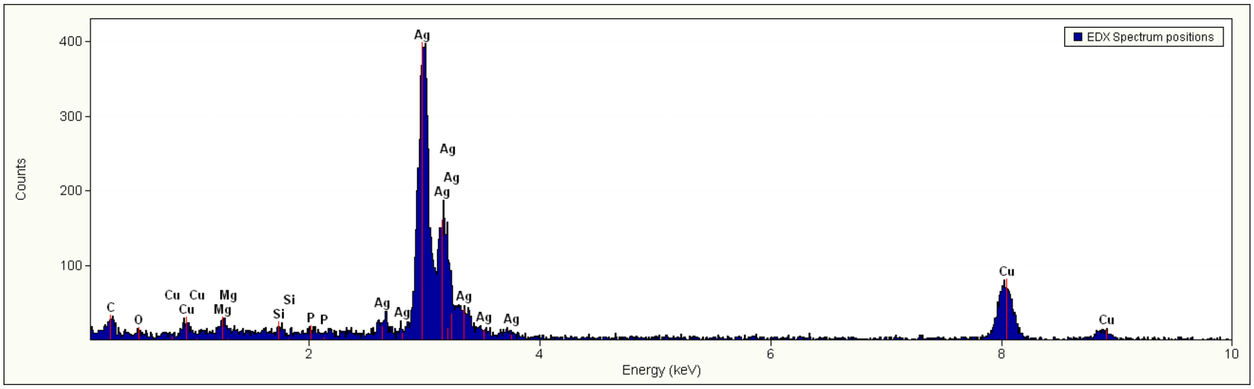
Spectrum Position O_7.



Spectrum Position O_8.

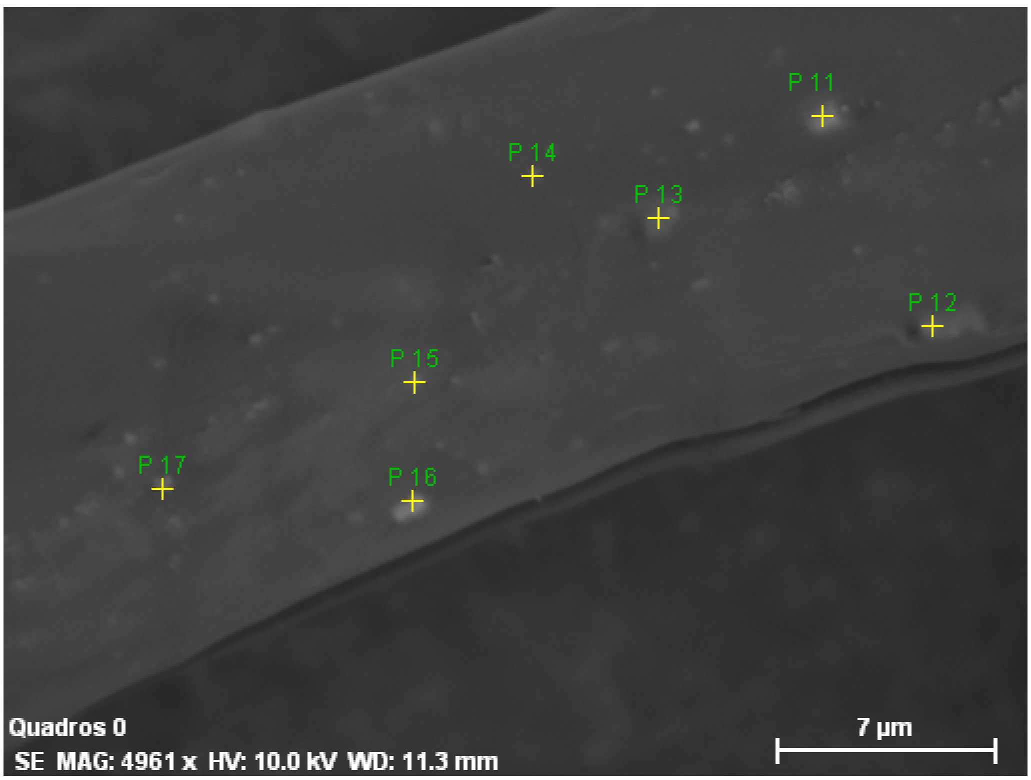


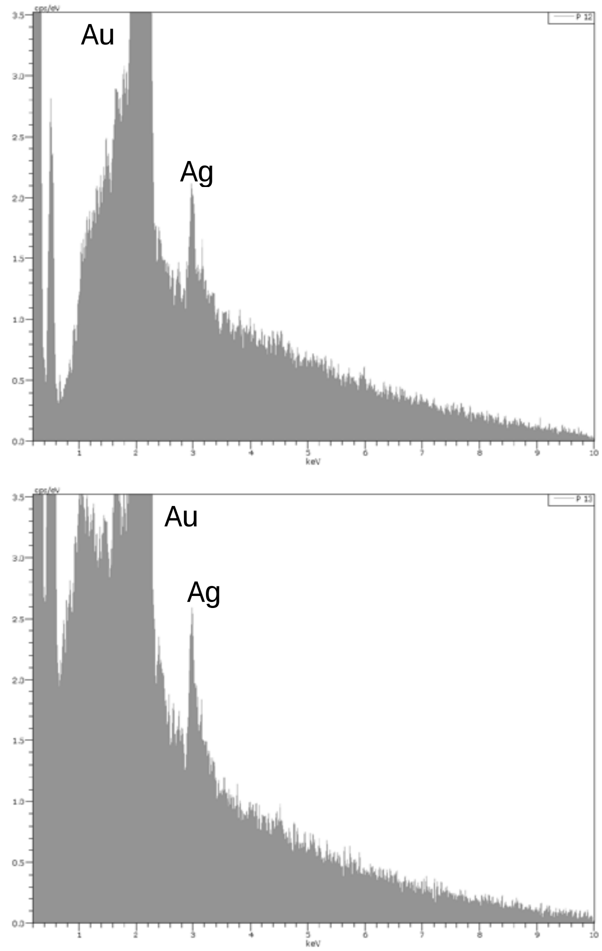
Spectrum Position O_9.



Spectrum Position O_{10}.

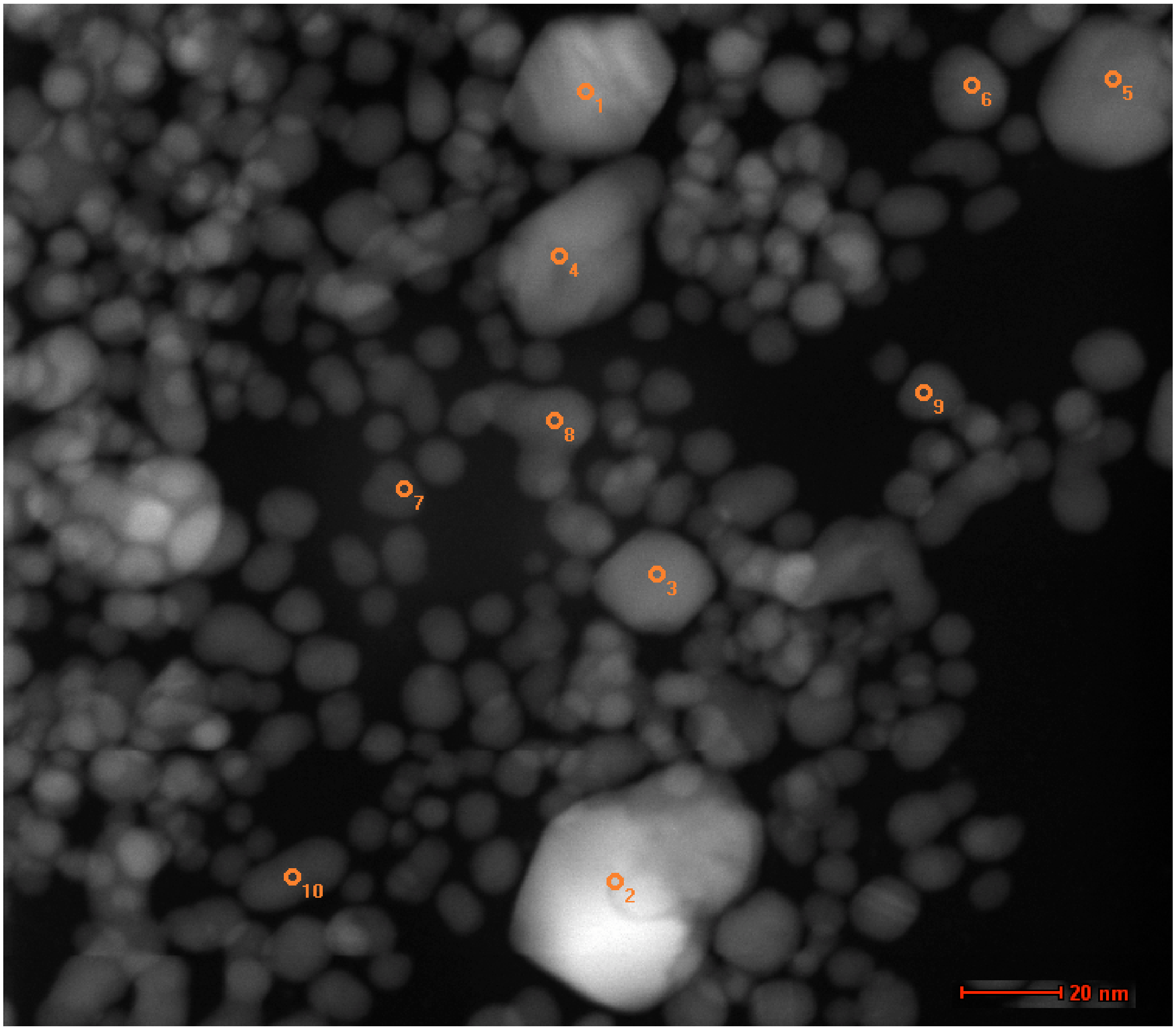
Baby blanket sample



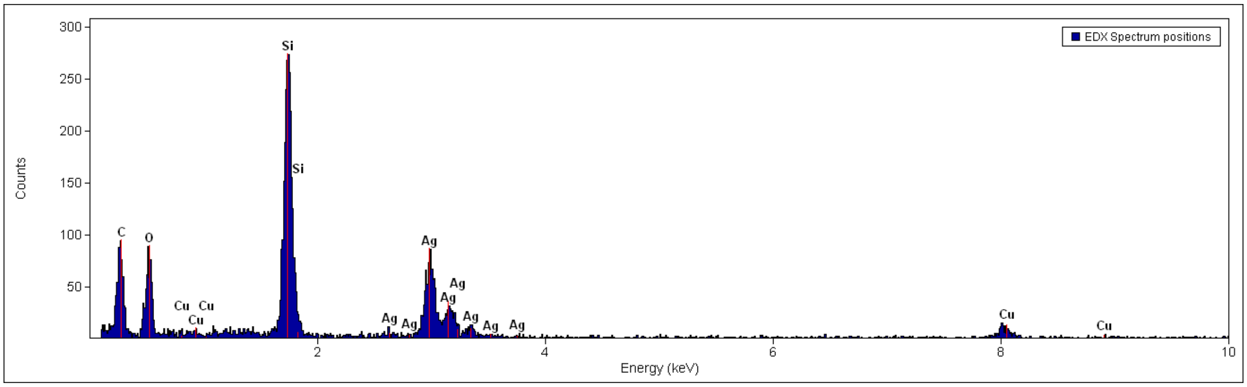


Spectrum Positions P12 (upper) and P13 (lower).

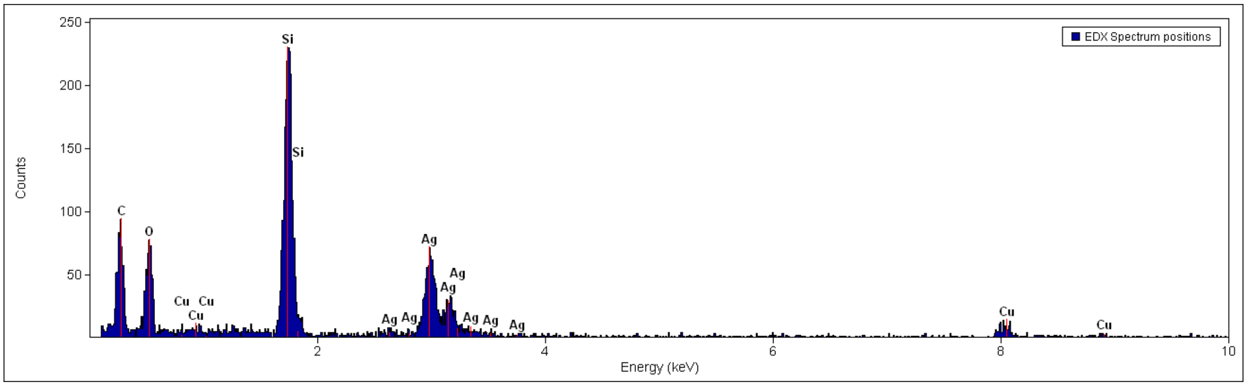
Baby blanket ash sample



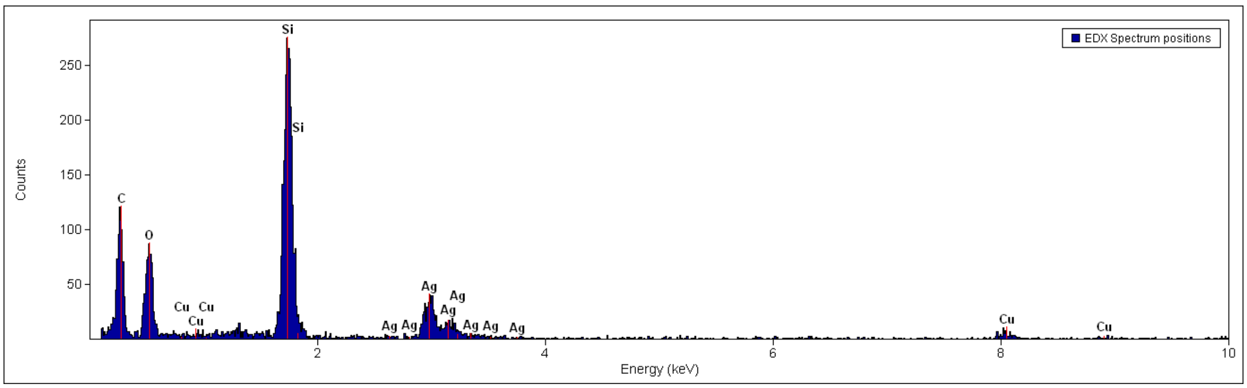
Location of EDS spectra.



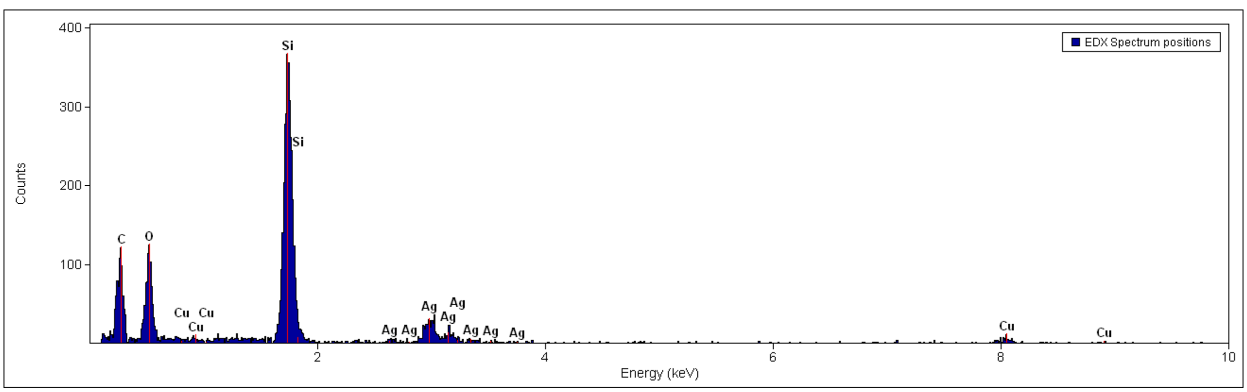
Spectrum Position O_1.



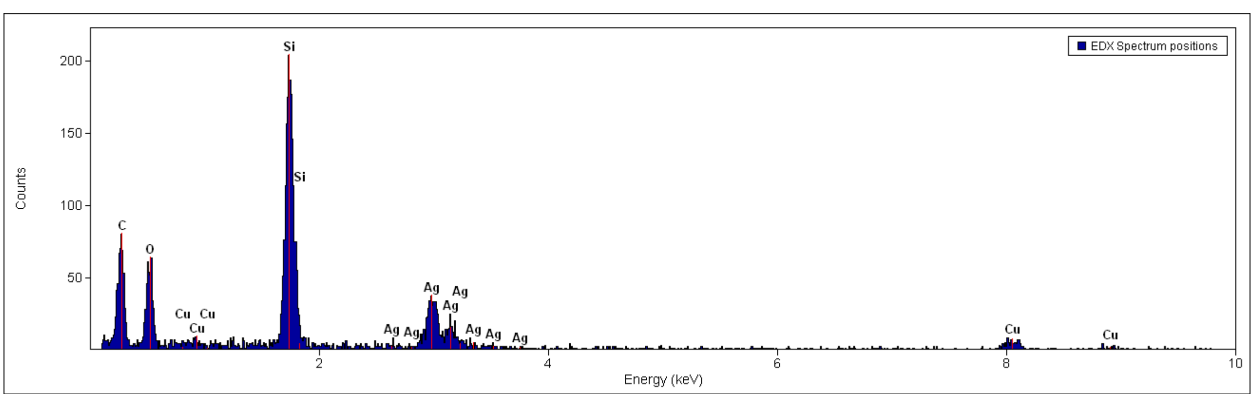
Spectrum Position O_2.



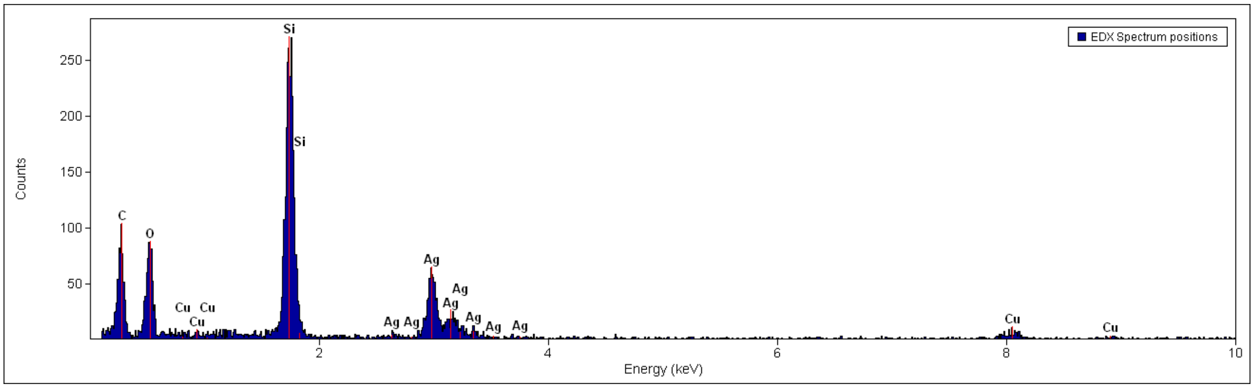
Spectrum Position O_3.



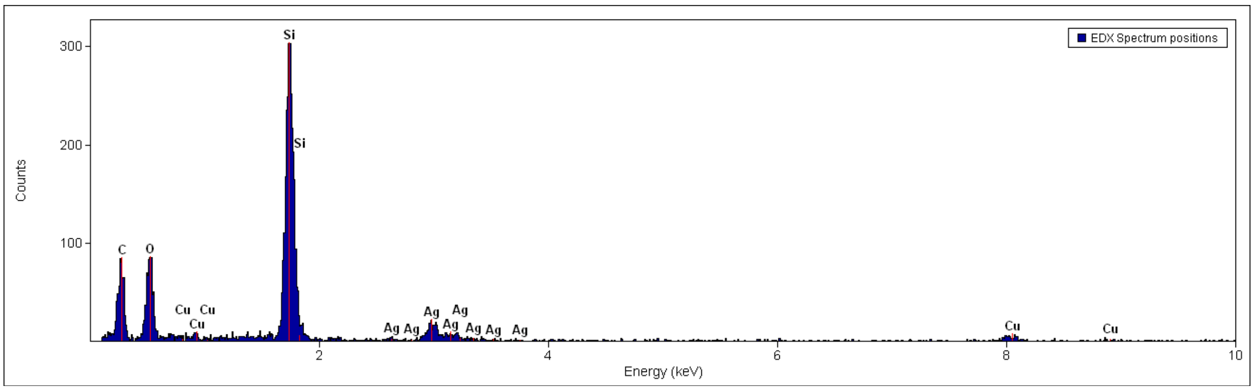
Spectrum Position O_4.



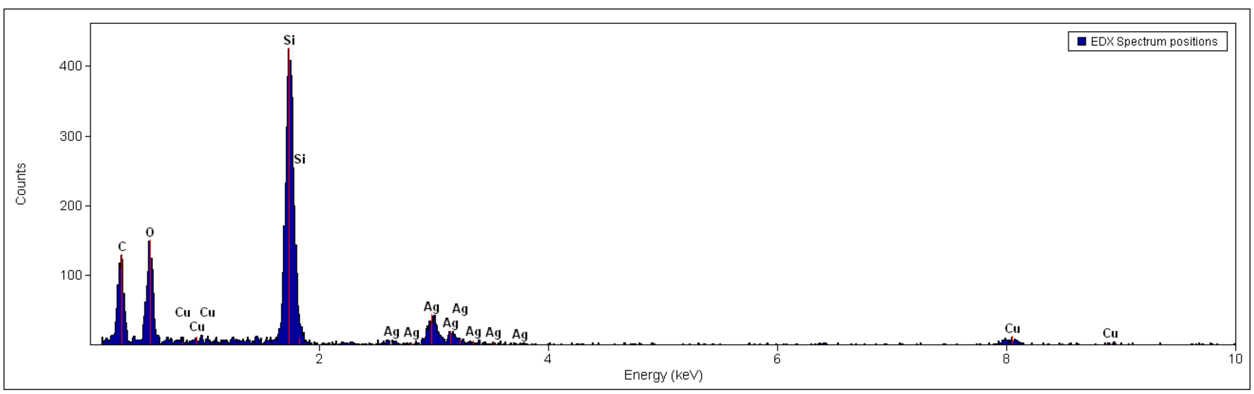
Spectrum Position O_5.



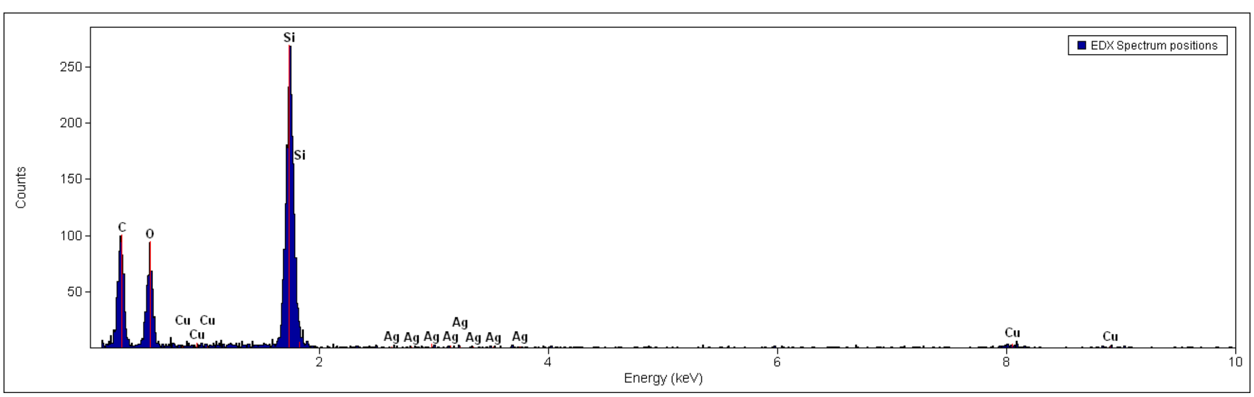
Spectrum Position O_6.



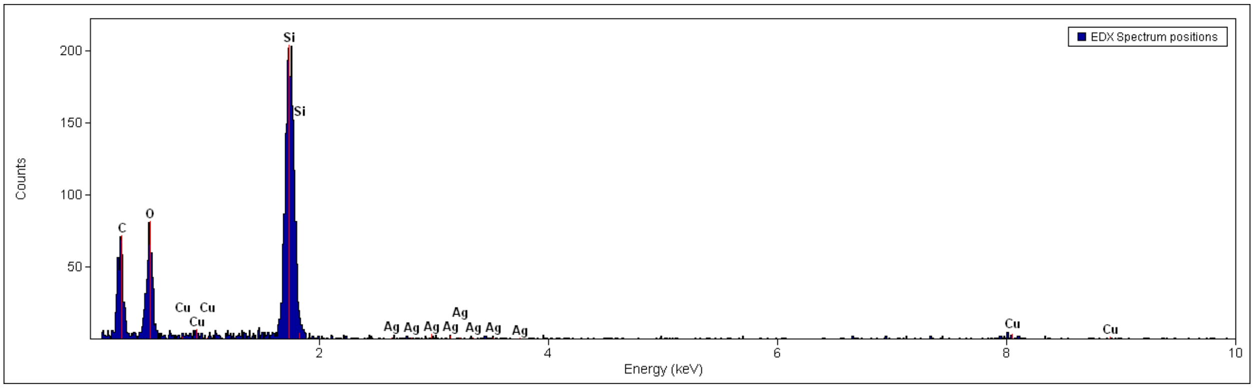
Spectrum Position O_7.



Spectrum Position O_8.



Spectrum Position O_9.



Spectrum Position O_{10}.

Appendix F: Leaching Media

1. 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.

2. Milk formula

We prepared milk formula ([redacted] Infant Formula, 360 g) according to the package instructions, using ultrapure water at 38^{o}C. The milk formula had a pH of 7.0.

3. Orange juice

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

4. Synthetic sweat

We used a synthetic sweat formulation suggested by Kulthong et al.[1], who assessed the silver nanoparticle release from a fabric into four sweat formulations. We chose the formulation described by the European Standard (EN1811-1999) because it released the highest amount of silver 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 media and corrected the pH to 6.5 using a solution of 0.2 N sodium hydroxide (NaOH).

5. 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 media, which had a pH of 7.8.

6. 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 media, which had a pH of 7.0.

7. Saline

Per EPA's recommendation, 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. The saline solution will be tested with one consumer product, the Precious Protechtor blanket. We used ultrapure water to prepare this media, which had a pH of 7.0.

8. ASTM F963-08 media (digestion substitute)

As with the saline solution, we have followed EPA's recommendation and prepared a 0.07 M hydrochloric acid solution to use as a leaching medium for comparison with other synthetic media. The hydrochloric acid solution will be tested with one consumer product, the [redacted] blanket. ASTM[4] recommends using this media "to simulate the situation in which materials stay 4 h in the alimentary tract after swallowing". We used ultrapure water to prepare this media, which had a pH of 1.4.