Release of silver from nanotechnology-based consumer products for children

Marina E. Quadros[1†‡], Raymond Pierson IV[1‡], Nicolle S. Tulve[2], Robert Willis[2], Kim Rogers[2], Treye A. Thomas[3], Linsey C. Marr[1]*

[1]Department of Civil and Environmental Engineering, Virginia Tech, 418 Durham Hall, Blacksburg, VA 24061, United States

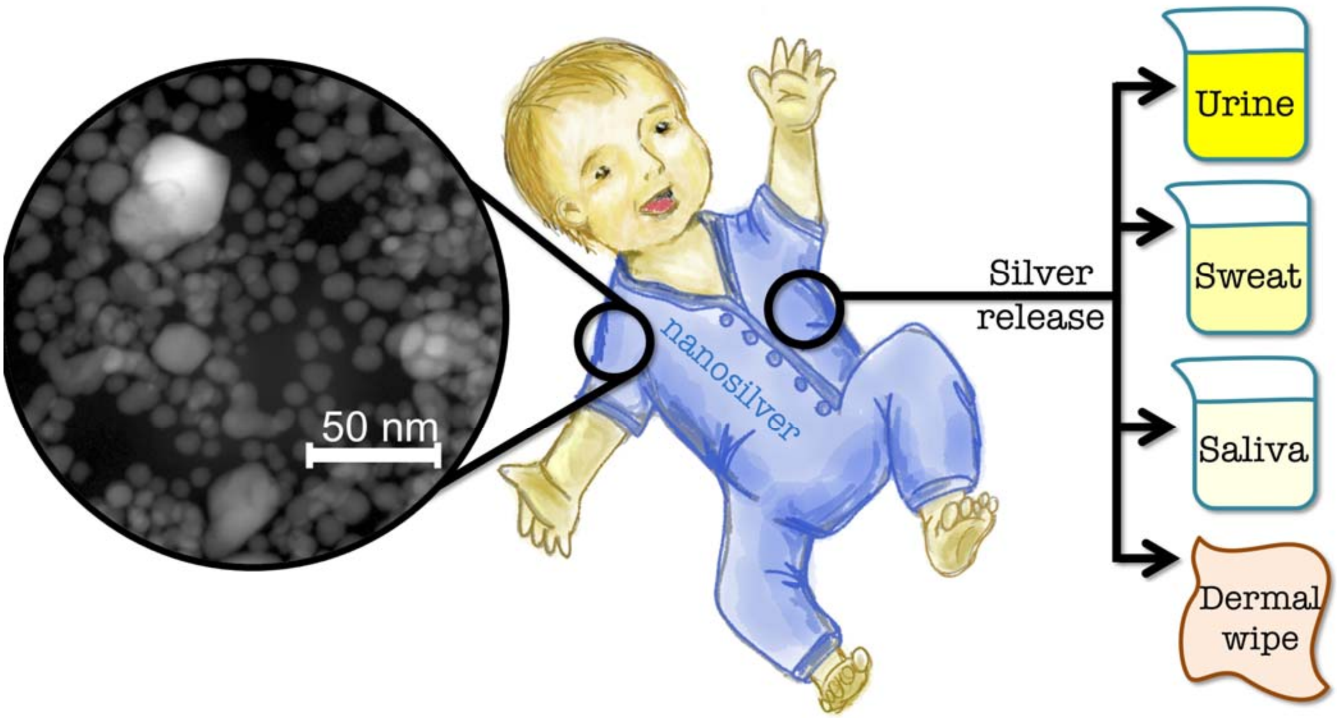
[2]National Exposure Research Laboratory, Office of Research and Development, United States Environmental Protection Agency, 109 TW Alexander Dr., Research Triangle Park, NC 27711, United States

[3]United States Consumer Product Safety Commission, 4330 East-West Highway, Bethesda, MD 20814, United States

Running title: Silver nanoparticles from children's consumer products

Keywords: children, consumer products, dissolution, exposure, leaching, nanoparticle, silver, nanosilver.

TOC ART


50 nm
nanosilver
Silver
release
Urine
Sweat
Saliva
Dermal
wipe

ABSTRACT

We assessed the potential for children's exposure to bioavailable silver during the realistic use of selected nanotechnology-based consumer products (plush toy, fabric products, breast milk storage bags, sippy cups, cleaning products, humidifiers, and humidifier accessory). We measured the release of ionic and particulate silver from products into water, orange juice, milk formula, synthetic saliva, sweat, and urine (1:50 product to liquid mass ratio); into air; and onto dermal wipes. Of the liquid media, sweat and urine yielded the highest amount of silver release, up to 38% of the silver mass in products; tap water yielded the lowest amount, ≤1.5%. Leaching from a blanket into sweat plateaued within 5 min, with less silver released after washing. Between 0.3 and 23 $\mu g\ m^{-2}$ of silver transferred from products to wipes. Aerosol concentrations were not significantly elevated during product use. Fabrics, a plush toy, and cleaning products were most likely to release silver. Silver leached mainly via dissolution and was facilitated in media with high salt concentrations. Levels of silver to which children may potentially be exposed during the normal use of these consumer products is predicted to be low, and bioavailable silver is expected to be in ionic rather than particulate form.

Introduction

Silver nanoparticles (nanosilver) are known for their broad-spectrum antimicrobial properties,[1-5] which have led to many applications in consumer products, such as disinfecting sprays, cosmetics, fabrics, and household appliances.[6,7] Such widespread use has led to concerns because exposure to silver is associated with chronic health effects, such as argyria,[8,9] and the safety of nanosilver has not been established. Ingested nanosilver has been shown to accumulate in various organs and to cause slight liver damage in rats.[10] Inhalation exposure to nanoparticles can lead to respiratory inflammation and cardiovascular disease.[9,11,12] Inhaled nanosilver has been shown to translocate to the brain and other organs in rats.[13] An *in vitro* study showed that nanosilver is toxic to lung cells.[14]

Compared to adults, children are at a higher risk for exposure because: (1) they have a higher metabolism and surface-area-to-mass ratio; (2) their organs and tissues are still under development; (3) they have more years ahead of them to develop health conditions from chronic exposure to emerging materials;[15] and (4) they have a higher tendency to place hands and objects in the mouth.[16] Guney and Zagury[17] recommended developing regulations on toxic substances in toys and children's products based on risk assessment, rather than just total contaminant content.

Although there have been studies of the toxic effects of silver nanoparticles and ions to human cells and the environment, a large gap in knowledge still exists concerning realistic human exposure scenarios during the use of nanosilver consumer products. Previous studies have shown that silver leaches from consumer products into water,[18-22] but more information is needed to describe the release of silver in exposure scenarios representing real-world use. Questions remain about the effects of ionic versus particulate silver, so it is important to distinguish between the two.[1,23,24] The objective of this work was to determine children's potential exposure to ionic and particulate silver from selected children's and household consumer products that employ silver nanotechnology. The ingestion, inhalation, and dermal routes of exposure were investigated. Specific objectives were to quantify the amount of silver released into media relevant to each product's normal, real-world, intended use and to determine how differences in liquid media composition control dissolution of silver from products. This work was initiated in response to the U.S. Consumer Product Safety Commission's (CPSC) interest in developing reliable methods for quantifying and characterizing silver release from children's consumer products. Results from this effort are also reported in a companion paper that focuses on characterization of nanosilver in selected children's consumer products.[25]

Experimental Methods

Products and Release Scenarios. We compiled an inventory of 82 consumer products that were claimed by the manufacturer to contain nanosilver or silver and that may be used by or around children.[25] From that inventory, we selected 13 products for testing, including one plush toy (teddy bear), three fabric products (baby blanket, sleepsuit, pair of baby scratch mitts), one set of breast milk storage bags, two sippy cups, three cleaning products (disinfecting spray, surface wipe, kitchen scrubber), two humidifiers claiming to contain silver to prevent biofilm formation in the water tank, and one humidifier accessory (a cube that can be placed in a humidifier's reservoir). Because some of the 13 products consisted of multiple pieces, they generated a total of 21 items for testing, of which 13 were shown to contain some form of silver (further described in the Supporting Information).[25] We analyzed these 13 items for their potential to release silver under conditions of normal, real-world use, defining release scenarios based on each product's intended use and whether it might come into contact with liquids (water, milk, saliva, sweat, or urine), touch the skin, or release silver-containing aerosols (Table 1).

Table 1. Release scenarios for each product reported to contain silver.

Product	Scenario
plush toy: exterior fur (new)[a]	leaching: tap water, saliva, sweat, urine dermal assay aerosol emissions
plush toy: exterior fur (aged)	leaching: tap water, saliva, sweat, urine
plush toy: interior foam[a]	leaching: tap water, saliva, sweat, urine
baby blanket[b] (new)	leaching: tap water, saliva, sweat, urine dermal assay aerosol emissions
baby blanket (aged)	leaching: tap water, saliva, sweat, urine
breast milk storage bags	leaching: milk formula
sippy cup #1: rubber ring	leaching: milk formula, orange juice[c]
sippy cup #1: transparent cap	leaching: milk formula, orange juice
sippy cup #2: spout cover	leaching: milk formula, orange juice
disinfecting spray	dermal assay aerosol emissions
surface wipes	dermal assay
kitchen scrubber	dermal assay
tabletop ultrasonic humidifier	leaching: tap water
manual ultrasonic humidifier[d]	leaching: tap water
humidifier accessory cube[e]	leaching: tap water aerosol emissions

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

[b]Because all three fabric products (baby blanket, sleepsuit, and scratch mitts) had approximately the same total silver concentration and were made by the same manufacturer, we concluded that the fabric used in all three was the same and subsequently examined release scenarios for only one product.

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

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

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

Silver Release into Liquid Media. The leaching assays consisted of soaking product samples in relevant liquid media under various conditions related to normal use. A detailed description of all leaching media is included in the Supporting Information. The leaching media included tap water from the town of Blacksburg, VA; synthetic sweat,[22] saliva,[26] and urine,[27] prepared and stored in a refrigerator at ~5°C; milk formula (Gerber Good Start Infant Formula), prepared according to the manufacturer's instructions using ultrapure water (18.2 MΩ cm, Barnstead); and orange juice (Kroger brand, no pulp). We weighed ~0.5-g pieces of each product, placed each of them in a 100-mL beaker, and added enough liquid media to achieve a 1:50 mass ratio between the product mass and leaching media.[28] For saliva, to simulate chewing, we placed 3–5 samples of 0.1–0.2 g of product into 2-mL bead-beating vials, added ~0.3 g of 1-mm glass beads and 1–1.5 mL of synthetic saliva, beat samples for 30 s using a bead beater (Mini-Beadbeart-1, Biospec) at 2500 rpm, and then combined them into one composite sample. We repeated this procedure twice more to produce three replicates. To each replicate, we added more saliva to obtain a 1:50 mass ratio of product to leaching media.

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

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

When soaking was completed, we removed 10-mL aliquots from the leachate, added 10% nitric acid (HNO_3 69%, reagent grade, Sigma-Aldrich) to dissolve any silver particles present, and analyzed the leachate for silver content using inductively coupled plasma mass spectrometry (ICP-MS, Thermo Electron X-Series, detection limit of 0.5 ppb).[20-22,25] Because fabric samples released fibers into the leaching media, we filtered the leachate through 0.45-μm PTFE syringe filters (Millipore Millex) before adding HNO_3. We digested the orange juice and milk formula samples using a modified version of the methods described by Benn and Westerhoff.[21] We added 10 mL of HNO_3 to 25-mL samples and heated them on a hot plate at ~90°C for ~30 min. After the samples had cooled, we added 5 mL of hydrogen peroxide (H_2O_2), returned samples to the hot plate, and added 1-mL aliquots of H_2O_2 until effervescence was minimal and sample volume was reduced below 25 mL. We then increased the sample volumes to 25 mL using ultrapure water (18.2 MΩ cm, Barnstead) and analyzed them by ICP-MS. To determine whether silver in the leachate was in ionic or particulate form, we filtered saliva, sweat, and urine samples that proved to contain silver using 3-KDa centrifugal filtering units (Millipore Amicon Ultra), acidified them with HNO_3, and analyzed the filtrate for silver by ICP-MS. These filtering units are designed for concentrating proteins ~1 nm or larger.[29]

We also compared our leaching media with two that have been used by the CPSC: a saline solution (9 g/L NaCl) as a surrogate for saliva, and a solution of hydrochloric acid (HCl, 0.07 M) to simulate ingestion.[28] We soaked samples from the blanket in these media under the same conditions previously described for sweat and urine.

Leaching Kinetics and Chemistry. We examined the kinetics of leaching and effects of product aging with the baby blanket, chosen for its high silver content and high repeatability in terms of total silver concentration upon digestion, which is indicative of a homogenous spatial distribution of silver within the product. We performed most additional experiments with the synthetic sweat formulation, due to its higher silver-leaching yield compared to other biologically relevant media, to simulate a worst-case scenario. To study the kinetics of leaching, we soaked triplicate samples of the baby blanket in synthetic sweat at a mass ratio of 1:50 for six time durations: 5, 15, 30, 60, 120, and 240 min. At the end of each period, we filtered samples to remove suspended fabric fibers (0.45-µm filter), acidified them with 10% HNO_3, and analyzed them by ICP-MS.

In the interest of determining how differences in liquid media composition affect dissolution of silver from a consumer product, we soaked blanket samples in various formulations of synthetic sweat, modified to omit one ingredient at a time (Table 2). We soaked samples for a period of 1 h at 37°C in a water bath. We also soaked samples in saline (185 mM of NaCl) and ultrapure water. We filtered, acidified, and analyzed samples as described previously.

Table 2. Sweat formulation descriptions, where X indicates the presence of an ingredient and '–' its absence.

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

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

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

To simulate product aging in the baby blanket and plush toy, we placed one piece of each product under a UV lamp (GE G8T5, 8W, 254 nm) for 1–2 weeks, then hung these samples on an outdoor clothes line to expose them to natural weathering for ~1 week (Blacksburg summer, no rain, average temperature 21–24°C, maximum temperature 32°C), and finally rubbed them against a concrete block for ~1 min before subjecting them to the same leaching experiments as the new, unaged products.

Characterization by Electron Microscopy. To determine the size and location of nanosilver in products, we characterized selected ones using an environmental scanning electron microscope (SEM) with electron dispersive X-ray spectrometry (EDS) capabilities (FEI Quanta 600 FEG), operated under high vacuum using backscattered and secondary electron detectors.[25]

Silver Release onto Skin. We assessed products that may come in contact with skin—the plush toy, baby blanket, disinfecting spray, surface wipes, and kitchen scrubber—for the potential for dermal exposure. On the basis of prior studies and recommendations, we identified wipes as the most reliable and feasible method for assessing the potential for dermal exposure to nanosilver.[30-32] We followed NIOSH Method 9102: Elements on Wipes,[33] that specifies the use of benzalkonium chloride moist towelettes, which were previously shown not to contain silver. For the baby blanket and the kitchen scrubber, we delimited three different areas on the products for wiping. Because we only had one plush toy, whose available surfaces had been mostly consumed by other experiments, we repeated wiping on the same areas (front and back of the toy) three times. For the disinfecting spray and surface wipes, we mimicked a situation in which a child may touch a surface after it had been cleaned using one of these products. We applied the spray and wipes onto three individual 1 ft. × 1 ft. vinyl floor tiles (Armstrong) and wiped the surfaces with towelettes, using horizontal and vertical strokes. Towelettes were digested in HNO_3 and H_2O_2 and analyzed for silver content by ICP-MS.

Silver Release from Humidifiers. We completely filled the water reservoir of each humidifier with tap water and let it sit at room temperature for 5–6 days, collected water samples from each basin, acidified the samples with 10% HNO_3, and analyzed them by ICP-MS. We also collected samples after ~17 days of soaking.

To assess the potential for inhalation exposure to silver, we measured the total silver concentration in the vapor produced by each humidifier. We filled the humidifier reservoirs with tap water and let them sit for 3 days. Using PVC reducing pipe and tubing, we routed the outlet of each humidifier through a sealed beaker submerged in ice, to promote condensation inside the beaker (~20 mL). The condensate was then acidified with 10% HNO_3 and analyzed by ICP-MS.

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

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

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

Results and Discussion

Silver Release into Liquid Media. Table 3 shows the total silver content in each product component and the amount that leached into liquid media.[25] Most fabric and plush toy samples released 1–6% of their total silver mass, although there were some outliers at <1% and >35% (plush toy's interior foam). As described later in this section, almost all of the silver released is thought to be in ionic rather than particulate form. Results for the fabric were in agreement with Kulthong *et al.*'s[22] findings that the amount of silver leaching from commercial fabrics into different sweat formulations ranged from nondetectable levels to 4% of the fabric mass. Even though the baby blanket had a higher total silver concentration than the plush toy, the fraction released was lower than the plush toy's interior foam in all media (Figure 1). The methods used to incorporate silver into the baby blanket's polyester fibers and the plush toy's interior low-resilience polyurethane "memory" foam likely differ. It appears that silver is more strongly impregnated into or bound to the blanket fibers than to the plush toy's foam. Silver release was generally low (<1 mg Ag/kg product) for the plastic samples (breast milk storage bags and sippy cups).

Table 3. Amount of silver in each product and amount leached into relevant liquid media (mean ± standard error).

product	silver content (mg Ag/kg product)[a]	liquid media	amount of silver leached	
			mg Ag/kg product	%
plush toy: interior foam	48.2 ± 5.0	tap water	0.24 ± 0.02	0.5 ± 0.0
		saliva	1.77 ± 0.03	3.7 ± 0.1
		sweat	18.5 ± 1.1	38.3 ± 2.4
		urine	17.4 ± 0.8	36.1 ± 1.6
plush toy: exterior fur	0.6 ± 0.1	tap water	ND[b]	-
		saliva	0.03 ± 0.001	5.6 ± 0.2
		sweat	0.14 ± 0.002	2.6 ± 0.6
		urine	ND	-
baby blanket	109.8 ± 4.1	tap water	1.6 ± 0.3	1.5 ± 0.3
		saliva	1.2 ± 0.1	1.1 ± 0.1
		sweat	4.8 ± 0.3	4.4 ± 0.3
		urine	3.7 ± 0.3	3.4 ± 0.3
		HCl	4.7 ± 0.0	4.4 ± 0.0
		Saline	4.0 ± 0.0	3.7 ± 0.0
sippy cup #1: rubber ring	24.3 ± 2.9	milk formula	ND	-
		orange juice	0.41 ± 0.01	1.7± 0.0
sippy cup #1: transparent cap	9.4 ± 1.0	milk formula	ND	-
		orange juice	0.07 ± 0.01	0.7 ± 0.1
sippy cup #2: spout cover	2.1 ± 1.5	milk formula	0.93 ± 0.02	43.8 ± 0.9
		orange juice	ND	-
breast milk storage bags	0.9 ± 0.6	milk formula	ND[c]	-

[a]Described in detail in Tulve et al.[25]

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

[c]Because the milk formula was placed inside the bags, the product was not immersed into the leaching medium as with other products, so the amount reported is the silver concentration in the milk formula, not the amount leached per mass of product.

The amount of silver leached from a product varied with the type of media. Figure 1 shows the amount of silver leached from the baby blanket and the plush toy into six different leaching media. The plush toy was not tested in HCl and saline. The release of silver from the plush toy's interior foam into sweat and urine was much higher than that of the other product components and liquid media tested. Across all products (Table 3), synthetic sweat and urine yielded the largest amount of silver (up to 38% of the amount present in the products), while tap water yielded the lowest amount (≤1.5%). This was likely due to the high concentration of chloride (Cl^-, 185–210 mM), which catalyzes silver dissolution[34-36] in these media. Silver release from the blanket into saline and HCl solutions was similar to that into urine and sweat.

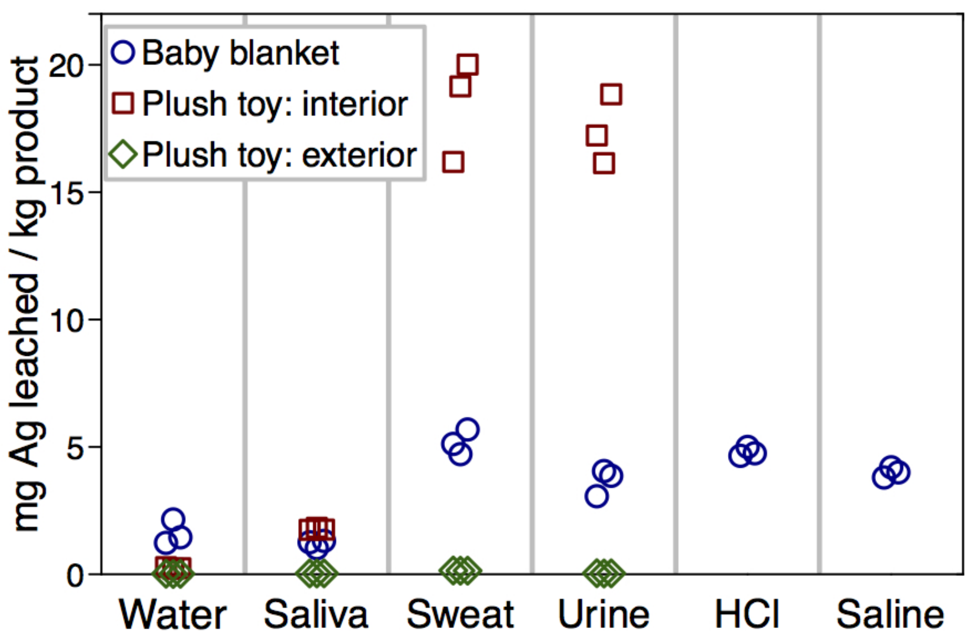


Figure 1. Amount of silver released into different leaching media (all data points shown). Data points are slightly offset to improve legibility.

Figure 2 shows the amount of silver released from fabric into various formulations of synthetic sweat. Silver dissolution can be limited at high pH and at low DO conditions because hydrogen ions and oxygen are consumed during the silver dissolution reaction,[34-36] but neither the pH nor the DO level changed during the soaking period, so these are not likely to have affected the results. The fraction of silver leached was slightly lower in the formulation lacking NaCl, indicating that other ingredients play a role in the dissolution reaction when NaCl is not present. According to a Tukey's HSD test ($\alpha = 0.05$), the only significantly different result is that with ultrapure water. The absence of urea and lactic acid individually did not affect leaching, but the absence of both (saline), resulted in slightly less leaching. This suggests the possibility that amine groups in urea and the carboxyl group in lactic acid might play competing roles in silver dissolution, in the absence of NaCl. Citrate, which contains three carboxyl groups, is a widely used stabilizing agent for silver nanoparticles.[36-38]

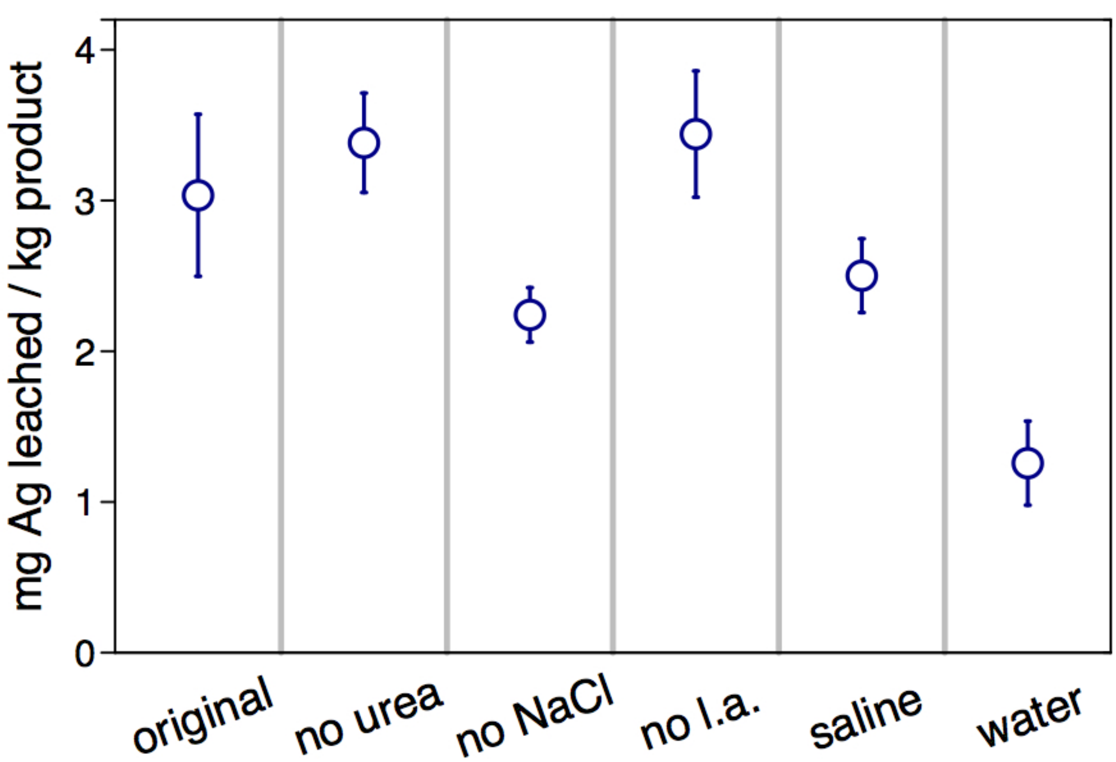


Figure 2. Silver release from fabric in various formulations of synthetic sweat. Average value of three data points and standard errors shown ("l.a." is lactic acid). The value for the original formulation shown here differs from that reported in Table 3 because this experiment was performed using a different blanket sample.

The average ionic fraction of silver in saliva, sweat, and urine for the plush toy's interior foam and baby blanket was 87.1 ± 2%. This fraction was not assessed for other products due to their low total silver concentration in the leachate. Considering that the 3-KDa filtering units used to separate ionic from particulate silver have absorbed up to 40% of ionic silver in an experiment performed by our group, using ultrapure water,[18] we can infer that most, if not all, of the silver released from these products was in dissolved form. Future studies using single-particle ICP-MS or field flow fractionation coupled with ICP-MS might be able to distinguish between particulate and dissolved silver with greater precision.

Leaching Kinetics During Product Use and Aging. Figure 3 shows the release of silver from the baby blanket into sweat over time. The amount of silver released reached a maximum in less than 5 min and then remained relatively constant; thus, extended exposure to sweat does not result in the release of more silver. Blanket samples that were soaked in synthetic sweat, rinsed with ultrapure water, and dried, with this cycle repeated three times, released less silver each time they were exposed to sweat. In each consecutive cycle, the samples released 4 ± 1, 0.39 ± 0.02, and 0.14 ± 0.01 mg/kg, totaling ~5% of the silver originally present in the fabric. For comparison, Benn and Westerhoff found that socks that were washed in ultrapure water in four 24-h cycles released silver in all washes, in some cases releasing almost all of their silver by the fourth wash.[21] Dissolution was not the only mechanism for silver release in their work because silver particles that became dislodged from the fabric were observed in the wash water. Their experiment did not aim to simulate the release of silver during product use, but to estimate how much silver would be released during washing.

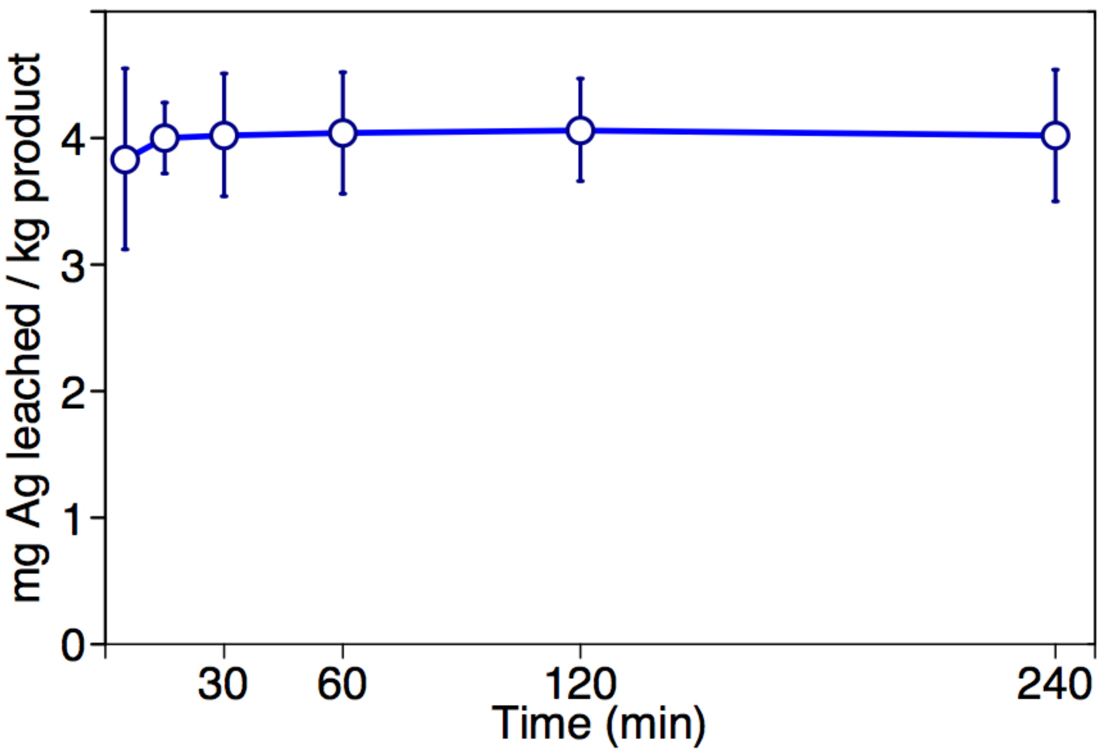


Figure 3. Silver release from fabric over time.

Blanket samples that had been previously soaked in synthetic sweat were coated with gold to enhance contrast and were visualized using SEM/EDS at 20 keV. Sparsely distributed silver particles ~500-nm in size were observed in backscattered mode (Figure 4) but not with the secondary electron detector. Since backscattered electrons travel from deeper within the sample than do secondary electrons, it is clear that these particles were inside the blanket's polyester fibers. A silver chloride aggregate was observed on the surface of a blanket fiber that had not been exposed to sweat.[25] Thus, it seems likely that silver dissolution was limited to the particles present on the surface or near the surface of the blanket fibers. Silver particles have been successfully grown within polyester fibers[39] or applied to polyester fibers after the removal of the fibers' surface finish with heat and caustic soda and before dying and finishing.[40] One implication of this result is that after an initial period of use, fabric products may no longer release measurable amounts of total silver because all silver on the surfaces of fibers may be depleted. We have not assessed whether such products maintain their antimicrobial properties after extended use.

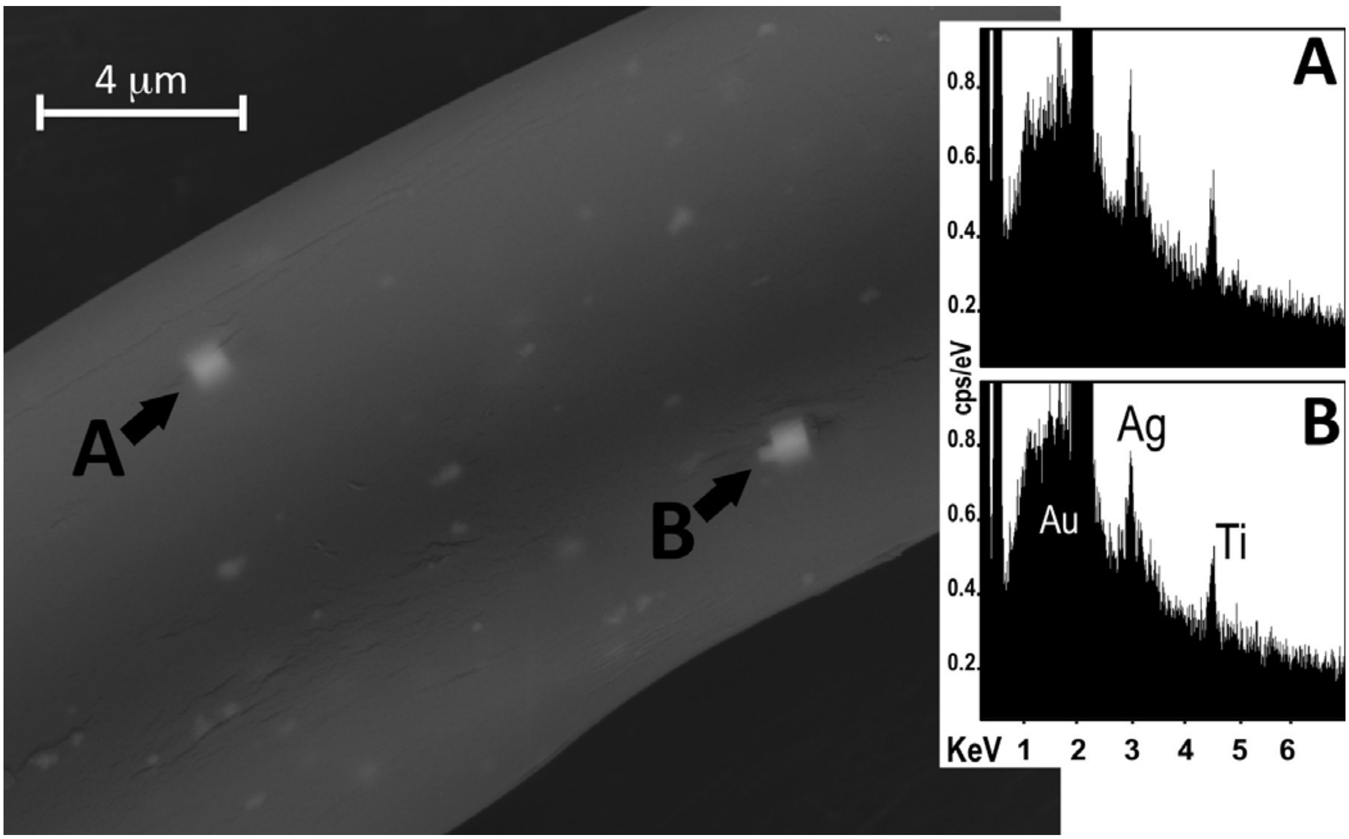


Figure 4. Silver particles (A and B) inside polyester fibers from the baby blanket observed in backscattered mode by SEM (left) and EDS spectra from particles A and B (right). The gold peak is from sample coating, and the titanium peak is likely associated with the smaller particles present on the fiber.

Figure 5 compares the silver released by new products versus those subjected to simulated aging. While no general trend is apparent, the aged samples soaked in sweat released more silver than new samples did. It is possible that fracturing of fibers during wear and tear contributed to increased silver release, and it is likely that the exposure of silver particles to UV radiation resulted in oxidation of the silver particles' surfaces, which is known to facilitate silver dissolution.[41]

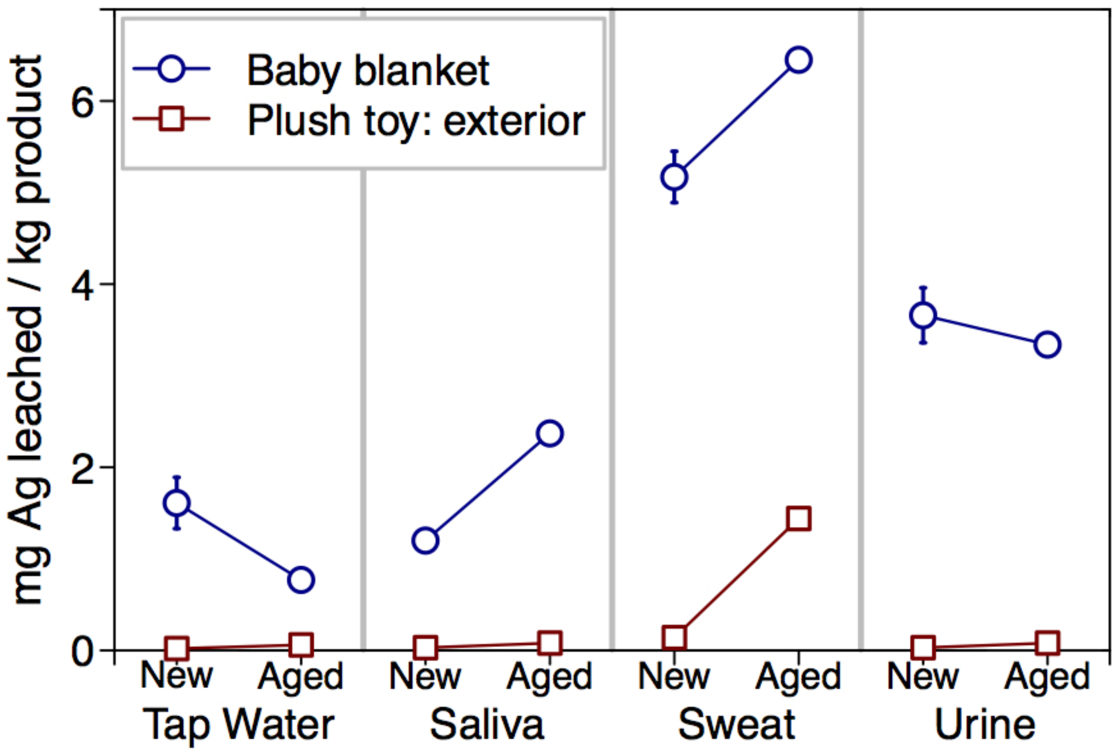


Figure 5. Comparison of silver leaching between new and aged products.

Silver Release onto Wipes. Table 4 shows the amount of silver that was transferred from products onto wipes, intended to assess the potential for dermal exposure. Values are shown in units of mass of silver transferred per area of product. The amount of silver transferred loosely follows the same ranking as the total amount of silver present in the products, except for the plush toy. It is possible that silver from the interior foam of this product migrated to the exterior fur and contributed to silver release onto wipes, that silver at the surface of the plush toy, though less concentrated, was less tightly bound to the fur fibers, or that the fibers' high surface area facilitated leaching. It is likely that the presence of chloride on the wipes used for this study facilitated the release of silver from the consumer products.

Table 4. Amount of silver transferred from surfaces onto dermal wipes (± standard error).

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

[a]Products were applied onto surface tiles, which were allowed to air-dry and were then wiped.

Silver Release from Humidifiers. Table S1 shows total silver concentrations in the reservoir water and in the vapor collected from each humidifier's output. We detected silver only in the reservoir water of the humidifier containing the silver accessory, at a very low concentration of 0.8 ± 0.2 ppb after 5 days of soaking, and not in the nanotechnology-based humidifiers. After 17 days of soaking, however, we no longer detected any silver in this sample. It is possible that over time, silver sorbed onto the humidifier reservoir's surfaces. The tabletop humidifier emitted 2.3 ± 0.7 ppb of silver in the condensed vapor, while the manual humidifier did not emit detectable levels of total silver. Benn and Westerhoff[20] reported concentrations in this range for two humidifiers: 0.19 (standard deviation not reported) and 1.1 ± 0.4 ppb.

Aerosol Release. Ambient aerosol concentrations were not significantly elevated above background levels (~3 - 6×10^{3} cm^{-3} for aerosols 14 - 750 nm and <150 cm^{-3} for aerosols 0.3 – 10 μm in diameter) during product use. If these products emit any form of silver-containing aerosols, the emission rates are very low.

Bioavailability of Nanosilver in Children's Consumer Products. Bioavailability of nanomaterials in environmental studies is a concept that does not yet have standard testing methods.[42-44] We assessed the bioavailability of silver to children by considering the concentration, size distribution, and location of silver within products,[25] as well as whether silver is released from the products into air, synthetic biological liquids, or onto skin. Based on these results and the intended use for each product, we ranked product categories according to their potential for silver bioavailability, from most likely to least likely to be a source of bioavailable silver: plush toy and fabric products (*e.g.*, teddy bear, clothing, blanket), cleaning products (*e.g.*, disinfecting spray and surface wipes), sippy cups, humidifiers, breast milk storage bag, and kitchen scrubber.

The levels of silver to which children may potentially be exposed during the normal use of these consumer products is predicted to be low, and bioavailable silver is expected to be in ionic rather than particulate form. A likely scenario is that areas of the fabric product that are exposed to sweat and urine will release small amounts of silver (<~5% for the products tested in this study) until the exposed silver particles become coated in silver chloride and cease releasing silver ions. The window of time in which –or likelihood that– all areas within a product become eventually exposed to sweat or urine has not been assessed and could extend beyond the product's lifetime.

To put our results into perspective, we compared the amount of silver released from products to the results of a study on the toxicity of ingested nanosilver in male rats.[10] Using three-quarters power scaling,[45] the equivalent dose for slight liver damage to be observed in a 10 kg child for short-term exposure to ingested nanosilver would be 1230 mg day^{-1}. In contrast, the amount of silver leached per kilogram of each product that we studied was at most 18.5 mg Ag kg^{-1} product. Even though actual doses cannot be estimated from our data, we can infer that the quantity of silver to which children would maximally be exposed is lower than the scaled dose at which slight liver damage would be expected.

There are currently no regulatory guidelines for silver in consumer products to serve as a comparison for this work. We have not studied the antimicrobial efficacy of these products before or after exposure to biological media, but we hypothesize that this protection is likely to be reduced after repeated wearing due to the observed decrease in silver solubility.

Further research is needed to understand the speciation of silver released into biologically relevant liquid media and the human body. Contradictory views on the toxicity of ionic versus nanoparticulate silver still exist. We recommend that cytotoxicity studies use low doses of particulate and ionic silver to assess realistic health effects associated with silver nanotechnology with a genetic toxicology approach focusing on changes in molecular mechanisms rather than median lethal dose (LD50) metrics.[46-48]

ASSOCIATED CONTENT

Supporting Information Available

Description of the leaching media and silver concentration in the humidifier's reservoir. This material is available free of charge via the Internet at http://pubs.acs.org/.

AUTHOR INFORMATION

Corresponding Author

* Phone: (540) 231-7095; fax (540) 231-7916; email: marinaeq@vt.edu. Department of Civil and Environmental Engineering, Virginia Tech, 418 Durham Hall, Blacksburg, VA 24061, United States

Present Addresses

†Institute for Critical Technology and Applied Science, Virginia Tech, 233 ICTAS I, Blacksburg, VA 24061, United States

Author Contributions

All authors contributed to the directions of the work, interpretation of results, and the preparation of this manuscript. All authors have given approval to the final version of the manuscript. ‡These authors collected the analytical data.

ACKNOWLEDGMENT

This work is part of an interagency agreement between the U.S. Environmental Protection Agency (EPA) and the U.S. Consumer Product Safety Commission (CPSC). The EPA, through its Office of Research and Development, partially funded and collaborated in the research described here under contract number RFQ-RT-10-00249 to Virginia Tech. Additional funding was provided by the National Science Foundation (NSF) under NSF Cooperative Agreement EF-0830093, Center for the Environmental Implications of NanoTechnology (CEINT) and Virginia

Tech's Institute for Critical Technology and Applied Science (ICTAS). This work has been subjected to EPA administrative review and approved for publication. The findings and conclusions in this paper are those of the authors and do not necessarily represent the views of the CPSC. Mention of trade names or commercial products does not constitute endorsement or recommendation for use, nor does it imply that alternative products are unavailable or unable to be substituted after appropriate evaluation.

The authors declare no competing financial interest.

References

1. Luoma, S. N. Silver nanotechnologies and the environment: Old problems or new challenges? Woodrow Wilson International Center for Scholars: September 2008, 2008; p 72.

2. Fabrega, J.; Luoma, S. N.; Tyler, C. R.; Galloway, T. S.; Lead, J. R., Silver nanoparticles: Behaviour and effects in the aquatic environment. Environ. Int. 2011, 37 (2), 517-531; DOI 10.1016/j.envint.2010.10.012.

3. Xiang, D. X.; Chen, Q.; Pang, L.; Zheng, C. L., Inhibitory effects of silver nanoparticles on H1N1 influenza a virus in vitro. J. Virol. Methods 2011, 178 (1-2), 137-142; DOI 10.1016/j.jviromet.2011.09.003.

4. Khaydarov, R. R.; Khaydarov, R. A.; Estrin, Y.; Evgrafova, S.; Scheper, T.; Endres, C.; Cho, S. Y., Silver nanoparticles: Environmental and human health impacts. In *Nanomaterials: Risks and benefits*, Linkov, I.; Steevens, J., Eds. Springer Sciences + Business Media: Faro, Portugal, 2009; pp 287-297.

5. Schacht, V. J.; Neumann, L. V.; Sandhi, S. K.; Chen, L.; Henning, T.; Klar, P. J.; Theophel, K.; Schnell, S.; Bunge, M., Effects of silver nanoparticles on microbial growth dynamics. J. Appl. Microbiol. 2013, 114 (1), 25-35; DOI 10.1111/jam.12000.

6. Quadros, M. E.; Marr, L. C., Environmental and human health risks of aerosolized silver nanoparticles. J. Air Waste Manage. Assoc. 2010, 60 (7), 770-781; DOI 10.3155/1047-3289.60.7.770.

7. Rejeski, D. An inventory of nanotechnology-based consumer products currently on the market. http://www.nanotechproject.org/inventories/consumer/ (accessed November 30th, 2008).

8. Hill, W. R.; Pillsbury, D. M., *Argyria: The pharmacology of silver*. The Williams & Wilkins company: 1939.

9. Drake, P. L.; Hazelwood, K. J., Exposure-related health effects of silver and silver compounds: A review. Ann. Occup. Hyg. 2005, 49 (7), 575-585; DOI 0003-4878.

10. Kim, Y. S.; Kim, J. S.; Cho, H. S.; Rha, D. S.; Kim, J. M.; Park, J. D.; Choi, B. S.; Lim, R.; Chang, H. K.; Chung, Y. H.; Kwon, I. H.; Jeong, J.; Han, B. S.; Yu, I. J., Twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in sprague-dawley rats. Inhalation Toxicol. 2008, 20 (6), 575-583; DOI 10.1080/08958370701874663.

11. Oberdorster, G.; Stone, V.; Donaldson, K., Toxicology of nanoparticles: A historical perspective. Nanotoxicology 2007, 1 (1), 2-25; DOI 10.1080/17435390701314761.

12. Oberdorster, G., Safety assessment for nanotechnology and nanomedicine: Concepts of nanotoxicology. Journal of Internal Medicine 2010, 267 (1), 89-105; DOI 10.1111/j.1365-2796.2009.02187.x.

13. Sung, J. H.; Ji, J. H.; Park, J. D.; Yoon, J. U.; Kim, D. S.; Jeon, K. S.; Song, M. Y.; Jeong, J.; Han, B. S.; Han, J. H.; Chung, Y. H.; Chang, H. K.; Lee, J. H.; Cho, M. H.; Kelman, B. J.; Yu, I. J., Subchronic inhalation toxicity of silver nanoparticles. Toxicol. Sci. 2009, 108 (2); DOI 452-461; 10.1093/toxsci/kfn246.

14. Soto, K. F.; Carrasco, A.; Powell, T. G.; Garza, K. M.; Murr, L. E., Comparative in vitro cytotoxicity assessment of some manufactured nanoparticulate materials characterized by transmission electron microscopy. J. Nanopart. Res. 2005, 7 (2-3), 145-169; DOI 10.1007/s11051-005-3473-1.

15. Becker, M.; Edwards, S.; Massey, R. I., Toxic chemicals in toys and children's products: Limitations of current responses and recommendations for government and industry. Environ. Sci. Technol. 2010, 44 (21), 7986-7991; DOI 10.1021/es1009407.

16. Xue, J.; Zartarian, V.; Moya, J.; Freeman, N.; Beamer, P.; Black, K.; Tulve, N.; Shalat, S., A meta-analysis of children's hand-to-mouth frequency data for estimating nondietary ingestion exposure. Risk Anal. 2007, 27 (2), 411-420; DOI 10.1111/j.1539-6924.2007.00893.x.

17. Guney, M.; Zagury, G. J., Toxic chemicals in toys and children's products. Environ. Sci. Technol. 2011, 45 (9), 3819-3819; DOI 10.1021/es200810s.

18. Quadros, M. E.; Marr, L. C., Silver nanoparticles and total aerosols emitted by nanotechnology-related consumer spray products. Environ. Sci. Technol. 2011, 45 (24), 10713-10719; DOI 10.1021/es202770m.

19. Hagendorfer, H.; Lorenz, C.; Kaegi, R.; Sinnet, B.; Gehrig, R.; Goetz, N. V.; Scheringer, M.; Ludwig, C.; Ulrich, A., Size-fractionated characterization and quantification of nanoparticle release rates from a consumer spray product containing engineered nanoparticles. J. Nanopart. Res. 2010, 12 (7), 2481-2494; DOI 10.1007/s11051-009-9816-6.

20. Benn, T.; Cavanagh, B.; Hristovski, K.; Posner, J. D.; Westerhoff, P., The release of nanosilver from consumer products used in the home. J. Environ. Qual. 2010, 39 (6), 8; DOI 10.2134/jeq2009.0363.

21. Benn, T. M.; Westerhoff, P., Nanoparticle silver released into water from commercially available sock fabrics. Environ. Sci. Technol. 2008, 42 (11), 4133-4139; DOI 10.1021/es7032718.

22. Kulthong, K.; Srisung, S.; Boonpavanitchakul, K.; Kangwansupamonkon, W.; Maniratanachote, R., Determination of silver nanoparticle release from antibacterial fabrics into artificial sweat. Part. Fibre Toxicol. 2010, 7; DOI 10.1186/1743-8977-7-8.

23. Klaine, S. J.; Alvarez, P. J. J.; Batley, G. E.; Fernandes, T. F.; Handy, R. D.; Lyon, D. Y.; Mahendra, S.; McLaughlin, M. J.; Lead, J. R., Nanomaterials in the environment: Behavior, fate, bioavailability, and effects. Environ. Toxicol. Chem. 2008, 27 (9), 1825-1851; DOI 10.1897/08-090.1.

24. Levard, C. m.; Hotze, E. M.; Lowry, G. V.; Brown, G. E., Environmental transformations of silver nanoparticles: Impact on stability and toxicity. Environ. Sci. Technol. 2012; DOI 10.1021/es2037405.

25. Tulve, N. S.; Stefaniak, A. B.; Quadros, M. E.; Rogers, K.; Mwilu, S.; LeBouf, R. F.; Schwegler-Berry, D.; Willis, R. D.; Thomas, T. A.; Marr, L. C., Potential exposure to silver nanoparticles in consumer products found in children's everyday environments. In prep.

26. Gal, J. Y.; Fovet, Y.; Adib-Yadzi, M., About a synthetic saliva for in vitro studies. Talanta 2001, 53 (6), 1103-1115; DOI 10.1016/S0039-9140(00)00618-4.

27. Mayrovitz, H. N.; Sims, N., Biophysical effects of water and synthetic urine on skin. Adv. Skin Wound Care 2001, 14 (6), 7; DOI 10.1097/00129334-200111000-00013.

28. ASTM, F963-08. Standard consumer safety specification for toy safety. West Conshohocken, PA, 2008; Vol. F963-08.

29. Erickson, H. P., Size and shape of protein molecules at the nanometer level determined by sedimentation, gel filtration, and electron microscopy. Biol. Proced. Online 2009, 11 (1); DOI 32-51; 10.1007/s12575-009-9008-x.

30. Fenske, R. A., Dermal exposure assessment techniques. Ann. Occup. Hyg. 1993, 37 (6), 687-706; DOI 10.1093/annhyg/37.6.687.

31. Ferguson, A. C.; Canales, R. A.; Leckie, J. O., Dermal exposure, uptake, and dose. In *Exposure analysis*, Ott, W. R.; Steinemann, A. C.; Wallace, L. A., Eds. CRC Press: Boca Raton, FL, 2007; pp 255 - 284.

32. Bai, Z. P.; Yiin, L. M.; Rich, D. Q.; Adgate, J. L.; Ashley, P. J.; Lioy, P. J.; Rhoads, G. G.; Zhang, J. F., Field evaluation and comparison of five methods of sampling lead dust on carpets. Aiha Journal 2003, 64 (4), 528-532.

33. NIOSH, 9102 - elements on wipes. NIOSH Manual of Analytical Methods (NMAM), Fourth Edition, 2003; p 5.

34. Kent, R. D.; Vikesland, P. J., Controlled evaluation of silver nanoparticle dissolution using atomic force microscopy. Environ. Sci. Technol. 2011; DOI 10.1021/es203475a.

35. Liu, J.; Hurt, R. H., Ion release kinetics and particle persistence in aqueous nano-silver colloids. Environ. Sci. Technol. 2010, 44 (6), 2169-2175; DOI 10.1021/es9035557.

36. Zhang, W.; Yao, Y.; Sullivan, N.; Chen, Y. S., Modeling the primary size effects of citrate-coated silver nanoparticles on their ion release kinetics. Environ. Sci. Technol. 2011, 45 (10), 4422-4428; DOI 10.1021/es104205a.

37. Dong, X. Y.; Ji, X. H.; Wu, H. L.; Zhao, L. L.; Li, J.; Yang, W. S., Shape control of silver nanoparticles by stepwise citrate reduction. J. Phys. Chem. C 2009, 113 (16), 6573-6576; DOI 10.1021/jp900775b.

38. Henglein, A.; Giersig, M., Formation of colloidal silver nanoparticles: Capping action of citrate. J. Phys. Chem. B 1999, 103 (44), 9533-9539; DOI 10.1021/jp9925334.

39. Silva, A. M. B.; de Araujo, C. B.; Santos-Silva, S.; Galembeck, A., Silver nanoparticle in situ growth within crosslinked poly(ester-co-styrene) induced by UV irradiation: Aggregation control with exposure time. J. Phys. Chem. Solids. 2007, 68 (5-6), 729-733; DOI 10.1016/j.jpcs.2007.03.052.

40. Ali, S. W.; Rajendran, S.; Joshi, M., Synthesis and characterization of chitosan and silver loaded chitosan nanoparticles for bioactive polyester. Carbohyd. Polym. 2011, 83 (2), 438-446; DOI 10.1016/j.carbpol.2010.08.004.

41. Gorham, J. M.; MacCuspie, R. I.; Klein, K. L.; Fairbrother, D. H.; Holbrook, R. D., UV-induced photochemical transformations of citrate-capped silver nanoparticle suspensions. J. Nanopart. Res. 2012, 14, (10); DOI 10.1007/s11051-012-1139-3.

42. SERDP and ESTCP. Expert panel workshop on research and development needs for understanding and assessing the bioavailability of contaminants in soils and sediments; Annapolis, Maryland, 2008;

43. Semple, K. T.; Doick, K. J.; Jones, K. C.; Burauel, P.; Craven, A.; Harms, H., Defining bioavailability and bioaccessibility of contaminated soil and sediment is complicated. Environ. Sci. Technol. 2004, 38 (12), 228A-231A;

44. Ehlers, L. J.; Luthy, R. G., Contaminant bioavailability in soil and sediment. Environ. Sci. Technol. 2003, 37 (15), 295A-302A;

45. *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose*; Office of the Science Advisor; Risk Assessment Forum; United States Environmental Protection Agency: Washington, DC, 2011; http://www.epa.gov/raf/publications/pdfs/recommended-use-of-bw34.pdf.

46. Arora, S.; Jain, J.; Rajwade, J. M.; Paknikar, K. M., Cellular responses induced by silver nanoparticles: In vitro studies. Toxicol. Lett. 2008, 179 (2), 93-100; DOI 10.1016/j.toxlet.2008.04.009.

47. Johnston, H. J.; Hutchison, G.; Christensen, F. M.; Peters, S.; Hankin, S.; Stone, V., A review of the in vivo and in vitro toxicity of silver and gold particulates: Particle attributes and biological mechanisms responsible for the observed toxicity. Crit. Rev. Toxicol. 2010, 40 (4), 328-346; DOI 10.3109/10408440903453074.

48. Mohamed, B. M.; Verma, N. K.; Davies, A. M.; McGowan, A.; Staunton, K. C.; Prina-Mello, A.; Kelleher, D.; Botting, C. H.; Causey, C. P.; Thompson, P. R.; Pruijn, G. J.; Kisin, E. R.; Tkach, A. V.; Shvedova, A. A.; Volkov, Y., Citrullination of proteins: A common post-translational modification pathway induced by different nanoparticles in vitro and in vivo. Nanomedicine 2012; DOI 10.2217/nnm.11.177.

Release of silver from nanotechnology-based consumer products for children

- SUPPORTING INFORMATION -

*Marina E. Quadros[1], Raymond Pierson[1], Nicolle S. Tulve[2], Robert Willis[2], Kim Rogers[3], Treye A. Thomas[4], Linsey C. Marr[1]**

[1]Department of Civil and Environmental Engineering, Virginia Tech, 418 Durham Hall, Blacksburg, VA 24061, United States

[2]National Exposure Research Laboratory, Office of Research and Development, United States Environmental Protection Agency, 109 TW Alexander Dr., Research Triangle Park, NC 27711, United States

[3]National Exposure Research Laboratory, Office of Research and Development, United States Environmental Protection Agency, 944 East Harmon Ave., Las Vegas, NV 89119, United States

[4]Consumer Product Safety Commission, 4330 East-West Highway, Bethesda, MD 20814, United States

*Corresponding author phone: (540) 231-6071, fax: (540) 231-7916, e-mail: lmarr@vt.edu

This supporting information document contains the following information:

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

Number of pages: 6

Number of figures: 0

Number of tables: 2

DESCRIPTION OF PRODUCTS TESTED

Table S1. Description of products and product parts with indication of silver detection. Further described in Tulve et al.[1]

product description	product parts tested	silver detected
1. plush toy	1. exterior fur (new)	yes
	2. exterior fur (aged)	yes
	3. interior foam	yes
2. baby blanket (new)	4. blanket (new)	yes
	5. blanket (aged)	yes
3. sleep suit	6. sleep suit	yes
4. baby scratch mitts	7. scratch mitts	yes
5. breast milk storage bags	8. breast milk storage bags	yes
6. sippy cup #1	9. ouside of cup	no
	10. inside of cup	no
	11. rubber ring	yes
	12. plastic rim	yes
	13. transparent cap	yes
7. sippy cup #2	14. ouside of cup	no
	15. inside of cup	no
	16. cap	no
	17. spout cover	no
8. disinfecting spray	18. liquid content	yes
9. surface wipes	19. wipes	yes
10. kitchen scrubber	20. scrubber	yes
11. tabletop ultrasonic humidifier	21. reservoir water	no
12. manual ultrasonic humidifier	22. reservoir water	no
13. humidifier accessory cube	23. reservoir water	yes

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

3. **Orange juice**

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

4. **Synthetic sweat**

We used a synthetic sweat formulation suggested by Kulthong et al.,[2] who assessed the release of silver nanoparticles from a fabric into four sweat formulations. We chose the formulation described by the European Standard (EN1811-1999) because it resulted in the highest amount of silver released in the Kulthong study.
The formulation of the synthetic sweat is:

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

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

5. **Synthetic urine**

We used a synthetic urine formulation described by Mayrovitz and Sims:[3]

- 25 g/L Urea (CH_4N_2O)
- 9 g/L Sodium chloride (NaCl)
- 3 g/L Ammonium chloride (NH_4Cl)
- 2.5 g/l Disodium hydrogen orthophosphate, anhydrous (Na_2HPO_4)

- 2 g/L Creatinine ($C_4H_7N_3O$)
- 3 g/L Sodium sulfite, hydrated (Na_2SO_3, $7H_2O$)

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

6. Synthetic saliva

We used a synthetic saliva formulation described by Gal et al.:[4]

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

We used ultrapure water to prepare this medium, which had a pH of 7.0. This medium was bubbled with air for ~ 2 min before use in order to saturate it with oxygen. Experiments using saliva were performed in a water bath at ~37ºC.

7. Saline

We prepared a simple saline solution (9 g/L NaCl in ultrapure water, pH 7.0) to use as a leaching medium for comparison with the synthetic saliva. We used ultrapure water to prepare this medium, which had a pH of 7.0. This medium was bubbled with air for ~ 2 min before use in order to saturate it with oxygen.

8. ASTM F963-08 media (digestion substitute)

As with the saline solution, we prepared a 0.07 M hydrochloric acid solution to use as a leaching medium for comparison with other synthetic media. ASTM[5] recommends using this medium "to simulate the situation in which materials stay 4 h in the alimentary tract after swallowing." We used ultrapure water to prepare this medium, which had a pH of 1.4 and bubbled it with air for ~ 2 min before use in order to saturate it with oxygen.

SILVER CONCENTRATION IN HUMIDIFIERS' RESERVOIR

Table S2. Silver concentrations in humidifier reservoir water after different periods of soaking.

product	soaking time (days)	silver concentration in reservoir (ppb)	silver concentration in vapor (ppb)
tabletop ultrasonic humidifier	6	ND[a]	2.3 ± 0.7
	18		
manual ultrasonic humidifier	6	ND	ND
	18	ND	
humidifier accessory cube (into a "non-nano" humidifier)	5	0.8 ± 0.2	ND
	17	ND	
non-nano humidifier	5	ND	ND

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

REFERENCES

Tulve, N. S.; Stefaniak, A. B.; Quadros, M. E.; Rogers, K.; Mwilu, S.; LeBouf, R. F.; Schwegler-Berry, D.; Willis, R. D.; Thomas, T. A.; Marr, L. C., Potential exposure to silver nanoparticles in consumer products found in children's everyday environments. In Prep.

Kulthong, K.; Srisung, S.; Boonpavanitchakul, K.; Kangwansupamonkon, W.; Maniratanachote, R., Determination of silver nanoparticle release from antibacterial fabrics into artificial sweat. *Part. Fibre Toxicol.* **2010,** *7*.

Mayrovitz, H. N.; Sims, N., Biophysical effects of water and synthetic urine on skin. *Adv Skin Wound Care* **2001,** *14*, (6), 7.

Gal, J. Y.; Fovet, Y.; Adib-Yadzi, M., About a synthetic saliva for in vitro studies. *Talanta* **2001,** *53*, (6), 1103-1115.

ASTM, F963-08. Standard Consumer Safety Specification for Toy Safety. In West Conshohocken, PA, 2008; Vol. F963-08.