Draft Decision Document

Proposed Conditional Registration of Nanosilva as a Materials Preservative in Textiles and Plastics

August 27, 2013

Environmental Protection Agency
Office of Pesticide Programs
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TABLE OF CONTENTS

LIST OF TABLES

EXECUTIVE SUMMARY

EPA is proposing to grant a conditional registration for the Nanosilva LLC product named "Nanosilva" with a product code of NSPW-L30SS (hereafter referred to as "Nanosilva") under section 3(c)(7)(C) of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). The Agency's basis for the conditional registration is that:

- Nanosilva contains nanosilver as an active ingredient and the nanosilver in Nanosilva is not in any currently registered pesticide;
- Use of Nanosilva will not cause unreasonable adverse effects on the environment during the period when newly required data are being developed;
- Insufficient time has elapsed for Nanosilva to generate and submit the newly required data; and
- Use of Nanosilva is in the public interest.

Nanosilva, which contains 1% nanosilver by weight, is the subject of an application submitted by Nanosilva LLC in August 2009 (MRID 47828900). Nanosilva LLC requested that the nanosilver in Nanosilva be registered as a new active ingredient because it was not an active ingredient in any currently registered pesticide product. Nanosilva is a silica-sulfur-nanosilver complex where the nanosilver active-ingredient is attached to crystalline silica via a thiolate bond. The diameter of the spherical silica core is 320 nm on average and the nanosilver particles that are attached to the silica core have mean diameters between 6.9 and 10.6 nm. Nanosilva is proposed to be incorporated into plastics and textiles to suppress the growth of bacteria, algae, fungus, mold and mildew, which cause odors, discoloration, stains, and deterioration. The plastics and textiles will contain less than 0.003% silver by weight.

EPA is proposing to make a registration decision for non-food contact uses of Nanosilva incorporated into plastic films, sheets, slabs, and molded parts, and textiles. Consistent with this, EPA is proposing to register Nanosilva for indoor use articles such as automotive parts, floor coverings, footwear, sportswear, uniforms and outdoor use articles such as furniture, decking, carpeting, and house siding.

EPA determined that workers, consumers, and the environment could be exposed to:

1) Silver ions released from Nanosilva;
2) the Nanosilva complex; and/or
3) Nanosilver that might break away from the Nanosilva complex.

In evaluating the risk from exposure to silver ions, EPA relied on the existing reregistration decision for silver which concluded that the human health or ecological risk from exposure to silver ions derived from plastics and textiles incorporating Nanosilva are not of concern.

In evaluating the risk from short-term exposure to Nanosilva, Nanosilva LLC submitted results from acute animal-toxicity tests completed using high-level doses of Nanosilva showing that

there were no mortalities or abnormalities in test animals after administration of Nanosilva by oral, dermal, and inhalation exposure routes. Nanosilva caused moderate irritation to the eyes of test animals, and was not a skin sensitizer. Based on these results, shipping containers filled with Nanosilva are required to carry a label stating "CAUTION" where contact with eyes or clothing should be avoided. Nanosilva LLC submitted a request to waive the required intermediate-term toxicity studies because silver at concentrations greater than the analytical detection limit were not found leaching from plastic coupons incorporating Nanosilva and because of the lack of toxicity noted in the acute animal-toxicity tests completed using high-level doses of Nanosilva.

To evaluate occupational exposure to the nanosilver that might break away from the Nanosilva complex, EPA calculated the daily-dose to workers assuming that all the silver in Nanosilva was freely available as nanosilver. EPA believes that this assumption overestimate the daily-dose of nanosilver that a person could potentially receive when working with Nanosilva. To evaluate consumer exposure to the nanosilver that might break away from plastics and textiles incorporating Nanosilva, Nanosilva LLC submitted studies showing that silver at concentrations greater than the analytical detection limit were not found leaching from plastic coupons and shirts incorporating Nanosilva. Thus, there is little exposure to nanosilver from plastics and textiles incorporating Nanosilva. EPA assumed that silver leaching from plastic coupons and shirts incorporating Nanosilva was at one-half the analytical detection limit and was in the form of nanosilver as found in Nanosilva.

There are no intermediate- or long-term human or environmental toxicity studies available for Nanosilva or for the nanosilver released from products incorporating Nanosilva. In the absence of intermediate-term toxicity studies, EPA evaluated the risk from occupational and consumer exposure using intermediate-term hazard data available in the scientific literature for nanosilver. Because of the potential difference between the nanosilver in the available toxicity data and the nanosilver in Nanosilva along with the lack of an acceptable developmental and reproductive toxicity study for nanosilver, the Agency used a maximum 10-fold database uncertainty factor when evaluating the risk from exposure to Nanosilva and the nanosilver released from products incorporating Nanosilva.

Impact to the environment was based on ecotoxicity studies available in the scientific literature for nanosilver and environmental exposure from indoor use was assessed assuming that 300 million people (U.S. population) each purchased one t-shirt containing Nanosilva and from outdoor use was assessed assuming that 1% of the projected yearly production of plastic lumber in the U.S. would contain Nanosilva.

Using robust toxicity data on nanosilver, conservative occupational exposure assumptions, leaching data showing minimal consumer exposure to nanosilver, and maximum values for risk uncertainty factors, EPA is able to determine that for the period of conditional registration, there is a low probability of adverse risk to human health and the environment from plastics and textiles incorporating Nanosilva. Thus, the Agency concludes that use of Nanosilva will not

cause unreasonable adverse effects on the environment during the period when newly required data are being developed.

As a condition of the proposed registration, EPA is proposing to require that Nanosilva LLC conduct studies during the period of conditional registration to better characterize the nanosilver in Nanosilva. In addition, EPA is proposing to require the following inhalation route-specific studies:

- 90-Day Inhalation Toxicity (Rat) (OCSPP 870.3465) modified to include *in vivo* bone marrow assay and functional observational battery, motor activity and detailed neuropathology
- Reproduction/Developmental Toxicity Screening Test (Modified OCSPP 870.3550/ OECD TG 421)

Although there is no risk concern for workers who use close-system loading when mixing and loading the Nanosilva liquid suspension, EPA is proposing to require these tests to confirm the adequacy of the 10-fold database uncertainty factor, to reduce the uncertainties related to differences in the physical properties of the nanosilver, and because there are currently no acceptable studies on the reproductive and developmental toxicity for nanosilver.

These studies must be completed within four years after issuing the registration. This time period was chosen to allow time for protocol reviews prior to initiation of the studies, completion of the studies, and Agency review of the study results. The Agency will evaluate these data as they are submitted to confirm the Agency's determination that the use of Nanosilva will not cause unreasonable adverse effects to human health and the environment. If Nanosilva LLC fails to take appropriate steps to initiate the required studies, or if Nanosilva LLC fails to submit the protocols or data, EPA is proposing to issue a notice of intent to cancel Nanosilva's registration under FIFRA section 6(e). In addition, the conditional registration for Nanosilva will automatically expire four years after being issued. Nanosilva LLC should request an amendment to remove the expiration date once they have submitted the required data if they wish to continue to sell and distribute Nanosilva in the United States.

EPA believes that the use of Nanosilva is in the public interest. Use of Nanosilva may lead to less environmental loading of silver as compared to currently registered products with the same use patterns. In addition, Nanosilva appears to offer prolonged ability to suppress the growth of odor and stain causing bacteria as compared to currently registered products containing silver salts with the same use patterns.

I. REGULATORY DECISION SUMMARY

EPA is proposing to grant a conditional registration for the Nanosilva LLC product named "Nanosilva" under section 3(c)(7)(C) of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). Nanosilva, which contains 1% nanosilver by weight, is the subject of an application submitted by Nanosilva LLC in August 2009 (MRID 47828900). Nanosilva LLC requested that the nanosilver in Nanosilva be registered as a new active ingredient because it was not an active ingredient in any currently registered pesticide product.

As described in the risk assessment sections of this document, EPA has determined that there is a low probability of adverse risk to human health and the environment from plastics and textiles incorporating Nanosilva during the period required for developing and submitting protocols for review, conducting the studies, and submitting the resulting data, as well as EPA's review of the submitted data. Thus, the Agency concludes that use of Nanosilva will not cause unreasonable adverse effects on the environment during the period when newly required data are being developed.

In August, 2009 Nanosilva LLC submitted acute toxicity studies required under 40 CFR part 161, a study that determined the amount of silver leaching from plastic coupons incorporating Nanosilva, and a request to waive required studies. The waiver request was based on the low exposure to Nanosilva given that the product was proposed to be embedded in plastic and the submitted leaching study for a plastic incorporating Nanosilva indicated that silver was not released at concentrations above the analytical detection limit. In May 2013, Nanosilva LLC also submitted a study showing that silver was not released at concentrations above the analytical detection limit from textiles incorporating Nanosilva. EPA's proposal is to grant the registration request with requirements for new data as terms and conditions on the Nanosilva registration. Nanosilva LLC, however, has not had sufficient time to generate and submit these newly required data.

For a variety of reasons, EPA has determined that use of Nanosilva is in the public interest. EPA believes that Nanosilva LLC's product may lead to less environmental loading of silver as compared to currently registered products containing silver salts with the same use patterns. In addition, Nanosilva appears to offer prolonged ability to suppress the growth of odor and stain causing bacteria as compared to currently registered products containing silver salts with the same use patterns. Moreover, because we can find that a section 3(c)(7)(C) registration is unlikely to cause unreasonable adverse effects on the environment during the time while Nanosilva LLC is generating the newly required data, allowing such registration to be granted before the newly-required data is generated allows the Nanosilva LLC product to compete with other like-situated products. Allowing Nanosilva LLC's product on the market pending generation of data allows Nanosilva LLC to participate in the plastics and textile economy along

with the other registrants with like-situated products, and allows consumers of these products a new choice among the potential like-situated products.

EPA intends to require Nanosilva LLC to conduct a number of studies as a condition of registration. These studies must be completed within a time duration of four years which was chosen to allow time for protocol reviews prior to initiation of the studies, completion of the studies, and Agency review of the study results. The Agency will evaluate these data as they are submitted during the period of the conditional registration to confirm the Agency's risk assessment. If Nanosilva LLC fails to take appropriate steps to initiate the required studies, or if Nanosilva LLC fails to submit the protocols or data, EPA has the authority to issue a notice of intent to cancel Nanosilva LLC's registration under FIFRA section 6(e). In addition, Nanosilva LLC's conditional registration for Nanosilva will automatically expire four years after being issued. Nanosilva LLC will have to submit an application to obtain an unconditional registration if they wish to continue to sell and distribute Nanosilva in the United States.

II. BACKGROUND

2.1 Regulatory History

In August 2009, Nanosilva LLC submitted an application for the registration of a new antimicrobial pesticide product named Nanosilva, a nanosilver-based product that was proposed for use as a materials preservative additive with no food contact uses. Nanosilva LLC's application claimed that Nanosilva was a new active ingredient not contained in any currently registered silver-based pesticide products and should be given a registration under FIFRA section 3(c)(5) (MRID 47828900).

2.2 FIFRA Scientific Advisory Panel (SAP) Meeting

In November, 2009 the Agency convened a meeting of the FIFRA Scientific Advisory Panel (SAP) to address a number of questions associated with assessing the hazard of and exposure to nanosilver and other nanoscale metal-based pesticides (FIFRA SAP, 2009). In general, the SAP advised that the toxicity of nanosilver could differ from and might be higher than other forms of silver (e.g., silver ions).

The SAP was unsupportive of bridging among silver-based materials with different properties. However, the SAP indicated that bridging would be appropriate for materials of similar size and essentially identical physical properties and that bridging between silver ions released from nanosilver and the existing database for silver ions is feasible (FIFRA SAP, 2009). The SAP cautioned about extrapolating from one nanosilver formulation to another when assessing hazards because differences in particle formulation (e.g., coating and inert ingredients) are likely to affect biological activity, among other things.

The SAP commented that not enough literature is available to draw any firm conclusions regarding human (occupational or consumer) and environmental exposures to nanosilver under realistic use scenarios. Potentially, three major routes exist for human exposure to nanoparticles: oral, inhalation, and dermal. Only a few studies in rodents are known that investigate the *toxicity* of nanosilver from exposure by these routes. Nor is there much information on the *level* of human exposure to nanosilver by these routes, for either workers or consumers using products containing nanosilver. The same situation exists for the environmental fate and transport of nanosilver. The ability to measure concentrations of nanosilver in the environment along with the environmental exposure pathways, bioavailability, toxicity, and potential impact of nanosilver on ecological systems are not well quantified. Furthermore, little or no information on the fate of nanosilver in soils and sediments was found. As a result, the SAP recommended a case-by-case approach to hazard and exposure assessment (i.e., product-by-product). The SAP also advised that existing requirements may have to be adjusted to obtain data appropriate to assess the fate, degradation, metabolism, mobility, dissipation, and accumulation of nanomaterials.

The SAP report further suggested that existing information on conventional silver could be useful but would not necessarily be sufficient in assessing potential nanosilver risks. The SAP recommended that the Agency treat nanosilver differently from its conventional silver counterpart in evaluating proposed nanosilver product applications (in terms of both data requirements and the conduct of risk assessments). Moreover, the Panel recommended that EPA require additional data on the physical chemistry, exposure potential, and the potential hazard to human health and the environment.

Historically, EPA has considered applications for pesticide products that claim to be identical or substantially similar in composition to a registered product as so-called “me-too registrations” under FIFRA registration authorities. Until recently, EPA generally has not focused on the size or surface coating of an ingredient as attributes relevant to determining if the product in an application is identical or substantially similar in composition to a registered pesticide product. However, a nanoscale ingredient may have properties that are different from those of conventionally-scaled ingredients and properties that differ from the atoms or molecules from which the nanoscale ingredient is constructed. Therefore, a nanoscale ingredient may also have different environmental health and safety properties. Accordingly, for a product containing an ingredient that is a nanoscale version of a conventionally-sized active or inert ingredient contained in an already-registered product or a different nanoscale version of a nanoscale material that is an active or inert ingredient in an already registered pesticide product, EPA necessarily will need to assess the nanoscale material and may require additional data to make the requisite statutory findings.

III. PRODUCT DESCRIPTION, TESTING, AND USE

3.1 Product Description

Nanosilva (NSPW-L30SS) is a liquid suspension containing silica-sulfur-nanosilver particulates where the nanosilver active-ingredient is attached to crystalline silica via a thiolate bond. The particles are formed by reacting silver nitrate and polyvinylpyrrolidone (PVP) with spherical silica particles that have been modified with thiol groups and are suspended in ethanol (Lee et al. 2007). The diameter of the spherical silica core particles are 320 nm on average (Lee et al., 2006). The nanosilver particles form with time and have an average diameter of 6.9 nm (minimum diameter of 3 nm and maximum diameter of 12 nm) after 10 minutes and 10.6 nm (minimum diameter of 5 nm and maximum diameter of 18 nm) after 2 hours (Lee et al., 2007). The liquid suspension contains 1% nanosilver by weight with average diameters between 6.9 and 10.6 nm (minimum diameter of 3 nm and maximum diameter of 18 nm), where the nanosilver surface is coated with sulfur and PVP, and is attached to silica.

The proposed labels states that Nanosilva (NSPW-L30SS), which contains 1% nanosilver by weight, is to be formulated into a polymeric intermediate known as a master batch. Nanosilva LLC proposed that the final product contain between 5 and 15% by weight of the polymeric intermediate or master batch that contains Nanosilva. Although not stated on the proposed label, the final product that a consumer may be exposed to will contain less than 0.003% nanosilver by weight. EPA is proposing that Nanosilva LLC state on the pesticide label that products incorporating Nanosilva contain a maximum of 0.003% nanosilver by weight.

3.2 Product Use

Nanosilva LLC is proposing that Nanosilva be incorporated into polymer and polymer based products to suppress the growth of bacteria, algae, fungus, mold and mildew, which cause odors, discoloration, stains, and deterioration. Nanosilva LLC proposed a wide range of non-food contact use categories for Nanosilva including house wares, building materials, bathroom fixtures and accessories, electronics and appliances, personal care products, automotive equipment, hospital and institutional facility equipment, sporting goods, and textiles.

3.3 Product Testing and Waivers

As an applicant for registration of a new active ingredient in 2009, Nanosilva LLC was required to submit all the applicable information and studies under 40 CFR part 161. Nanosilva LLC submitted results from testing of Nanosilva to determine the product identity and composition, physical and chemical properties, and acute toxicity as summarized in Table 1. Some of these tests were completed using standard EPA test guidelines[1] prior to consulting with EPA. Although

[1] Available at http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm

these test results provided useful information for Nanosilva, the guidelines on which they are based have not been adapted generally for use with nanoscale particles. EPA anticipates that these guidelines will require revision going forward in terms of their application to nanoscale materials. As a result, it is recommended that future applicants for products containing nanoscale materials consult with the EPA prior to performing any tests.

Table 1 – Product Testing for the Nanosilva Liquid Suspension

Guideline Number	Guideline Name	Required under 40 CFR	Nanosilva LLC Submission (MRID)
Product Identity and Composition			
830.1550	Product identity and composition	161.155	47828901
830.1600	Description of materials to produce product	161.160	47828902
830.1620	Description of production process	161.162	47828903
830.1650	Description of formulation process	161.165	47828904
830.1670	Discussion of formulation of impurities	161.167	47828905
830.1700	Preliminary analysis	161.170	47828906
830.1750	Certified limits	161.175	47828907
830.1800	Enforcement analytical method	161.180	47828908
Physical and Chemical Properties			
830.6302	Color	161.190	47828909
830.6303	Physical state	161.190	47828910
830.6304	Odor	161.190	47828911
830.6317	Storage stability	161.190	47828912
830.6320	Corrosion characteristics	161.190	47828912
830.7000	pH	161.190	47828913
830.7100	Viscosity	161.190	47828914
830.7200	Melting point/melting range	161.190	
830.7220	Boiling Point/Boiling Range		47828915
830.7300	Density	161.190	47828916
830.7520	Particle size, fiber length, and diameter distribution	Not Required, information in Lee et al., 2006 and 2007	
830.7840	Water solubility	161.190	47828917
Health Effects (Toxicology)			
870.1100	Acute Oral Toxicity	161.340	47828918
870.1200	Acute Dermal Toxicity	161.340	47828919
870.1300	Acute Inhalation Toxicity	161.340	47828920
870.2400	Acute Eye Irritation	161.340	47828921
870.2500	Acute Dermal Irritation	161.340	47828922
870.2600	Skin Sensitization	161.340	47828923

In addition to the test results listed in Table 1, Nanosilva LLC submitted the following study to support the registration of Nanosilva:

Plastic Leaching Study

- Leaching protocol for Nanosilva treated LLDPE polymer in food and food simulated matrices as functions of time, temperature and chemistry of the matrix with determined migration values (MRID 47828925)

Nanosilva LLC also submitted a request to waive the following data:

1. Hydrolysis (OPPTS 835.2120)
2. Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids (OPPTS 850.1010)
3. Fish Acute Toxicity Test, Freshwater and Marine (OPPTS 850.1075)
4. Avian Acute Oral Toxicity Test (OPPTS 850.2100)
5. 90-Day Dermal Toxicity (OPPTS 870.3250)
6. Prenatal Developmental Toxicity Study (OPPTS 870.3700)
7. Bacterial Reverse Mutation Test (OPPTS 870.5100)
8. Detection of Gene Mutations in Somatic Cells in Culture (OPPTS 870.5300)
9. In Vitro Mammalian Cytogenetics (OPPTS 870.5375)
10. Mammalian Bone Marrow Chromosome Aberration Test (OPPTS 870.5385)
11. Immunotoxicity (OPPTS 870.7800)

Waivers for the above studies were requested because of the lack of acute toxicity noted in the six acute toxicity studies (MRID 47828918 through 47828923), because Nanosilva has a specific gravity greater than water, because Nanosilva has low solubility in water as determined by the water column generator method (MRID 47828917), and due to the non-leaching characteristic of Nanosilva because silver at concentrations greater than the analytical detection limit were not detected in leaching studies conducted with a plastic incorporating Nanosilva (MRID 47828925). As detailed in Appendix A, the data requirements listed above in items 1 through 11 are either waived or satisfied. In general, the data requirement is met or satisfied because:

- certain guideline studies are not appropriate for nanosilver and where appropriate are being modified and required as terms and conditions on the registration (i.e., Reproduction/Developmental Toxicity Screening Test)
- certain environmental data are satisfied because the environmental hazard labeling statement was determined using literature studies completed with nanosilver
- Dermal guideline studies are not appropriate given there was no risk concern
- *in vitro* mutagenicity study requirements are satisfied and have shown that nanosilver is possibly mutagenic
- the immunotoxicity study is initially waived and only triggered after the potential immune toxicity of nanosilver is determined during the 90-day inhalation toxicity study required as a term and condition on the registration

In response to EPA's review of the leaching and product chemistry studies, Nanosilva LLC subsequently submitted the following documents:

Nanosilver Content of Plastic Coupons Containing Nanosilva

- Determination of Silver Content and Silver Recovery Rate for NSPW-L30SS (MRID 48652901)

Description of Production and Formulation Process

- Lee, J.M., Kim, D.W., Jun, Y.D., Oh, S.G. 2006. Preparation of silica-silver heterogeneous nanocomposite particles by one-pot preparation strategy using polyol process: Size-controlled immobilization of silver nanoparticles. *Materials Research Bulletin* 41:1407-1416.(MRID 48379901 and 48379903)

- Lee, J.M., Kim, D.W., Kim, T.H., Oh, S.G. 2007. Facile route for preparation of silica-silver heterogeneous nanocomposite particles using alcohol reduction method. *Materials Letters* 61:558-1562. (MRID 48379902 and 48379904)

Textile Leaching Study

- NanoSilva LLC prepared and submitted on August 20, 2012 a draft protocol titled "The Quantification and Characterization of Silver Released from Textiles Treated with NanoSilva (NSPW-L30) as a Results of Washing"

- The Quantification and Characterization of Silver Released from Textiles Treated with NSPW-L30SS As a Result of Simulated Laundering Conditions. Study Number: 110112.0001 Revision 2 (MRID 49045301)

IV. HAZARD CHARACTERIZATION OF NANOSILVER

Nanosilver is a broad spectrum antimicrobial agent that works by releasing ionic silver but also exhibits particle-specific effects (Wang et al. 2013). In November, 2009 the Agency convened a meeting of the FIFRA Scientific Advisory Panel (SAP) to address a number of questions associated with assessing the hazard of and exposure to nanosilver and other nanoscale metal-based pesticides (FIFRA SAP, 2009). In general, the SAP advised that the toxicity of nanosilver could differ from and might be higher than other forms of silver (e.g., silver ions). The Panel agreed with EPA that particle size has a substantial impact on particle properties, including rate and concentration of silver ion release, where the effects of size are generally most observable for particles with dimensions below 20 nm and largely below 10 nm (2009 SAP, p. 6). In addition to size, other properties such as shape, charge, and surface coating are also likely to impact the biological response to nanosilver.

4.1 Acute Human Toxicology of Nanosilva

Nanosilva LLC submitted results from guideline acute animal-toxicity tests completed using high-level doses of a liquid suspension containing Nanosilva with 1% nanosilver by weight. As outlined in Table 2, there were no mortalities or abnormalities noted in test animals after administration of Nanosilva by oral, dermal, and inhalation routes at dose levels of up to 5,000 mg/kg, and 2.05 mg/L, respectively; Nanosilva caused moderate to no irritation to skin or eyes at dose levels of up to 0.1 mL and 0.5 mL, respectively; and was not a skin sensitizer. According to the Agency's Toxicity Category system, which is used for product labeling purposes, shipping containers filled with Nanosilva are required to carry a label stating "CAUTION" where contact with eyes or clothing should be avoided.

Table 2 – Acute Toxicity Profile for the Nanosilva Liquid Suspension

Study	Toxicity Category
Acute Oral Toxicity	Category IV No mortality or abnormalities after dose of 5,000 mg/kg
Acute Dermal Toxicity	Category IV No mortality or abnormalities after dose of 5,000 mg/kg
Acute Inhalation Toxicity	Category IV No mortality or abnormalities after dose of 2.05 mg/L
Acute Eye Irritation	Category III Moderate to not irritating after dose of 0.1 mL
Acute Dermal Irritation	Category IV Mild or slight irritation after dose of 0.5 mL
Skin Sensitization	Not a sensitizer

4.2 Subchronic and Chronic Toxicological Studies Available for Nanosilver

There are no repeated-dose subchronic or chronic toxicity studies available for Nanosilva or the nanosilver in Nanosilva. However, there are repeated-dose toxicity studies available in the scientific literature for nanosilver. Since nanosilver might be released from Nanosilva and articles incorporating Nanosilva, the Agency considers the scientific literature studies on nanosilver toxicity relevant. The Agency believes the studies described in the following sections are sufficient for assessing the risks from the use of Nanosilva during generation of the requisite data.

4.2.1 Oral

There are currently three studies in the scientific literature that investigate the oral toxicity of nanosilver in rats and two studies completed with mice. The first reported findings after 28 days of repeated administration of carboxymethyl cellulose-coated nanosilver with average diameter of 60 nm (minimum diameter of 53 nm and maximum diameter of 71 nm) to four-week old male and female Sprague-Dawley rats (n = 10 per dose) (Kim, et al, 2008). There were liver effects (dilation of the central vein, bile-duct hyperplasia and increased foci), a coagulative effect on peripheral blood, and an increase in serum alkaline phosphatase (ALP) and cholesterol. There was a dose-dependent increase in silver distribution in many tissues (liver, kidneys, stomach, brain, lungs, testes, and blood) and a two-fold higher accumulation in the kidneys of female rats when compared with male rats was also reported for all dose groups. A no observed adverse effect level (NOAEL) of 30 mg/kg/day (lowest dose level) was reported based on the observed liver effects and increase in alkaline phosphatase (ALP) and cholesterol at 300 mg/kg/day (mid dose level). The second study was performed by the same group using four-week old Fisher rats (n= 10 per dose) for 90 days (Kim et al., 2010). This study involved repeated administration of carboxymethyl cellulose-coated nanosilver with average diameter of 56 nm (minimum diameter of 25 nm and maximum diameter of 125 nm) to rats and reported similar findings as the 28-day study including gender-related distribution of silver in the kidneys and a reported NOAEL of 30 mg/kg/day. However, intestinal pigmentation from exposure to nanosilver was reported, which was not observed in the 28 day study.

The third study involved feeding nanosilver stabilized with polyvinylpyrrolidone with average diameter of 14 nm and silver acetate to four-week old male (n= 6 per dose) and female (n=10 per dose) Wistar Hannover Galas rats for 28 days (Hadrup et al. 2012a). Consistent to the studies above, investigators did not find increases in ALP or cholesterol for nanosilver at doses of up to 9 mg/kg/day. They also reported no observable effects on the microbiological status of the rat's gastrointestinal tract caused by ingesting nanosilver. However, Hadrup et al., (2012a) did report effects for silver acetate at a dose of 14 mg/kg/day including an increase in ALP, decrease in plasma urea, and lower thymus weights. Hadrup et al. (2012b) compared the neurotoxic effects, *in vivo* and *in vitro*, of polyvinylpyrrolidone stabilized nanosilver with average diameter of 14 nm and silver acetate. Following 28 days of oral administration, nanosilver (4.5 and 9

mg/kg/day) and silver acetate (9 mg/kg/day) significantly increased the concentration of dopamine in the brains of Wistar female rats, while the brain concentration of 5-hydroxytryptamine (5-HT) was increased only by nanosilver at a dose of 9 mg/kg/day. However, in the 14-day range-finding study, the brain dopamine concentration decreased in rats treated with nanosilver at doses of 2.25 and 4.5 mg/kg/day. Three solutions consisting of 1) nanosilver, 2) an ionic silver solution obtained by filtering a nanosilver suspension, and 3) silver acetate were tested in neuronal-like PC12 cells *in vitro.* Nanosilver did not induce necrosis; however, cell viability was decreased and apoptosis (involving both the mitochondrial and the death receptor pathways) was found with all three solutions where silver acetate was relatively more potent.

There is one study for the repeated administration of nanosilver with average diameter of 42 nm (minimum diameter of 25 nm and maximum diameter of 55 nm) to mice over 28 days (Park, et al., 2010). The study reported that, after oral administration of nanosilver at the dose levels of 0.25 mg/kg/day, 0.5 mg/kg/day, or 1.0 mg/kg/day, the serum enzyme levels of alkaline phosphatase (ALP) and aspartate transaminase (AST) were significantly elevated in both male and female mice in the high dose group. The level of alanine transaminase (ALT) was also elevated in the high dose females. Histopathological analysis was performed in the high-dose groups and revealed that tissue change (i.e., slight cell infiltration) was observed in the cortex of the kidneys in both male and female mice, but no other histopathological changes were found in the portions of liver or small intestines that were examined. A NOAEL of 0.5 mg/kg/day was reported, based on the observed findings of elevated ALP, AST and ALT and the histopathological changes in the kidneys at the 1.0 mg/kg/day dose level.

Liu et al. (2013) reported that nanosilver did not affect spatial cognition or hippocampal neurogenesis in adult male ICR mice (n=15 per dose, n=10 for control). Adult mice were administered nanosilver with average diameter of 51.4 nm by DLS and 26.3 nm (size range from 10 to 70 nm) by TEM via intraperitoneal injection, at doses of 0, 10, 25, or 50 mg/kg, once a day in the morning for 7 consecutive days. Another group of mice received scopolamine (3mg/kg) as a positive control for the behavioral studies. Investigators used the Morris water maze (MWM) test for spatial cognition along with bromodeoxyuridine to detect proliferating cells. The test results showed that both reference memory and working memory were not impaired in nanosilver exposed groups, compared with the control group, and no differences were revealed in hippocampal progenitor proliferation, new born cell survival, or differentiation in nanosilver treatment groups.

4.2.2 Inhalation

The subchronic inhalation toxicity of nanosilver was determined in six-week old male and female Sprague-Dawley rats (n=10 per dose) exposed to nanosilver with average diameters between 18 to 19 nm (minimum diameter of 2 nm and maximum diameter of 65 nm) in a whole

body inhalation chamber for 13 weeks (90-day) using OECD test guideline 413 (Sung et al., 2009). Male and femal rats exposed to the high-dose level of 515 $\mu g/m^3$ (3.03×10^6 particles/cm^3) showed toxic effects in the liver (bile-duct hyperplasia) and lungs (chronic alveolar inflammation and macrophage accumulation in the lungs of males and females, and erythrocyte aggregation). A follow-on study was performed to determine lung-function recovery of five-week old male and female Sprague-Dawley rats (n= 17 male and 12 female per dose) after exposure to nanosilver with average diameters between 14 to 15 nm (minimum diameter of 4 nm and maximum diameter of 47 nm) in a whole body inhalation chamber for 12 weeks (Song et al., 2012). The pathological findings in the lungs were very similar to those observed in Sung et al. (2009) even though there were slight differences in particle size and concentration. During the 12-week exposure period, there was a decrease in lung function of male animals exposed to all doses of nanosilver including a decrease in tidal and minute volume, and peak expiratory flow. After a 12-week recovery period, the male animals in the middle- (117 $\mu g/m^3$ or 1.41×10^6 particles/cm^3) and high-dose (381 $\mu g/m^3$ or 3.24×10^6 particles/cm^3) groups did not achieve complete recovery of lung function. Remarkably, no adverse lung function effects were observed for female animals. The decrease in lung function was matched by histopathological observations of mixed cell infiltrate perivascular and chronic alveolar inflammation in male animals but these effects were also observed in female animals even though they had no measurable decrease in lung function. Although female animals showed gradual clearance of silver from lung tissues and decreased inflammation after 12 weeks of recovery, persistent inflammation in lung tissues was observed in the high-dose male animals throughout the 12-week recovery period. This lack of recovery indicates permanent or persistent organ damage. Significant increases in the amount of silver in tissues, such as lungs, liver, olfactory bulb, brain, kidneys, and blood, were also reported. Females had two to three times more silver accumulation in their kidneys than males.

Liu et al. (2012) investigated the effects of nanosilver on hippocampal synaptic plasticity and spatial cognition in adult male Wistar rats (n=8 per dose). Uncoated nanosilver with average diameter of 244.5 nm (size range from 33 to 380 nm) by Dynamic Light Scattering (DLS) and with sizes from 50 to 100 nm by Transmission Electron Microscopy (TEM) was administered to rats by nasal drops at doses of 3 mg/kg and 30 mg/kg once every two days for 14 consecutive days. Spatial cognition as determined by the Morris water maze (MWM) test showed that animals receiving nanosilver exhibited lower learning ability and memory retention, which was more prominent in the high-dose group (30 mg/kg). This finding was also supported by impaired synaptic plasticity as determined by long-term potentiation (LTP) recording for these same animals. Further, the quantity of reactive oxygen species (ROS) in hippocampal homogenate was significantly increased in nanosilver treated groups, and edema, nuclear shrinking phenomenon, and necrobiosis were shown in hematoxylin and eosin (HE) stains of pyramidal neurons in the PP (perforant path) and DG (dentate gyrus) regions of hippocampus.

4.2.3 Dermal

There are only two dermal toxicity studies available for nanosilver. One study was performed to determine liver, skin and spleen pathologies of five to six week old male guinea pigs (n=6) after exposure to nanosilver (concentrations 100 µg/ml, 1000 µg/ml, and 10,000 µg/ml) with a particle size of less than 100 nm based on daily rub exposure to aqueous solution using $AgNO_3$ as a positive control (Korani et al, 2011). The nanosilver suspension was applied by daily rubbing to an area of 5 cm by 5 cm on the back of the animal with no wipe off or removal of chemical mentioned. The applied dose in mg/kg was not determined in the study. According to email contact with authors, animals were restrained for 5 hours after application of chemical, however, the method of restraint was not discussed. During the 13 week study, dose dependent and nanosilver specific effects were seen in the liver, the spleen and on the skin. Skin effects included decreased thickness of the epidermis and dermis, inflammation, increased levels of round cells, acidophilic cytoplasm in muscle fibers and increased levels of macrophages in the endomysium. Liver effects included overproduction of Kupffer cells and degeneration of hepatocytes in a dose dependent fashion and necrosis at the 10,000 µg/ml concentration. In the liver, red capsules were thinner, inflammation, accumulation of red blood cells, and white pulp atrophy along with hepatic cord destruction in a dose dependent fashion. It does not appear that gauze or other dressing was used to cover the dosed area. No hematology, clinical chemistry or urinalysis was performed. Only the liver, spleen and skin were examined histopathologically.

Another study was performed to determine the effect of skin exposure to nanosilver (size = 20 and 50 nm, concentration = 0.34, 3.4 and 34 µg/ml aqueous solution) in 2 female pigs (Samberg et al, 2010). A 500 µL nanosilver suspension was placed on one of 14 spots on the back of a a hair-clipped pig, allowed to air dry, then covered with a Hilltop chamber (occlusion pad) and secured with non irritating tape. This was followed by a body stocking covering the dorsum of each pig. Actual exposure was 0.6, 6, and 60 ng/mm^2 and total exposure was 0.17, 1.7 and 17 µg per dosing period. No gross pathological effects were noticed on the porcine skin. A concentration, but not particle size or washing state, dependent effect was seen on the dermal layers under microscopic investigation. Low dose effects were slight intra and intercellular epidermal edema. Intermediate effects were a more focal intra and intercellular epidermal edema alongside focal dermal and epidermal inflammation. The highest dose caused severe edema, with severe focal dermal inflammation, epidermal hyperplasia and parakeratosis. Precautions were taken to prevent oral dosing, including restraint/anesthesia and multiple covering layers. Only effects relating to the skin were examined.

None of the above studies are an acceptable substitute for a dermal subchronic study or a dermal irritation study. In no case was a full gross or microscopic histopathology panel performed, even in the cases where dose dependence effects of nano-silver particles was seen. None of these studies used the same size particles, coating type or washing state, making it potentially difficult to generalize information from them onto other products. In Korani et al. (2011), the study

lacked any clinical chemistry, hematology or urinalysis of the guinea pigs. This study also could not draw NOAEL/LOAEL conclusions given that effects were seen at all levels of dosing. In Samberg et al. (2010), no systemic histopathology would have been possible, as multiple dose concentrations were tested on the same animal. This study would not suffice as a dermal irritant/sensitizer as there is no induction or recovery period.

In addition, there is one published report on nanosilver that is available for use by the Agency concerning nanosilver used in wound dressings for patients suffering from burns. In this report, a burn patient using nanosilver coated wound dressing developed clinical signs of argyria and elevated serum liver enzymes indicative of liver toxicity along with elevated silver concentrations in blood and urine (Trop, 2006). This study indicates to the Agency that nanosilvers can be systemically absorbed when a large area of the skin barrier is severely compromised.

In the absence of any such dermal toxicity studies, the Agency normally uses extrapolation from another route of exposure (usually oral). However, use of the oral endpoint for evaluating dermal toxicity requires knowledge regarding the amount absorbed through the skin. This information is typically provided by a dermal penetration study using whole animals (usually rats), which allows determination of the fraction of topically applied dose that is available for systemic absorption (i.e., the dermal absorption factor or DAF). There are *in vitro* techniques available that allow determination of dermal penetration of chemicals through isolated animal or human skin. However, the Agency does not rely on *in vitro* dermal absorption study data as the sole basis for deriving a DAF because standardized *in vitro* test methodology is not currently available. Without detailed, standardized methodology for *in vitro* absorption studies, the Agency has observed variation in test results among laboratories. As a result, the Agency assumes a default DAF of 100% when no *in vivo* data are available. Therefore, if *in vivo* dermal penetration studies are available, either those studies alone or in combination with *in vitro* studies are used for deriving a DAF of less than 100%.

Here, there is no guideline or scientific literature study conducted in animals for the *in vivo* dermal absorption of the nanosilver in Nanosilva or of nanosilver available to the Agency. However, there is a human clinical study, which is observational, that examined silver levels in serum and urine after application of burn wound cream containing silver sulfadiazine nanosilver (Wan et al., 1991). EPA used this information to derive a conservative DAF of 6.7% for nanosilver. A study completed by Brandt et al. (2012) demonstrated that nanosilver and silver sulfadiazine had similar skin absorption characteristics in mice after normalizing for silver dose. In addition an *in vitro* study with nanosilver in human skin is available in the scientific literature indicating that nanosilver penetration was very low for both intact and abraded skin at 0.00066% and 0.0033%, respectively (Larese et al., 2009).

4.2.4 Reproductive and Developmental Toxicity of Nanosilver

There are studies showing significant, dose-dependent increases in the concentration of silver in the testes of rats after oral ingestion, inhalation, and injection of nanosilver (see Section 4.3); however, there are few studies available on the reproductive and developmental toxicity of nanosilver.

There is an *in vitro* study investigating the toxicity of 15 nm nanosilver on spermatagonia isolated from 6-day old mouse testes and immortalized with SV40 large T antigen (Braydich-Stolle et al., 2005). In this transformed (i.e. immortalized) cell line, nanosilver and silver ions caused altered cellular morphology, decreased mitochondrial activity (as indicated by MTS assay), and increased apoptosis at doses up to 10 μg/ml; however, the affects from nanosilver were greater than observed for silver ions. The conservative interpretation of this data is that nanosilver that reaches the testes may be able to cause decreased fertility due to toxicity to spermagonia and this effect would be more severe for nanosilver than for silver ion.

Austin et al. (2011) investigated the distribution of citrate coated nanosilver with diameters between 30 and 60 nm and silver nitrate in pregnant mice (n= 6 to 12 per dose) and developing embryos. Nanosilver suspensions and silver nitrate were administered by intravenous injection (i.v.) on gestation days (GD) 7, 8, and 9 at nanosilver concentrations of 0, 0.4, and 0.73 mg/kg/day. Austin et al. (2011) reported a significant increase in nanosilver content as compared to silver nitrate treated animals in nearly all tissues; nanosilver accumulation was significantly higher in liver, spleen, lung, tail (injection site), visceral yolk sac, and endometrium. Nanosilver was identified in vesicles in endodermal cells of the visceral yolk sac. This study demonstrated that nanosilver distributed to major maternal organs and extra-embryonic tissues, but the authors stated that very little silver reached developing embryos and no adverse morphological effects on the developing embryos were observed.

The teratogenicity potential of nanosilver in pregnant rats was investigated by Mahabady et al. (2012). Nanosilver of unknown size and surface coating was administered to pregnant rats via intraperitoneal injection (i.p.) on GD 8 and 9. Fetuses collected on GD 20 from animals that received nanosilver were reported to have reduced weight and length but no effect on the skeletal system as compared to animals treated with a saline control. Because Mahabady et al. (2012) did not report key information about the nanosilver particles size and surface coatings, it is not possible to compare these results to the nanosilver in any other study or in any nanosilver containing product.

4.2.5 Mutagenicity of Nanosilver

Currently, there are no studies in the scientific literature that investigate the potential of nanosilver to cause cancer (i.e., carcinogenicity). However, the potential of nanosilver to induce changes in genetic material (i.e., mutagenicity, genotoxicity) has been investigated *in vitro* using

traditional mutagenicity tests including the bacterial reverse mutation assay (*i.e.,* Ames test), the mouse lymphoma forward mutation test, the mammalian cell chromosome aberration test in Chinese hamster ovary cells, and the mouse lymphoma Comet assay for oxidative damage. Additionally, nanosilver was investigated in an *in vitro* human lymphoblastoid micronucleus assay as well as and in an *in vivo* rat bone marrow micronucleus assay. Results from these assays are summarized below:

In vitro reverse gene mutation assay in bacterial cells: The mutagenicity of nanosilver (average diameter of 10 nm) suspended in a 1% citric acid solution was determined at concentrations of up to 500 µg/plate in bacterial cells using the Ames test (Kim et al., 2012). Although cytotoxicity was observed at 31.25 µg/plate, nanosilver did not induce a mutagenic effect in the histidine-requiring strains of *Salmonella typhimurium* TA98, TA100, TA1538 and TA1537 or in tryptophan-requiring Escherichia coli WP2uvrA with or without the metabolic activation system (±S9). A similar result was obtained by Li et al.(2012) for nanosilver with an average diameter of 5 nm (size range from 4 to 12 nm) prepared in TEM for the primary particles and for particles with a diameter of 1608.7 ± 175.4 nm, prepared in culture media. In this test, there was no increase in revertant mutant colonies of the standard *S. typhimurium* tester strains, which included the strain used to detect oxidative damage (TA102) up to cytotoxic concentration (31.25-62.5 µg/plate -S9; 125-250 µg/plate +S9). However, Li et al. (2012) cautioned that because of cytotoxicity and the physical properties of nanosilver, this test system may lack the sensitivity required to detect the mutagenic action of the test material.

In vitro forward gene mutation assay in mouse lymphoma L5178Y/$TK^{+/-}$ cells with a Comet Assay: The mutagenicity of nanosilver, which had an average diameter of 5 nm (size range from 4 to 12 nm) prepared in TEM for the primary particles and a diameter of 1608.7 ± 175.4 nm in the culture media, was evaluated in mouse lymphoma L5178Y at the $TK^{+/-}$ locus and the modes of action was assessed using standard alkaline and enzyme-modified Comet assays with a gene expression analysis (Mei et al., 2012). Nanosilver induced dose-dependent cytotoxicity and mutagenicity with a marked increase in the mutation frequency at 4 and 5 µg/mL (<50% cell survival at ≥5 µg/mL) where nanosilver had a clastogenic mode of action. Subsequent testing revealed no evidence of DNA damage (Comet test) but oxidative damage (modified Comet test), confirmed by gene expression analysis, which showed an expression pattern consistent with production of reactive oxygen species (ROS).

In Vitro Chromosome Aberration Test: The clastogenic effect of nanosilver (average diameter of 10 nm) suspended in a 1% citric acid at concentrations of less than 31.25 µg/mL was determined in Chinese hamster ovary cells (CHO-k1) after 6- and 24-hour exposures ±S9 using OECD Test Guideline 473 (Kim et al. 2012). Nanosilver did not induce any statistically significant increase in the number of cells with chromosome aberration, polyploidy, or endoreduplication when compared with the control group at concentrations causing approximately 50% cytotoxicity.

In Vitro Mammalian Cell Micronucleus Test: An *in vitro* mammalian cell micronucleus test (OECD Test Guideline 487) was used to determine the genotoxicity of nanosilver, which had an average diameter of 5 nm (size range from 4 to 12 nm) by TEM for the primary particles and a diameter of 1608.7±175.4 nm prepared in culture media. Nanosilver at concentrations of up to 30 µg/mL induced a significant and dose-related increase in micronuclei in human lymphoblastoid TK6 cells, indicating to the investigator that nanosilver has weakly positive genotoxic potential (Li et al, 2012).

In Vivo Micronucleus Assay: In contrast to the above *in vitro* findings, oral administration of carboxymethyl cellulose-coated nanosilver with average diameter of 60 nm (minimum diameter of 53 nm and maximum diameter of 71 nm) caused overt toxicity but failed to induce an increase in micronucleated polychromatic erythrocytes (MN PCEs) in the bone marrow of male and female rats after 28 days of treatment at doses 0, 30, 300, and 1,000 mg/kg/day (Kim, et al, 2008). This study, which was based on OECD Test Guideline 474, reported that there was no statistically significant treatment-related increase in MN PCEs when compared to the negative control. This study indicates that nanosilver is neither clastogenic nor aneugenic *in vivo*, although a limitation of this study is that no measurements were performed to determine if nanosilver reached the bone marrow.

Overall the results suggest that of the *in vitro* tests discussed above, the data from the Ames and chromosome aberration assays indicate that nanosilver is not expected to be mutagenic while the mammalian cell micronucleus and mouse lymphoma with comet assay suggest that nanosilver may have mutagenic potential. This is consistent with a recent review article which stated that *in vitro* data point to possible mutagenic properties of nanosilver (Bartłomiejczyk et al., 2013). However, the lack of genotoxicity from the *in vivo* study suggests that the mutagenic potential observed in some of the *in vitro* studies may be intrinsic to nanoparticles but may not be expressed in whole animals or that nanosilver may be subjected to first-pass effects such as biliary elimination. At this time, there is inadequate information to assess mutagenic (and hence carcinogenic) potential of nanosilver due to differences in results between the *in vitro* studies and *in vivo* study, and the limitations of the only available *in vivo* study.

4.2.6 Silver Ions

Humans may also be exposed to silver ions that would be released by Nanosilva. Conventional silver, and the silver ions it releases, are defined as pesticides under FIFRA. The SAP concluded that the hazards of silver ions would be the same, whether they came from conventional silver or from silver nanoparticles. With respect to silver ions, the Agency evaluated the toxicity and exposure to silver ions and determined that unreasonable adverse effects from use of silver containing products are unlikely (U.S. EPA, 1993).

4.3 Absorption, Distribution, Metabolism, and Excretion (ADME) of Nanosilver

Studies were completed using either injection or oral administration of nanosilver to laboratory animals to determine the absorption, distribution, metabolism, and excretion (ADME) of nanosilver in whole animals. Based on the studies described in the following sections the Agency believes biliary or fecal excretion of nanosilver is the primary elimination pathway. Silver is found primarily in the liver, spleen, and kidneys, but also in the thymus, brain, heart, lungs and testes of animals dosed with nanosilver, silver nitrate, and silver acetate, where the organs of animals dosed with silver nitrate and silver acetate contained greater amounts of silver than in animals dosed with nanosilver. Animals can clear silver from blood and most organs given enough time but retain silver in the testes and brain. Animals treated with silver nitrate, silver acetate, and nanosilver all contain silver granules with dimensions on the nanoscale. However, it is unclear if intact nanosilver is absorbed into tissues or if nanosilver dissolves into ionic silver before being absorbed into tissues and forming nanoscale granules.

4.3.1 Injection of Nanosilver

The translocation, distribution and accumulation of silver after a single subcutaneous injection at 62.8 mg/kg of body weight of nanosilver with diameters between 50 and 100 nm and microsilver with diameters between 2,000 and 20,000 nm was determined in Wistar female rats (n = 30 per group) (Tang et al, 2009). The silver content of feces between 2 and 24 weeks after injection was significantly higher than in urine for both the nanosilver and microsilver injected animals, which suggests that both nanosilver and microsilver were eliminated through biliary excretion. Although there was no significant difference between the amount of silver at the injection site or in excrements after administration of nanosilver or microsilver, the amount of silver in organs was significantly greater for nanosilver. Animals injected with nanosilver were found to contain significantly more silver in the liver, kidney, spleen, brain, lung and blood than in animals injected with microsilver. Histopathological observations found that nanosilver injected animals contained elemental silver spheres that were absent from the microsilver treated animals. The elemental silver spheres were observed in different kinds of cells, such as renal tubular epithelial cells and hepatic cells. Moreover, these elemental silver spheres also induced blood-brain barrier (BBB) destruction and astrocyte swelling, and caused neuronal degeneration.

The serum kinetics, tissue distribution, and excretion of silver after single injections of 0.5 mg/kg and 5 mg/kg of citrate coated nanosilver with average diameter of 7.9±0.95 nm was determined in SPF New Zealand White rabbits (n = 4) (Lee et al, 2012). There was no significant general toxicity reported in either the 0.5 or 5 mg/kg treatment groups. Accumulation of silver was observed in all the tested organs including liver, kidney, spleen, lung, brain, testes, and thymus, where the liver and spleen contained the greatest amount of silver. As with the rat study above, the amount of silver in feces between 1 and 28 days after injection was significantly greater than

in urine, which suggests biliary excretion of silver is the major route of elimination after injection of nanosilver.

Blood kinetics, tissue distribution, and organ accumulation of silver after daily intravenous injections of between 23.8 and 27.6 mg/L over 5 consecutive days of nanosilver with average diameters of 20, 80, and 110 nm was determined in six week old male Wistar rats (n=21 in treatment groups; n= 2 in control) (Lankveld et al., 2010). The concentration of silver in blood rapidly decreased during the initial 10 minutes following both single and repeated injection of nanosilver and then remained stable for up to one hour after injection. Silver was distributed to all organs evaluated including the liver, lungs, spleen, brain, heart, kidneys and testes, regardless of the size of nanosilver injected. After injecting 20 nm diameter nanosilver, silver was found to be distributed mainly in the liver, followed by kidneys and spleen, whereas after injection of the 80 and 110 nm diameter nanosilver, silver was distributed mainly to spleen followed by liver and lung. Thus, there was a size dependent tissue distribution. Repeated administration of nanosilver resulted in accumulation of silver in liver, lung and spleen, indicating that these organs may be potential target organs for toxicity after repeated exposure.

4.3.2 Oral Administration of Nanosilver

The organ distribution and cellular localization of silver was determined following 28 day repeated oral administration at 9.0 mg/kg/day of polyvinylpyrrolidone stabilized nanosilver with average diameter of 14 ± 4 nm and silver acetate to four week old female Wistar Hannover Galas rats (n = 9 for nanosilver and n = 7 for silver acetate) (Loeschner et al., 2011). Although the distribution of silver in organs for animals treated with nanosilver and silver acetate was similar, the concentration of silver in the organs treated with silver acetate was greater than it was for nanosilver treated animals. This was in agreement with the higher fecal excretion of nanosilver as compared to silver actetate. Besides the intestinal system, the largest silver concentrations were detected in the liver and kidneys; however, silver was also found in the lungs and brain. Remarkably, silver containing granules in the same size range as that of the administered nanosilver were observed in the ileum and kidney tissues of rats exposed to nanosilver and silver acetate. Using transmission electron microscopy (TEM), sulfur and selenium containing granules were detected in the ileum of animals exposed to nanosilver and silver acetate, and were mainly located in the basal lamina of the ileal epithelium and in lysosomes of macrophages within the lamina propria. The results of the present study demonstrate that the organ distribution and form of silver was similar when nanosilver or silver acetate were administered orally to rats.

The toxicokinetics and tissue distribution of silver was determined following 28 day oral gavage at 90 mg/kg/day of nanosilver with average diameter of 17.7±3.3 nm by TEM, polyvinylpyrrolidone coated nanosilver with average diameter of 12.1±8.0 nm by TEM, and 9 mg/kg/day of silver nitrate to six-week-old male pathogen free Sprague Dawley rats (n = 5 per group) (Van der Zande et al., 2012). Greater than 99% of the silver administered to the rats was

excreted in their feces indicating that only a small fraction of silver from nanosilver and silver nitrate was absorbed. After normalizing for gavage dose, the concentration of silver in the blood of the silver nitrate treated animals was significantly higher than for the nanosilver treated animals at all time points during exposure. This clearly illustrates a much higher uptake of silver when silver nitrate was administered as compared to nanosilver. One day after the oral gavage, a significant reduction in blood silver concentration was observed. One week after the oral gavage, the concentration of silver in blood was reduced to nondetectable levels indicating rapid clearance of silver from the blood for both nanosilver and silver nitrate treated animals. After normalizing for gavage dose, silver was observed in all examined organs with the highest levels in the liver and spleen where animals treated with silver nitrate had accumulated significantly more silver than in animals treated with nanosilver. Silver was cleared from most organs eight weeks after the final gavage, but remarkably not from the brain and testes, where between 94 and 100% of the silver was still present in the brain compared to the amount one day after the last gavage of nanosilver and silver nitrate, respectively. Using single-particle inductively coupled plasma mass spectrometry; nanosilver was detected one day after the final gavage in the liver, spleen, lungs, and gastrointestinal contents of nanosilver gavaged rats. Remarkably, nanosilver was also detected in the liver, spleen, lungs, and gastrointestinal contents one day after the final gavage from silver nitrate treated rats demonstrating the *in vivo* formation of nanosilver from silver nitrate. Blood enzyme levels were not significantly different from untreated animals indicating that there was no acute hepatotoxicity observed. Also, there was no indication that nanosilver caused nonspecific immune responses based on immunotoxic responses.

4.4 Safety Factor for Infants and Children (FQPA Safety Factor)

Under the Food Quality Protection Act (FQPA), P.L. 104-170, which was enacted in 1996 to amend the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug and Cosmetic Act (FFDCA), the Agency was directed to "ensure that there is a reasonable certainty that no harm will result to infants and children" from aggregate exposure to a pesticide chemical residue. The law further states that in the case of threshold effects, for purposes of providing this reasonable certainty of no harm, "an additional tenfold margin of safety for the pesticide chemical residue and other sources of exposure shall be applied for infants and children to take into account potential pre- and post-natal toxicity and completeness of the data with respect to exposure and toxicity to infants and children. Notwithstanding such requirement for an additional margin of safety, the Administrator may use a different margin of safety for the pesticide residue only if, on the basis of reliable data, such margin will be safe for infants and children."

There is no food use proposed for Nanosilva at this time, and therefore, no FFDCA aggregate exposure to Nanosilva residue is required.

4.5 Antimicrobial Resistance

Silver is currently used as a broad spectrum antiobiotic in wound dressings. There is a concern that increasing use of silver, such as nanosilver for preserving plastics, may result in more bacteria developing resistance to silver and limit its use as an antibiotic agent for wound care (Gupta and Silver, 1998). In the wound care setting, a recent review by Chopra (2007) concluded that the threat of bacterial resistance to silver in the clinical setting is low. However, Chopra (2007) cautioned against use of wound dressings that release sublethal levels of silver over a long period of time allowing bacteria to develop resistance.

In terms of environment, a recent study involved releasing 1 mg/L of nanosilver into microcosms containing estuary water overlying estuarine sediment cores (Bradford et al., 2009). The study found no impact to the microbial community over a 30 day monitoring period. Evidence for antibacterial resistance was also evaluated during this study and no increase in antibiotic resistance to the bacterial population in the sediment was found (Mühling et al. 2010). Wigginton et al. (2010) suggested that the lack of antimicrobial effect in the microcosm was expected given that bacterial proteins efficiently bind to nanosilver.

The Agency concludes that while development of antibacterial resistance due to the use of nanosilver in Nanosilva might be possible, the likelihood is low that the levels used in products incorporating Nanosilva will lead to the development of silver resistant microbes who will spread throughout the environment and results in widespread bacterial resistance to silver.

4.6 Point of Departure Selections and Target Margin of Exposure

The currently available oral toxicity studies indicate that nanosilver causes liver and kidney toxicity in laboratory animals where silver is distributed to all organs and tissues with accumulation of silver in the brain and male animal testes. Inhalation toxicity studies also identified liver toxicity as well as lung effects including chronic alveolar inflammation. There were potential neurotoxic effects identified with increases in neurotransmitter concentrations and loss of spatial cognition; however, these same effects were not observed in a follow-on study. EPA believes that these neurotoxicity studies are inadequate in their assessment of behavioral effects and do not use optimal methods to evaluate the potential toxicity to the nervous tissue structure and function. The *in vitro* Ames and chromosome aberration assays indicate that nanosilver is not expected to be mutagenic while the mammalian cell micronucleus and mouse lymphoma with comet assay suggest that nanosilver may have mutagenic potential. However, the lack of genotoxicity from the *in vivo* study indicates that there is inadequate information to assess mutagenic (and hence carcinogenic) potential of nanosilver. Finally, there is not enough information on the reproductive and developmental toxicity for nanosilver at this time.

Together, these studies indicate to the Agency that, if sufficient quantities of nanosilver break away from Nanosilva, and if such nanosilver displays toxicity similar to the nanosilver used in

the oral and inhalation route-specific studies, then route-specific exposure to Nanosilva derived nanosilver may result in adverse health effects.

4.6.1 Point of Departures for Nanosilva

The toxicological point of departure (POD) is the lower confidence bound on the lowest experimental dose from a dose response study that showed an effect. This dose is determined from dose-response data and marks the beginning of extrapolation to determine the risk associated with environmentally relevant human exposures. Commonly, this is a NOAEL from a laboratory animal toxicity study, which represents the dose at which no adverse affects were observed in laboratory animals. Oral or dermal subchronic and chronic toxicology studies are not available for Nanosilva or the nanosilver that might break away from articles incorporating Nanosilva. In place of these studies, the Agency is determining NOAELs and LOAELs from subchronic inhalation and oral toxicity studies found in the scientific literature for nanosilvers to evaluate the effects that could occur from exposure to the nanosilver present in Nanosilva.

As stated in Section 2.2, the FIFRA SAP was unsupportive of bridging among silver-based materials with different properties. However, the SAP indicated that bridging would be appropriate for materials of similar size and essentially identical physical properties and that bridging between silver ions released from nanosilver and the existing database for silver ions is feasible (FIFRA SAP, 2009). The SAP cautioned about extrapolating from one nanosilver formulation to another when assessing hazards because differences in particle formulation (e.g., coating and inert ingredients) are likely to affect biological activity, among other things.

The oral toxicity study by Hardrup et al. (2012a) used PVP coated nanosilver with average diameter of 14 nm, which is similar to the diameter and surface coating of the nanosilver in Nanosilva. However, EPA cannot determine a NOAEL or LOAEL from this study because histopathological patterns indicative of distinct adverse effects at the highest nanosilver dose of 9 mg/kg/day were not identified. The oral toxicity study by Park et al. (2010) used uncoated nanosilver with an average diameter of 42 nm, which is different from the diameter and surface coating of the nanosilver in Nanosilva. In 2011, EPA used the NOAEL reported in the Park et al. (2010) study as the POD for evaluating the oral and dermal toxicity of the nanosilver in HeiQ AGS-20 (U.S. EPA, 2011a). EPA reviewed this study again as part of this assessment and now concludes that although the results of the Park et al. 2010 study showed evidence of changes in clinical chemistry, it lacks histological support for the effects used as the basis for the study NOAEL/LOAELs. In the absence of histopathological findings, the clinical chemistry changes observed are insufficient evidence of an adverse effect.

The oral toxicity studies by Kim et al. (2008) and Kim et al. (2010) used CMC coated nanosilver with average diameter of 56 and 60 nm, respectively, which is different from the diameter and surface coating of the nanosilver in Nanosilva. These studies were stated as being completed

according to OECD guidelines and identified histopathological patterns in the liver that were indicative of distinct adverse effects. EPA has determined that the NOAEL is 30 mg/kg/day from the 28-day Kim et al. (2008) study based on significant increases in alkaline phosphatase and cholesterol, significant changes in hematology, and accompanied by histopathological evidences of liver toxicity (bile-duct hyperplasia around central vein, infiltration of inflammatory cells, and dilation of the central vein) at the LOAEL dose of 300 mg/kg/day. A NOAEL for the 90-day Kim et al. (2010) cannot be established because of adverse histological patterns evident at the lowest dose of 30 mg/kg/day. EPA has determined that the LOAEL from the 90-day Kim et al. (2010) study is 30 mg/kg/day based on histopathological evidences of liver toxicity (bile-duct hyperplasia with focal, multifocal, or lobular necrosis) in both males and females.

The inhalation toxicity studies by Sung et al. (2009) and Song et al. (2012) used uncoated nanosilver with average diameters of 18 to 19 nm and 14 to 15 nm, respectively, which is similar to the diameter and but different than the surface coating of the nanosilver in Nanosilva. EPA has determined that the NOAEL is 133 $\mu g/m^3$ from the 90-day Sung et al. (2009) study based on the minimal bile-duct hyperplasia, perivascular mixed cell infiltrate and chronic alveolar inflammation and macrophage accumulation in the lungs in both male and female rats, and decreases in physiological measures of lung function (respiratory minute volume, tidal volume, and peak inspiratory flow) in the male rats. EPA has determined that the NOAEL is 49 $\mu g/m^3$ from the 12-week Song et al. (2012) study based on adverse histopathological patterns in lung tissue observed at the 117 $\mu g/m^3$ dose. In 2011, EPA used the NOAEL reported in the Sung et al. (2009) study as the POD for evaluating the inhalation toxicity of the nanosilver in HeiQ AGS-20 (U.S. EPA, 2011a). EPA compared the Sung et al. (2009) study to the Song et al. (2012) study and now concludes that the newly available Song et al. (2012) study is more appropriate for evaluating inhalation exposure to nanosilver.

Based on the above analysis, EPA has determined that the NOAEL of 30 mg/kg/day from 28-day oral toxicity study by Kim et al., 2008 is the POD for short-term oral exposures (< 30 days) to the nanosilver in Nanosilva (Table 3). EPA has also determined that the LOAEL from the 90 day oral toxicity study by Kim et al., 2010 is the POD for intermediate-term oral exposures (1 to 6 months) to the nanosilver in Nanosilva. EPA has determined that the NOAEL of 49 $\mu g/m^3$ derived from the Song et al. (2012) study is the POD for short- and intermediate-term inhalation exposures to the nanosilver in Nanosilva. These studies were conducted using OECD guidelines and the effects are consistent across studies, and therefore the NOAELs/LOAELs are protective of effects seen in other studies.

Table 3 – Toxicity Endpoints for Use in Nanosilver Risk Assessment

Dietary Risk Assessment – No Dietary Exposures				
Nondietary Risk Assessments				
Exposure Scenario	**Point of Departure (POD)**	**Uncertainty Factor**	**Target MOE for Risk Assessment**	**Study**
Incidental Oral (short-term)	NOAEL = 30 mg/kg/day	$UF_A = 10$ $UF_H = 10$ $UF_D = 10$	Target MOE = 1000	Kim et al. (2008)
Incidental Oral (Intermediate-term)	LOAEL = 30 mg/kg/day	$UF_A = 10$ $UF_H = 10$ $UF_D = 10$ $UF_L = 3$	Target MOE = 3000	Kim et al. (2010)
Dermal (short -term)	NOAEL = 30 mg/kg/day DAF = 6.7%	$UF_A = 10$ $UF_H = 10$ $UF_D = 10$	Target MOE = 1000	Kim et al. (2008)
Dermal (Intermediate-term)	LOAEL = 30 mg/kg/day DAF = 6.7%	$UF_A = 10$ $UF_H = 10$ $UF_D = 10$ $UF_L = 3$	Target MOE = 3000	Kim et al. (2010)
Inhalation (short- and intermediate- term)	NOAEL = 49 $\mu g/m^3$	$UF_A = 10$ $UF_H = 10$ $UF_D = 10$	Target MOE = 1000	Song et al. (2012)
Cancer (oral, dermal, inhalation)	At this time there is inadequate information to assess carcinogenic potential of nanosilver due to differences in results between the *in vitro* studies and *in vivo* study, and the limitations of the only available *in vivo* study.			

There are no acceptable dermal toxicity studies on nanosilver available to the Agency. In the absence of any such dermal toxicity studies, the Agency normally uses extrapolation from another route of exposure (usually oral) and assumes a default dermal absorption factor (DAF) of 100% when no *in vivo* data are available (see Section 4.2.3). The available human *in vivo* study indicating absorption of nanosilver is 6.7% and the *in vitro* data indicating absorption of nanosilver from intact and abraded human skin is substantially below 0.1% provide scientific support for setting a conservative DAF of 6.7% for the nanosilver that might break away from the Nanosilva. EPA has determined that the dermal toxicity for the nanosilver in Nanosilva will be evaluated using the oral POD of 30 mg/kg/day and a DAFs of 6.7%. In 2011, EPA used a DAF of 0.1% that was based on human clinical study by Moiemen et al., (2011) for evaluating the dermal toxicity of the nanosilver in HeiQ AGS-20 (U.S. EPA, 2011a). EPA reviewed this study again as part of this assessment and now concludes that the 0.1% DAF was an underestimate because it was based on the Moiemen et al. (2011) study which only reported silver concentrations in the blood of patients. The 6.7% DAF is based on the observational study by Wan et al. (1991) which reported silver concentration in serum and eliminated through urine.

There are currently no acceptable studies on the reproductive and developmental toxicity for nanosilver. However, there were dose-dependent increases in the concentration of silver in the testes of rats after oral ingestion, inhalation and injection of nanosilver and, in another study, nanosilver was distributed to major maternal organs and extra-embryonic tissues although no adverse morphological effects on the developing embryos were observed. Therefore, EPA believes that the oral NOAEL and LOAEL of 30 mg/kg/day and inhalation NOAEL of 49 $\mu g/m^3$ are expected to be protective of developmental and reproductive effects of nanosilver.

The study completed by Hadrup et al. (2012b), which reported significant increases in neurotransmitter concentrations (e.g., dopamine) after oral administration of nanosilver to rats at concentrations of up to 9 mg/kg/day, lacks histological support for determining NOAEL/LOAELs. The study by Liu et al. (2013) reported no effects on the spatial cognition or hippocampal activity of mice after injecting nanosilver into the body cavity (i.e., intraperitoneal injection) at concentrations of up to 50 mg/kg. Therefore, EPA believes that the oral NOAEL and LOAEL of 30 mg/kg/day are expected to be protective of neurotoxic effects of nanosilver.

The effects on spatial cognition and hippocampal activity observed by Liu et al. (2012) after administering nasal drops containing nanosilver at concentrations of 3 and 30 mg/kg to rats suggest possible neurotoxic effects from the inhalation of nanosilver. However, EPA believes that the doses used in the Liu et al. (2012) study were greater than the maximum dose used in the inhalation toxicity study by Song et al. (2012). Therefore, EPA believes that the inhalation NOAEL of 49 $\mu g/m^3$ based on the Song et al. (2012) study is expected to be protective of neurotoxic effects of nanosilver.

The available *in vitro* studies suggest that nanosilver may have mutagenic potential. However, the one available *in vivo* micronucleus assay performed on rats after oral administration of nanosilver at concentrations of up to 1,000 mg/kg/day (Kim, et al, 2008) indicated that nanosilver is neither clastogenic nor aneugenic *in vivo*; however, there was no positive proof that nanosilver reached the bone marrow in the micronucleus assay. Therefore, EPA believes that the oral short-term NOAEL and intermediate-term LOAEL of 30 mg/kg/day are expected to be protective of the potential mutagenic effects of nanosilver.

4.6.2 Target Margin of Exposure

The target margin of exposure (MOE) is based on uncertainty factors. There are two standard uncertainty factors that account for potential interspecies extrapolation and intraspecies variation. The first is a 10 fold uncertainty factor (UF_A) assigned to account for extrapolation of laboratory animal data to humans (interspecies). The second is a 10 fold uncertainty factor (UF_H) assigned to account for variations in susceptibility within the human population (intraspecies). In addition to the two standard uncertainty factors given above, there is a third uncertainty factor (UF_D)

which accounts for the incomplete characterization of nanosilver toxicity (database). In this case, the Agency has determined that the inhalation and oral toxicity data for nanosilver is sufficient to asses the potential health effects caused by nanosilver released from Nanosilva (Table 3). However, as the SAP concluded, there is uncertainty on how particle parameters such as size, surface charge and coating affect the toxicity potential of nanosilver. In addition, the database is incomplete with respect to reproductive and developmental effects. Therefore, the Agency is using the maximum 10 fold database uncertainty factor to cover the missing information on reproductive and developmental effects and the potential for differences between the nanosilver in the available toxicity data (U.S. EPA, 2002). Thus, the target MOE is:

Target MOE: 10 (UF_A) × 10 (UF_H) × 10 (UF_D) = 1,000

The target MOE of 1,000 is for evaluating short-term (< 30 days) and intermediate-term (1 to 6 months) continuous daily inhalation exposures to Nanosilva because the inhalation POD was based on a 90 day duration study by Song et al. (2012). The target MOE of 1,000 is for evaluating the short-term exposure (< 30 days) continuous daily oral exposures to Nanosilva because the oral POD was based on a 28-day oral toxicity study by Kim et al., 2008.

The oral POD that was based on the LOAEL from the 90 day oral toxicity study by Kim et al., 2010 has an additional 3 fold uncertainty factor (UF_L) to account for the lack of NOAEL. The target MOE for evaluating intermediate-term exposure (1 to 6 months) continuous daily oral exposures to Nanosilva is:

Target MOE (intermediate-term oral): 10 (UF_A) × 10 (UF_H) × 10 (UF_D) × 3(UF_L) = 3,000

The maximum recommended long-term target MOE is 3,000 (U.S. EPA, 2002), which has already been established for intermediate-term exposures. Therefore, a long-term target MOE cannot be determined from the existing toxicity database.

4.6.3 Margin of Exposure

The margin of exposure (MOE) is used to determine if exposure to a chemical can be expected to cause an adverse effect. The MOE is calculated by dividing the POD by the estimated daily dose to which humans will be exposed as expressed by the following:

MOE = POD / Daily Dose

After calculating a MOE from the POD and daily dose, EPA evaluates the risk from exposure to a pesticide by comparing the calculated MOE to a target MOE (U.S. EPA, 2002). If a calculated MOE is equal to or greater than a target MOE, EPA may conclude that exposure to the pesticide is unlikely to pose a risk concern and, therefore, will not cause unreasonable adverse effects for

that specific exposure scenario. If a calculated MOE is less than a target MOE, then EPA may have a risk concern. However, the MOE analysis is not the only factor the Agency uses when determining if there is a risk concern from exposure to a pesticide. The Agency also considers other scientific evidence in a weight of evidence evaluation such as the severity of toxic effects, the controls used to minimize exposures, and the population exposed to the pesticide. A risk concern, however, is not the equivalent of a determination that the potential risk constitutes an unreasonable adverse effect. Rather, where EPA finds a risk concern, EPA will generally: (1) require protective measures or use restrictions to mitigate the risk; (2) further refine its risk assessment analysis, particularly if conservative assumptions were used to produce the initial assessment; or (3) explicitly analyze any potential benefits of the pesticide to determine whether, on balance, those benefits outweigh the identified risk.

The Agency's MOE approach for nanosilver uses mass-based metrics, both for determining the POD and for calculating exposure. The Agency is aware of the ongoing debate within the scientific community that metrics other than mass (such as particle number or surface area) may be more suitable for assessing nanosilver risks and therefore acknowledges the potential for limitations of mass-based risk estimates.

V. OCCUPATIONAL AND CONSUMER RISK ASSESSMENT

Based on the proposed use pattern of Nanosilva in plastics and textiles, EPA anticipates humans could be exposed to the following substances:

1) Silver ions released from Nanosilva;
2) Nanosilva complex; and/or
3) Nanosilver that might break away from the Nanosilva complex.

Because the levels of silver in plastics and textiles incorporating Nanosilva are lower than for other currently registered products which use silver ions and because the Agency determined that unreasonable adverse effects from use of silver containing products are unlikely (U.S. EPA, 1993), EPA concluded that the risk from exposure to silver ions derived from plastic and textile products incorporating Nanosilva is not of concern. Nanosilva LLC has submitted a number of studies and other information to support its application for registration, including information relevant to assessing the toxicity of and exposure to Nanosilva. In addition, EPA has also reviewed data and information on nanosilver from the scientific literature. This section discusses EPA's assessment of the potential risks to human health from the use of Nanosilva. The first section addresses potential levels of occupational exposure and risk to workers who handle the Nanosilva liquid suspension. The final sections address potential levels of consumer exposure and risk to children who come in contact with plastics and textiles incorporating Nanosilva.

5.1 Occupational Risk Assessment for Mixing and Loading the Nanosilva Liquid Suspension

EPA expects that occupational inhalation and dermal exposures to Nanosilva and the nanoparticles that might break away from Nanosilva are likely to occur during the following use scenarios:

1. Mixing and loading of Nanosilva during preparation of a master batch
2. Mixing, loading, and applying the Nanosilva containing master batch during treatment of plastics and textiles
3. Handling plastics and textiles incorporating Nanosilva

EPA evaluates the risk of occupational exposures from mixing, loading, and applying or handling pesticide products. Although exposure to Nanosilva during subsequent work activities involving plastics and textiles incorporating Nanosilva can occur in occupational settings (items 2 and 3), the risk to workers for these scenarios is unlikely to exceed the risk during mixing and loading Nanosilva during preparation of the master batch (item 1). Therefore, only the mixing and loading of Nanosilva during preparation of a master batch is evaluated in the subsequent sections.

5.1.1 Occupational Exposure when Preparing the Master Batch Containing Nanosilva

A master batch consists of polymer pellets (i.e., plastic pellets) containing Nanosilva which are subsequently used in treating plastics (MRID 48652901). A master batch is prepared by blending a polymer and the Nanosilva liquid suspension using a mixer and compounding this mixture using an extruder. Workers may be exposed to Nanosilva and nanosilver particles that break away from the Nanosilva complex while loading the Nanosilva liquid suspension into the master batch mixer.

Nanosilva LLC did not submit data or information quantifying the amount of Nanosilva that workers are exposed to during loading of the Nanosilva liquid suspension into a master batch mixer. To calculate worker exposure, the Agency is using the standard occupational handler unit exposure values for mixing and loading of liquids, as shown in Table 4, which are from the Antimicrobial Exposure Assessment Task Force II (AEATF II) liquid pour human exposure monitoring study (U.S. EPA, 2012a) and the Pesticide Handlers Exposure Database (PHED) (U.S. EPA, 1998).

Table 4 – Unit Exposures for Mixing and Loading of Liquids

Personal Protective Equipment (PPE) Level	Exposure Route	Unit Exposure	Data Source
No respirator	Inhalation	0.21 (µg/m^3/lb)	AEATF II
Engineering Control – Closed System Loading	Inhalation	0.016 (µg/m^3/lb)	PHED
No gloves, use of long-sleeve shirt, long pants, shoes plus socks	Dermal	10 (mg/lb)[A]	AEATF II
Gloves, long-sleeve shirt, long pants, shoes plus socks	Dermal	1.2 (mg/lb)[B]	AEATF II
Engineering Control – Closed System Loading	Dermal	0.0086 (mg/lb)	PHED

[A]Arithmetic mean for conventional liquid pour scenario
[B]Estimated from the no gloves unit exposure using a glove protection factor of 10.

The inhalation unit exposure for mixing and loading of liquids using engineering controls (closed system loading) as listed in PHED is 0.083 mg/lb. This value was calculated based on the air concentrations measured in workers' breathing zone during the handling of a pesticide, the time that elapsed during the sampling, an assumed breathing rate of 29 liters per minute, and the amount of the pesticide that was handled during the sampling period. When the PHED unit

exposures were initially calculated, they were expressed in units of mg/lb for comparisons to PODs that were expressed in units of mg/kg/day. Because the POD for inhalation exposure to nanosilver is expressed as a daily-average air concentration of $\mu g/m^3$, the PHED unit exposure was converted to a daily-average air concentration with units of $\mu g/m^3$ per lb of pesticide handled per day. This was done by dividing the air concentrations listed in the PHED by the amount of pesticide handled while accounting for the time elapsed during sampling and an eight hour work day. This yielded an inhalation unit exposure of 0.016 $\mu g/m^3$ per lb of pesticide and it represents an eight hour time weighted average (Table 4). This unit exposure is the arithmetic mean of 27 PHED monitoring events (formerly called replicates) that represent inhalation exposure during the mixing and loading of pesticide containing liquids while using closed-system loading.

5.1.2 Occupational Margins of Exposure

As detailed in the following sections, EPA evaluated the risk to workers who load the Nanosilva liquid suspension into a master batch mixer.

Occupational Inhalation Daily-Exposure and MOEs

The inhalation exposure to the nanosilver in the Nanosilva liquid suspension was calculated using the following:

> **Inhalation Daily Exposure** = Amount of nanosilver in Nanosilva handled per day × Inhalation Unit Exposure

Where:

- Amount of nanosilver in Nanosilva handled per day is 1.2 lb/day. This value was calculated assuming that the Nanosilva liquid suspension was mixed into a low density polyethylene plastic using an extruder with the maximum screw diameter of 8 inches (Chanda and Roy, 2007) which yields a plastic production rate of 5,000 lb/hour (Flite Technology, 2012). Assuming an 8 hour work day yields a plastics production volume of 40,000 lb per day at each treatment facility. The maximum amount of nanosilver applied to plastics or textiles is 0.003% by weight. Thus, the amount of nanosilver that is handled per day is 40,000 lb/day × 0.003% ÷ 100% = 1.2 lb/day.
- The unit exposure for inhalation is 0.21 $\mu g/m^3$/lb when no respirator is worn and 0.016 $\mu g/m^3$/lb when closed-system loading is used (see Table 4).

Table 5 – Occupational Handlers Inhalation MOEs for the Nanosilver in the Nanosilva Liquid Suspension

Mix/Load Nanosilva Liquid Suspension	Amount of Plastics Treated per day (lb)	Application Rate[A] (by wieght)	Amount of Nanosilver Handled per day (lb)	Inhalation Unit Exposure ($\mu g/m^3/lb$)	Daily Exposure[C] ($\mu g/m^3$)	MOE[D]
Without Respirator	40,000	0.003%	1.2	0.21	0.25	200
Closed System Loading (Without Respirator)	40,000	0.003%	1.2	0.016[B]	0.02	2,500

A. Maximum amount of nanosilver in plastics incorporating Nanosilva
B. PHED unit exposure value converted to air concentration units based on mean 8 hour TWA
C. Daily Exposure = Amount nanosilver Handled × Unit Exposure
D. MOE = POD / Daily Exposure (rounded to two significant digits)

The MOEs shown in Table 5 were calculated by dividing the inhalation exposure for nanosilver by the POD of 49 $\mu g/m^3$, which is the NOAEL from a 90-day inhalation toxicity study for nanosilver (Song et al., 2012). This assumes that all the nanosilver in Nanosilva is freely available after inhalation and behaves like the nanosilver used in the Song et al. (2012) study even though the nanosilver in Nanosilva is attached to a silica core particle through a thiolate bond and is covered with sulfur and polyvinylpyrrolidone. When no respirator is worn the MOE is 200, which is based on the AEATF II unit exposure data, and is less than the target MOE of 1,000 indicating the risk from short- and intermediate-term exposures is of concern. The MOE is 2,500 for workers who use closed-system loading when mixing and loading the Nanosilva suspension, which is based on PHED unit exposure data, and is greater than the target MOE of 1,000 indicating the risk from short- and intermediate-term exposures is not of concern. These MOEs indicate that the risk concern for inhalation exposures to the nanosilver in Nanosilva is mitigated when workers use closed-system loading during mixing and loading of the Nanosilva liquid suspension.

Occupational Dermal Daily-Dose and MOEs

The dermal dose from exposure to the nanosilver in the Nanosilva liquid suspension was calculated using the following:

Dermal Daily Dose = (Amount of nanosilver in Nanosilva handled per day × Dermal Unit Exposure × Dermal Absorption Factor) / Body Weight

Where:

- Amount of nanosilver in Nanosilva handled per day is 1.2 lb/day (Table 5).

- The dermal unit exposures are 10 mg/lb when no gloves are worn, 1.2 mg/lb when gloves are worn, and 0.0086 mg/lb when closed-system loading is used (Table 4).
- The dermal absorption factor (DAF) for nanosilver is 6.7% (see Section 4.2.3).
- The body weight of an adult is 80 kg (U.S. EPA, 2011b).

Table 6 – Occupational Handlers Dermal MOEs for the Nanosilver in the Nanosilva Liquid Suspension

Mix/Load Nanosilva Liquid Suspension	Amount of Nanosilver Added or Handled[A] (lb)	Dermal Unit Exposure (mg/lb)	Exposure[B] (mg/day)	Daily Dose[C] (mg/kg/day)	MOE[D]
Without Gloves	1.2	10	12	0.010	3,000
With Gloves	1.2	1.2	1.4	0.0012	25,000
Closed System Loading	1.2	0.0086	0.01	0.0000086	>1,000,000

A. Based on the same assumptions as those used for the inhalation MOE (see Table 5)
B. Dermal Exposure = Amount of nanosilver Handled × Dermal Unit Exposure
C. Dermal Dose = (Dermal Exposure × Dermal Absorption Factor)/ Body Weight
D. Dermal MOE = POD / Daily Dose (rounded to two significant digits)

The MOEs shown in Table 6 were calculated by dividing the dermal dose by the POD of 30 mg/kg/day, which is the NOAEL from a 28-day oral toxicity study (Kim et al., 2008) and the LOAEL from a 90 day oral toxicity study (Kim et al., 2010). This assumes that all the nanosilver in Nanosilva is freely available after dermal contact and behaves like the nanosilver used in the Kim et al. (2008 and 2010) studies even though the nanosilver in Nanosilva is attached to a silica core particle through a thiolate bond and is covered with sulfur and polyvinylpyrrolidone. The MOEs for dermal exposure are 3,000 when no gloves are worn and 25,000 when gloves are worn, which are based on the AEATF II unit exposure data, and are both greater than the target MOE of 3,000 indicating that the risk from short- and intermediate-term occupational dermal exposures are not of concern. The MOE is greater than 1,000,000 when closed-system loading is used, which is based on the PHED unit exposure data, and is greater than greater than the target MOE of 3,000 indicating that the risk from short- and intermediate-term occupational dermal exposures are not of concern.

5.1.3 Conclusions for Occupational Risk

The MOE for occupational inhalation exposure to the nanosilver in the Nanosilva liquid suspension is 2,500 if workers use close-system loading when mixing and loading the Nanosilva liquid suspension, which is greater than the target MOE of 1,000 indicating the risk from short- and intermediate-term inhalation exposures is not of concern. The MOE for dermal exposure to the nanosilver in the Nanosilva liquid suspension is greater than 1,000,000 if workers use closed-system loading when mixing and loading the Nanosilva liquid suspension, which is greater than the target MOE of 3,000 indicating that the risk from short- and intermediate-term dermal exposures is not of concern. Therefore, EPA proposes to require that closed-system loading as defined in 40 CFR part 170.240(d)(4) be used when mixing and loading the Nanosilva liquid suspension. The proposal to require closed-system loading is based on unit exposure data from PHED. The MOEs calculated using unit exposure data from AEATF II were shown for comparison purposes only and were not relied on to determine that Nanosilva will not cause unreasonable adverse effects to human health.

EPA does not typically consider long-term occupational exposures to materials preservatives used to treat plastics or textiles because application of these chemicals does not typically occur on a daily basis for more than 6 months.

There are several uncertainties in the occupational risk assessment. The exposure analysis assumes that all the nanosilver in Nanosilva is freely available even though the nanosilver in Nanosilva is attached to a silica core particle through a thiolate bond and the nanosilver in Nanosilva is covered with sulfur and polyvinylpyrrolidone. The PODs used were based on inhalation and oral toxicity studies completed using nanosilver, not Nanosilva or the nanosilver that might break away from Nanosilva, and thus these PODs may not equal PODs determined from testing conducted with Nanosilva or the nanosilver found in Nanosilva. Because the nanosilver in the toxicity database is different from the nanosilver in Nanosilva and because the database is incomplete with respect to reproductive and developmental effects, the Agency used a maximum 10-fold database uncertainty factor when evaluating the risk from occupational exposure to Nanosilva. There are also uncertainties in extrapolating from effects observed after feeding test-animals nanosilver (i.e., oral route) to the effects that might be observed after applying nanosilver to the skin of test animals (i.e., dermal route).

5.1.4 Occupational Exposure and Health Data Requirements

EPA is not proposing to require that Nanosilva LLC conduct an indoor applicator study to quantify the unit exposure values during mixing and loading of the Nanosilva liquid suspension into the master batch mixer. This is because closed-system loading, such as lock and load containers, where the Nanosilva liquid suspension is transferred to a plastic extruder through piping minimizes occupational exposure to Nanosilva to the greatest extent possible.

Although there is no risk concern for workers who use close-system loading when mixing and loading the Nanosilva liquid suspension, EPA is proposing to require an inhalation route-specific subchronic test (OCSPP 870.3465) to confirm the Agency's determination that the risks to workers who mix and load Nanosilva are not unreasonable. Also, because workers of child bearing age could handle Nanosilva during mixing and loading, EPA is proposing to require a modified reproduction/developmental toxicity screening test since this data is currently missing from the toxicity database. In sum, EPA is proposing to require these tests to confirm the adequacy of the 10 fold database uncertainty factor, to reduce the uncertainties related to differences in the physical properties of the nanosilver, and because there are currently no acceptable studies on the reproductive and developmental toxicity for nanosilver.

In summary, the following guideline studies are proposed to be required for Nanosilva:

- 90-Day Inhalation Toxicity (Rat) (OCSPP 870.3465) modified to include *in vivo* bone marrow assay and functional observational battery, motor activity and detailed neuropathology
- Reproduction/Developmental Toxicity Screening Test (Modified OCSPP 870.3550/ OECD TG 421)

5.2 Consumer Risk Assessment for Products Incorporating Nanosilva

EPA expects consumer exposures to Nanosilva, the silver ions derived from the Nanosilva complex, and the nanoparticles that break away from the Nanosilva complex could potentially occur during the following use scenarios:

1. Incidental oral exposure to plastics and textiles incorporating Nanosilva
2. Dermal exposure to plastics and textiles incorporating Nanosilva

Nanosilva is proposed to be mixed into polymer and polymer based products to suppress the growth of bacterial, algae, fungus, mold and mildew, which cause odors, discoloration, stains, and deterioration of plastics and textiles. Because plastics incorporating Nanosilva could be subsequently used to manufacture children's toys and textiles worn by children, it is assumed that children will be exposed to products containing Nanosilva.

While children younger than 6 months may potentially be exposed to plastics and textiles incorporating Nanosilva, it is believed that exposure for children older than 6 months will be equivalent, if not greater, due to behavioral and anatomical/physiological development; therefore, EPA assessed exposure to children older than 6 months. EPA's recently revised its standard operating procedures (SOPs) for residential pesticide exposure assessments (U.S. EPA, 2012b). Based on the combined quantitative and qualitative analysis of the index lifestage, the Agency has determined that the 1 < 2 year old lifestage represents the most appropriate index

lifestage for children for most exposure scenarios. In 2011, EPA evaluated the exposure to textiles containing the nanosilver based pesticide HeiQ AGS-20 using children in the 2 to 3 year old lifestage based on the Agency's determination, at that time, that the 2 to 3 year old child was likely the most vulnerable subpopulation from chewing on and wearing textiles treated with HeiQ AGS-20 (U.S. EPA, 2011a). After issuing the registration decision for HeiQ AGS-20, questions were raised about the use of the 2 to 3 year old lifestage. In light of those questions and in light of the newly revised SOPs, in this case, EPA is evaluating exposure to children in the 6 to < 12 month, the 1 to < 2 year, and 2 to <3 year old age range from plastic toys and flooring and textiles incorporating Nanosilva for transparency.

5.2.1 Consumer Exposures to Plastics and Textiles Incorporating Nanosilva

Leaching studies are required to determine the amount and form of silver that consumers will be exposed to when in contact with plastics and textiles incorporating nanosilver. These studies typically involve immersing products incorporating nanosilver in biological fluids such as simulated saliva solutions for extended periods of time at physiological temperatures (i.e., 98.6 degrees Fahrenheit or 37 degrees Celsius) and measuring the amount and form of silver released to those fluids. Such "leaching studies" were submitted by Nanosilva LLC using food contact solutions for plastic coupons incorporating Nanosilva and using laundry detergent and simulated saliva for shirts incorporating Nanosilva.

Plastics Leaching Study

Nanosilva LLC submitted a leaching study on the migration of Nanosilva from a linear low-density polyethylene (LLDPE) plastic (MRID 478289-25). The study was based on Food and Drug Administration methodology to determine the migration of chemicals from plastics which contact food. The leaching study involved 44 LLDPE coupons with 14 being ashed to determine silver content and 28 used in the leaching tests. The 28 plastic coupons were prepared with four Nanosilva master batch concentrations where seven coupons each contained 0, 2.5, 5, and 10%, respectively. These master batch concentrations corresponded to nanosilver concentrations of 0, 5, 10, and 20 mg/kg (supplementary information provided on 17 December 2010). The coupons each had a surface area of approximately 112 cm^2, a volume of approximately 16.3 mL and an approximate mass of 15g. Each coupon was exposed to 50 mL of the fluids listed in Table 7 at temperatures of 40 and 100 degrees Celsius for periods of up to 240 hours (10 days). The amount of nanosilver present in the coupons was 0, 75, 150, and 300 µg, respectively. Given that coupons were in contact with 50 mL of fluid, the maximum silver concentrations that could be obtained from each coupon was 0, 1500, 3000, and 6000 µg/L, respectively.

In general, the concentration of silver in the fluids contacting plastic incorporating Nanosilva was low with most values below the analytical detection limit. The analysis method, inductively coupled plasma atomic emission spectroscopy (ICP-AES), employed an acid digestion and thus the silver concentrations are for total silver content. The Nanosilva LLC leaching study reported

method detection limits that ranged from 0.8 to 2 µg/L, with the exception of the Oil trial where the detection limit was 240 µg/L for the times of less than 240 hours. However, because there was no method detection limit study provided to support these values, EPA is using the silver detection limit of 5 µg/L, which is the value reported as the estimated detection limit in U.S. EPA Method 6010C (U.S. EPA, 2007a) and in the Nanosilva LLC submitted leaching study (Table 1, Page 30).

Table 7 – Concentration of Silver in Fluid Contacting the Nanosilva Containing Plastic Coupons at 40 degrees Celsius

Fluid	Maximum Silver Concentration Detected (µg/L)	Silver Matrix Spike Recovery (%)
Low pH, pH 2.0 using 10% nitric acid in water	15 of 15 samples < 5 µg/L	93 to 110
High pH, pH 8.0 using 0.1N sodium hydroxide in water	15 of 15 samples < 5 µg/L	100
Alcohol, 10% ethanol in water	14 of 15 samples < 5 µg/L (11 µg/L for the 5% coupon after 24 hr)	34 to 120
Salt, 10% sodium phosphate monobasic in water	13 of 15 samples < 5 µg/L (9.9 µg/L for the 10% coupon after 24 hr)	80 to 174
Oil, 10% olive oil in water	12 of 12 samples < 240 µg/L 3 of 3 samples < 5 µg/L	5.7 to 7.8
Salt and Sugar, 10% sodium phosphate monobasic and 10% sucrose in water	30 of 30 samples < 5 µg/L (14 µg/L for the 0% coupon after 168 hr at 40°C)	13.6 to 92
Salt and Sugar after scrubbing the plastic coupon	13 of 15 samples < 5 µg/L (27 µg/L for the 5% coupon after 48 hr)	3.6 to 6.8

Given that EPA is evaluating human exposure to plastics incorporating Nanosilva which occur at physiological temperatures (i.e., 98.6 degrees Fahrenheit or 37 degrees Celsius), the leaching results for the trials conducted at 100 degrees Celsius are not addressed here. The 100 degrees Celsius conditions are more representative of microwaving plastics than of contact with humans. Silver was found above the detection limit during 4 out of the 105 coupon leaching trials conducted at 40 degrees Celsius where the maximum silver concentration of 27 µg/L was found after 48 hours in a plastic coupon containing 5% Nanosilva master batch (10 mg/kg nanosilver) (Table 7). A value of 27 µg/L represents 0.9% of the maximum concentration of silver that could have leached from the 5% Nanosilva containing coupon. However, there was no clear

correlation between Nanosilva content and the duration or temperature of each experimental trial. In one analysis, silver was found in the salt and sugar fluid after 168 hours at 40 degrees Celsius in a coupon that did not contain Nanosilva (0% nanosilver) suggesting that the detections of silver may have been due to contamination from sources of silver other than from Nanosilva. Interpretation of these results is further complicated by the wide range in silver matrix spike recoveries determined for the alcohol, salt, oil, and salt and sugar fluids. This range in silver matrix spike recoveries may indicate that nanosilver released from the coupons was not detectable by the analytical method.

The average concentration of silver was 3.3 µg/L, as calculated for the trials conducted at 40 degrees Celsius. This was based on the concentrations reported and assuming that all results reported as being below the detection limit had a value of one-half the detection limit (i.e., 2.5 µg/L), which is consistent with how EPA assigns values to non-detect results when evaluating pesticide residues in food (US EPA, 2000). This average excludes the concentrations reported for the oil trial because the detection limit for this trial was reported as 240 µg/L. The average concentration of 3.3 µg/L was determined for conditions where the plastic coupon incorporating Nanosilva was in contact with fluids having low and high pH, high salt and sugar content, and after mechanical abrasion of plastic incorporating Nanosilva. Thus, this average concentration represents a range of conditions that are expected to occur during residential use of plastics incorporating Nanosilva. However, EPA is only evaluating risk from human contact with plastics incorporating Nanosilva, and therefore, only the results for low and high pH fluids, which are more representative of biological fluids, are included in this assessment. The concentration of silver in the low and high pH fluids were all below the analytical detection limit of 5 µg/L meaning that EPA used 2.5 µg/L as the concentration of silver released from plastics incorporating Nanosilva. Since this value is below the detection limit, the form of silver is unknown and was assumed to be in the form of nanosilver as found in Nanosilva. Using the assumed concentration of 2.5 µg/L equates to silver releases of 0.17, 0.08, 0.042% from the coupons containing 5, 10, and 20 mg/kg of nanosilver, respectively. Nanosilva LLC is proposing that plastic products incorporating Nanosilva contain a maximum of 0.003% nanosilver by weight or 30 mg/kg of nanosilver. Assuming that a leaching study conducted using a coupon containing 30 mg/kg of nanosilver would also result in silver releases below the detection limit would result in a silver release rate of 0.028% for a coupon containing 30 mg/kg of nanosilver. However, Nanosilva LLC did not submit leaching results for coupons containing 30 mg/kg of nanosilver.

The leaching study submitted by Nanosilva LLC for plastic coupons incorporating Nanosilva was initially determined to be unacceptable by EPA because less than 0.8% of the silver in the plastic coupons was recovered during the ashing process, the wide range in silver matrix-spike recoveries, and the lack of validation data for the silver analysis. Nanosilva LLC subsequently completed an additional study to determine the silver content of identically prepared plastic

coupons (MRID 48652901). As per EPA recommendation, Nanosilva LLC employed the non-destructive Neutron Activation Analysis (NAA) to determine the silver content of the Nanosilva liquid suspension, a master batch containing 2% Nanosilva, and plastic coupons containing 5% of the Nanosilva master batch. Using NAA, Nanosilva LLC determined the amount of silver in coupons was within 99% of the amount added.

The available leaching study for plastic coupons containing Nanosilva was conducted using a protocol that was not reviewed by EPA prior to conducting the study. The leaching study did not employ physiological fluids such as artificial saliva, however, the study was completed at the physiological temperature of 40 degrees Celsius. The coupons tested contained nanosilver at concentrations of 5, 10, and 20 mg/kg, which are less than the maximum nanosilver concentration of 30 mg/kg that Nanosilva LLC is proposing for the Nanosilva pesticide label. Even though these issues preclude EPA from accepting the leaching study as final, the study does provide a reasonable first estimate for the amount of silver transferred to the mouth while mouthing and transferred to skin while contacting plastics incorporating Nanosilva.

Textile Leaching Study

Nanosilva LLC prepared and submitted a draft protocol titled "The Quantification and Characterization of Silver Released from Textiles Treated with Nanosilva (NSPW-L30) as a Results of Washing" with the stated purpose to quantify and characterize silver released from textiles incorporating Nanosilva during laundering. The protocol was based on methods developed by Geranio et al. (2009) and Lorenz et al. (2012) who modified the ISO Colour Fastness test (ISO, 1997) to determine the amount and form of silver released to the environment when washing textiles containing silver and nanosilver. Because EPA must also evaluate the release of nanosilver when children chew and mouth textiles incorporating Nanosilva, EPA recommended including an additional test using simulated human saliva. EPA does not anticipate dermal exposure will result in a risk concern for textiles incorporating Nanosilva, and therefore a separate leaching study involving simulated human sweat was not recommended. EPA will use the results of the ISO Colour Fastness test completed with simulated human saliva to evaluate the dermal exposure to nanosilver from textiles incorporating Nanosilva.

Nanosilva LLC completed and submitted the modified ISO Colour Fastness test (MRID 49045301) using shirts incorporating Nanosilva. The washing tests were conducted on shirts composed of polyethylene terephthalate (PET), which incorporated Nanosilva into the PET yarn for a final nanosilver content of 0.00262% or 26.2 mg/kg per shirt. For the detergent wash test, a section was cut from each of three shirts and washed separately in 150 mL of distilled water with commercial laundry detergent and 10 hard rubber balls with diameter of 10 mm for 30 minutes at 40 degrees Celsius. The detergent wash was followed by two rinse cycles with deionized water. The concentration of silver in the wash and rinse water were determined after filtering samples through a 0.45 µm pore size filter using inductively coupled plasma mass spectrometry (ICP-

MS). The concentration of silver retained on the filter was also determined by ICP-MS. These samples were acid digested prior to analysis and thus the silver concentrations are for total silver content and do not distinguish between silver ions, nanosilver, or the Nanosilva complex.

Although the Nanosilva wash test study reported an ICP-MS detection method of 0.0094 µg/L for aqueous samples and 0.94 µg/kg for filter samples, EPA determined that the detection limit for aqueous samples was 10 µg/L and 1,000 µg/kg for solids samples. EPA determined these detection limits based on a statistical analysis of seven ICP-MS calibration curves and the analysis results from 100 µg/L quality control samples. The concentration of silver in the wash and rinse water from the detergent wash test for three shirt sections was below the analytical detection limit of 10 µg/L (Table 8). The concentration of silver retained on 0.45 µm pore size filters was also less than the analytical detection limit of 1,000 µg/kg.

Table 8 – Concentration of Silver Released from Shirts Incorporating Nanosilva during Modified ISO Colour Fastness Test

Number of Shirt Sections	Wash Medium	Concentration of Silver	
		Wash/Rinse Water (µg/L)	0.45 µm Pore Size Filter (Particles with Diameters >0.45 µm) (µg/kg)
3	Distilled Water with Detergent	< 10	< 1,000
9	Simulated Human Saliva	< 10	< 1,000

A separate study was conducted using three sections from each of three shirts and washed separately in 150 mL of simulated human saliva with 10 hard rubber balls with diameter of 10 mm for 45 minutes at 40 degrees Celsius. The concentration of silver in the simulated saliva was determined after filtering through a 0.45 µm pore size filter using ICP-MS where the concentration of silver in the saliva for nine shirt sections was below the analytical detection limit of 10 µg/L (Table 8). The concentration of silver retained on filters was also less than the analytical detection limit of 1,000 µg/kg.

Given that none of the silver concentrations in detergent or saliva solutions were above 10 µg/L and none of the filters contained silver at a concentration above 1000 µg/kg, EPA evaluated the amount of silver released from textiles incorporating Nanosilva by replacing the non-detect values with half the detection limit, 5 µg/L for liquid samples and 500 µg/kg for solids samples (U.S. EPA, 2000). The amount of silver released from textiles was calculated based on the volume of detergent and rinse water, and saliva along with the mass of the 0.45 µm filters used for each test (Table 9).

Table 9 – Amount of Silver Released from Shirts Incorporating Nanosilva

Wash Medium	Volume/Mass	Concentration	Amount of Silver (µg)	Total Silver Potentially Released	
				(µg)	%
Distilled Water with Detergent	190 mL wash/rinse water	5 µg/L[B]	0.95	1.05	1.6
	0.2 g filters[A]	500 µg/kg[B]	0.1		
Simulated Human Saliva	150 mL Saliva	5 µg/L	0.75	0.81	0.9
	0.12 g filter	500 µg/kg	0.06		

[A]There was a 0.1 g filter used for the detergent wash and another 0.1 g filter for the rinse.
[B] Set to half the detection limit

The initial amount of silver in textiles washed with distilled water and detergent was 66.54 µg, therefore the amount of silver released during the detergent wash was:

$$\text{Silver released in detergent wash} = \frac{1.05 \mu g}{66.54\ \mu g} \times 100\% = 1.6\%$$

The initial amount of silver in textiles washed with simulated human saliva was 94.9 µg, therefore the amount of silver released during the saliva wash was:

$$\text{Silver released in saliva wash} = \frac{0.81 \mu g}{94.9\ \mu g} \times 100\% = 0.9\%$$

The value of 1.6% will be used in evaluating releases to the environment from wash water and the value of 0.9% will be used in calculating oral and dermal exposures to textiles incorporating Nanosilva. Since these releases were determined using concentrations that were below the ICP-MS detection limit, the form of silver is unknown. EPA will assume the form of silver is identical to the nanosilver present in Nanosilva in the absence of further information.

The results of these studies demonstrate that PET shirts which incorporate Nanosilva at 26.2 µg/kg of nanosilver do not release silver at concentrations above the analytical detection limit. The ISO Colour Fastness test is thought to represent aggressive washing conditions with one wash cycle representing up to five domestic or commercial laundering cycles when the multiple test is employed. The amount of silver released during one ISO Colour Fastness test is believed

to exceed the daily exposure to nanosilver from a treated textile because the ISO Colour Fastness test involves immersing the textile in water containing detergents or simulated human saliva and hard rubber balls followed by mechanical agitation for 30 to 45 minutes. Thus, results from studies which are based on the ISO Colour Fastness test will be used to determine the daily dose of nanosilver for children who chew and mouth, adults who wear, and workers who manufacture items from nanosilver treated textiles even though this likely overestimates the daily dose of nanosilver.

5.2.2 Consumer Margins of Exposure to Plastics Incorporating Nanosilva

EPA expects that consumers will be exposed to plastics incorporating Nanosilva by the routes of incidental oral and dermal exposures. As detailed in the following sections, EPA evaluated the risk to children, as the likely most vulnerable subpopulation, from mouthing toys, and mouthing and crawling on flooring incorporating Nanosilva.

Consumer Incidental Oral Daily-Dose and MOE for Toys Incorporating Nanosilva

Incidental oral exposures to toys incorporating Nanosilva were calculated using the following:

Incidental Oral Exposure = Surface Residue (mg/cm^2) × Saliva Extraction Efficiency (%) × Surface Area of Toy Mouthed per day (cm^2)

Where:

- Surface area of a toy mouthed per day is 800 cm^2 for 6 to < 12 month old children, 560 cm^2 for 1 to < 2 year old children, and 396 cm^2 for 2 to < 3 year old children. These values were calculated assuming that a child mouths an area of 10 cm^2 each time they contact a toy, that 6 to < 12 month old children contact objects 20 times per hour, 1 to < 2 year old children contact objects 14 times per hour, and 2 to < 3 year old children contact objects 9.9 times per hour (U.S. EPA, 2011a) for 4 hours per day.

The concentration of silver detected in low and high pH fluids, which are more representative of biological fluids, contacting Nanosilva containing coupons represents both the surface residue and the saliva extraction efficiency. The concentration of silver in the low and high pH fluids were all below the analytical detection limit of 5 µg/L for coupons containing 5, 10, and 20 mg/kg of Nanosilva. EPA is assuming that non-detectable concentrations of silver would be found in a similar test conducted with coupons containing 30 mg/kg of Nanosilva. EPA is using 2.5 µg/L, which is one-half the detection limit of 5 µg/L, as the concentration of silver released from plastic incorporating Nanosilva. Using the concentration of silver, the volume of the leachate (50 mL), and the surface area of the test coupons, the amount of available nanosilver at the coupon surface is:

Surface residue and saliva extraction: $\frac{0.0025\ mg}{L} \times \frac{50\ mL}{} \times \frac{L}{1000\ mL} \times \frac{}{112\ cm^2} = \frac{0.00000112 mg}{cm^2}$

The incidental oral exposure for toys incorporating Nanosilva is:

Incidental oral exposure for 6 to < 12 month old children: $\frac{1.12\times10^{-6}\ mg\ nanosilver}{cm^2} \times \frac{800\ cm^2}{day} = \frac{0.000893\ mg\ nanosilver}{day}$

Incidental oral exposure for 1 to < 2 year old children: $\frac{1.12\times10^{-6}\ mg\ nanosilver}{cm^2} \times \frac{560\ cm^2}{day} = \frac{0.000625\ mg\ nanosilver}{day}$

Incidental oral exposure for 2 to < 3 year old children: $\frac{1.12\times10^{-6}\ mg\ nanosilver}{cm^2} \times \frac{396\ cm^2}{day} = \frac{0.000442\ mg\ nanosilver}{day}$

The incidental oral daily-dose was calculated from the incidental oral exposure using the following:

Incidental Oral Daily Dose = Exposure / Body Weight

Where:

- Exposure is determined in the calculations above.
- The body weight of a child with age between 6 and < 12 months is 9.2 kg, between 1 and < 2 years is 11.4 kg, between 2 and < 3 years is 13.8 (U.S. EPA, 2011b).

Table 10 – Incidental Oral MOEs for Children Exposed to Plastic Toys Incorporating Nanosilva

Age Range of Child	Exposure[A] (mg/day)	Daily Dose[B] (mg/kg/day)	MOE[C]
6 to < 12 months	8.93×10^{-4}	9.7×10^{-5}	310,000
1 to < 2 years	6.25×10^{-4}	5.5×10^{-5}	550,000
2 to < 3 years	4.42×10^{-4}	3.2×10^{-5}	940,000

A. Exposure = Surface Residue (mg/cm^2) × Saliva Extraction Efficiency × Surface Area of Toy Mouthed (cm^2)
B. Dose = [Exposure (mg/day)] / Body Weight (kg)
C. MOE = POD / Daily Dose (rounded to two significant figures)

The MOEs in Table 10 are for incidental oral exposures and were calculated from the incidental oral dose using the POD of 30 mg/kg/day, which is the NOAEL from a 28-day oral toxicity study (Kim et al., 2008) and the LOAEL from a 90 day oral toxicity study (Kim et al., 2010). The MOEs for incidental oral exposures are 310,000 for 6 to < 12 month old children, 550,000 for 1 to < 2 year old children, and 940,000 for 2 to <3 year old children, which are greater than the target MOE of 3,000 indicating that the risk for short- and intermediate-term exposure to children who mouth plastic toys containing Nanosilva is not of concern.

Consumer Dermal Daily-Dose and MOEs to Flooring Incorporating Nanosilva

The dermal exposure to flooring incorporating Nanosilva was calculated using the following:

Dermal Exposure = Surface Residue (mg/cm^2) × Transfer factor from flooring to skin (%) × Body surface area in contact with floor (cm^2/day)

Where the:

- Body surface area contacting flooring is 4,500 cm^2/day for children between 6 and < 12 months of age, 5,300 cm^2/day for children between 1 and < 2 years of age, and for a 2 to < 3 year old is 6,100 cm^2/day (U.S. EPA, 2011b). These are the total surface area of children and don't account for the portion of child covered with clothing.

The concentration of silver detected in fluids contacting Nanosilva containing coupons (Table 7) represents both the surface residue and the fraction of Nanosilva transferred from flooring to skin. The concentration of silver detected in low and high pH fluids, which are more representative of biological fluids, were all below the analytical detection limit of 5 µg/L for coupons containing 5, 10, and 20 mg/kg of Nanosilva. EPA is assuming that non-detectable concentrations of silver would be found in a similar test conducted with coupons containing 30 mg/kg of Nanosilva. EPA is using 2.5 µg/L, which is one-half the detection limit of 5 µg/L, as the concentration of silver released from plastic incorporating Nanosilva. Using the concentration of silver, the volume of the leachate (50 mL), and the surface area of the test coupons, the amount of available nanosilver at the coupon surface is:

Surface Residue × Transfer factor from flooring to skin: $\frac{0.0025\ mg}{L} \times \frac{50\ mL}{1000\ mL} \times \frac{}{112\ cm^2} = \frac{0.00000112\ mg}{cm^2}$

The dermal exposure for flooring incorporating Nanosilva is:

Dermal Exposure for 6 to < 12 month old children:

$$\frac{1.12\times10^{-6} mg}{cm^2} \times \frac{4500\ cm^2}{day} = \frac{0.00502\ mg\ nanosilver}{day}$$

Dermal Exposure for 1 to < 2 year old children:

$$\frac{1.12\times10^{-6} mg}{cm^2} \times \frac{5300\ cm^2}{day} = \frac{0.00592\ mg\ nanosilver}{day}$$

Dermal Exposure for 2 to < 3 year old children:

$$\frac{1.12\times10^{-6}mg}{cm^2} \times \frac{6100\ cm^2}{day} = \frac{0.00681\ mg\ nanosilver}{day}$$

The dermal daily-dose was calculated from the dermal exposure using the following:

Dermal Daily Dose = Exposure × Dermal absorption factor (%) / Body Weight

Where:

- Exposure is determined in the calculations above.
- The DAF is 6.7% (see Section 4.2.3).
- The body weight of a child with age between 6 and < 12 months is 9.2 kg, between 1 and < 2 years is 11.4 kg, between 2 and < 3 years is 13.8 (U.S. EPA, 2011b).

Table 11 – Dermal MOEs for Children Exposed to Flooring Incorporating Nanosilva

Age Range of Child	Dermal Exposure[A] (mg/day)	Daily Dose[B] (mg/kg/day)	MOE[C]
6 to < 12 months	5.02×10^{-3}	3.7×10^{-5}	820,000
1 to < 2 years	5.92×10^{-3}	3.5×10^{-5}	860,000
2 to < 3 years	6.81×10^{-3}	3.3×10^{-5}	910,000

A. Exposure = Surface Residue (mg/cm^2) × Floor to Skin Transfer Factor (%) × Skin Surface Area of Child (cm^2)

B. Daily Dose = [Exposure (mg/day)] × Dermal absorption factor (%) / Body Weight (kg)

C. MOE = POD / Daily Dose (rounded to two significant figures)

The MOEs in Table 11 are for dermal exposures to flooring incorporating Nanosilva and were calculated from the dermal dose using the POD of 30 mg/kg/day, which is the NOAEL from a 28-day oral toxicity study (Kim et al., 2008) and the LOAEL from a 90 day oral toxicity study (Kim et al., 2010). The MOEs for dermal exposures listed in Table 11 are 820,000 for 6 to < 12 month old children, 860,000 for 1 to < 2 year old children, and 910,000 for 2 to < 3 year old children, which are greater than the target MOE of 3,000 indicating that the risk for short- and intermediate-term exposure to children who contact flooring incorporating Nanosilva is not of concern.

Consumer Incidental Oral Daily-Dose and MOEs to Flooring Incorporating Nanosilva

The incidental oral exposure to nanosilver in flooring incorporating Nanosilva was calculated using the following:

Incidental Oral Exposure = Surface Residue (mg/cm^2) × Transfer factor from flooring to skin (%) × Surface area of hand that contacts the floor and the child's mouth (cm^2/event) × Frequency of hand-to-mouth contacts (events/hr) × Saliva extraction efficiency (%) × Exposure time (hr/day)

Where the:

- Surface area of the hands that contact both the floor and the child's mouth is 10 cm^2/event for children from 1 to < 2 years of age (U.S. EPA, 2012b).
- Frequency of hand-to-mouth events is 19 events/hr for 6 to < 12 month old children, 20 events/hr for 1 to < 2 year old children, and 13 events/hr for 2 to <3 year old children (U.S. EPA, 2011b).
- Saliva extraction efficiency from hand to mouth is 50% (U.S. EPA, 2012b)
- Exposure time is 101 minutes per day for 6 to < 12 month old children, 126 minutes per day for 1 to < 2 year old children, and 108 minutes/day for 2 to <3 year old children which are the mean times for "Doers" in kitchens and bathrooms (Table 16-15, U.S. EPA, 2011b)

The concentration of silver detected in fluids contacting Nanosilva containing coupons (Table 7) represents both the surface residue and the fraction of Nanosilva transferred from flooring to skin. The concentration of silver detected in low and high pH fluids, which are more representative of biological fluids, were all below the analytical detection limit of 5 µg/L for coupons containing 5, 10, and 20 mg/kg of Nanosilva. EPA is assuming that non-detectable concentrations of silver would be found in a similar test conducted with coupons containing 30 mg/kg of Nanosilva. EPA is using 2.5 µg/L, which is one-half the detection limit of 5 µg/L, as the concentration of silver released from plastic incorporating Nanosilva. Using the concentration of silver, the volume of the leachate (50 mL), and the surface area of the test coupons, the amount of available nanosilver at the coupon surface is:

Surface Residue × Transfer factor from flooring to skin: $\frac{0.0025\ mg}{L} \times \frac{50\ mL}{} \times \frac{L}{1000\ mL} \times \frac{}{112\ cm^2} = \frac{0.00000112\ mg}{cm^2}$

The incidental oral exposure for flooring incorporating Nanosilva is:

Incidental Oral Exposure for 6 to < 12 month old children: $\frac{1.12\times10^{-6} mg}{cm^2} \times \frac{10\ cm^2}{event} \times \frac{19\ events}{hr} \times \frac{50\%}{100\%} \times \frac{101\ min}{day} \times \frac{hr}{60\ min} = \frac{0.000178\ mg\ nanosilver}{day}$

Incidental Oral Exposure for 1 to < 2 year old children: $\frac{1.12\times10^{-6} mg}{cm^2} \times \frac{10\ cm^2}{event} \times \frac{20\ events}{hr} \times \frac{50\%}{100\%} \times \frac{126\ min}{day} \times \frac{hr}{60\ min} = \frac{0.000234\ mg\ nanosilver}{day}$

Incidental Oral Exposure for 2 to < 3 year old children: $\frac{1.12\times10^{-6}mg}{cm^2} \times \frac{10\ cm^2}{event} \times \frac{13\ events}{hr} \times \frac{50\%}{100\%} \times \frac{108\ min}{day} \times \frac{hr}{60\ min} = \frac{0.000131\ mg\ nanosilver}{day}$

The incidental oral daily-dose was calculated from the dermal exposure using the following:

Incidental Oral Daily-Dose = Exposure / Body Weight

Where:

- Exposure is determined in the calculations above.
- The body weight of a child with age between 6 and < 12 months is 9.2 kg, between 1 and < 2 years is 11.4 kg, between 2 and < 3 years is 13.8 (U.S. EPA, 2011b).

Table 12 – Incidental Oral MOEs for Children Exposed to Flooring Incorporating Nanosilva

Age Range of Child	Oral Exposure[A] (mg/day)	Daily Dose[B] (mg/kg/day)	MOE[C]
6 to < 12 months	1.8×10^{-4}	1.9×10^{-5}	>1,000,000
1 to < 2 years	2.3×10^{-4}	2.1×10^{-5}	>1,000,000
2 to < 3 years	1.3×10^{-4}	0.95×10^{-5}	>1,000,000

A. Exposure = Surface Residue (mg/cm^2) × Transfer factor from flooring to skin (%) × Surface area of hand that contacts the floor and the child's mouth (cm^2/event) × Frequency of hand-to-mouth contacts (events/hr) × Saliva extraction efficiency (%) × Exposure time (hr/day)

B. Dose = [Exposure (mg/day)] / Body Weight (kg)

C. MOE = POD / Daily Dose

The MOEs in Table 12 are for incidental oral exposures to flooring incorporating Nanosilva and were calculated from the incidental oral dose using the POD of 30 mg/kg/day, which is the NOAEL from a 28-day oral toxicity study (Kim et al., 2008) and the LOAEL from a 90 day oral toxicity study (Kim et al., 2010). The MOEs for incidental oral exposures are greater than 1,000,000 for 6 to < 12 month old, 1 to < 2 year old children, and 2 to < 3 year old children, which are greater than the target MOE of 3,000 indicating that the risk for short- and intermediate-term exposure to children who mouth their hands after contacting flooring incorporating Nanosilva is not of concern.

5.2.3 Consumer Margins of Exposure to Textiles Incorporating Nanosilva

EPA expects that consumers will be exposed to textiles incorporating Nanosilva by the routes of inhalation, dermal, and incidental oral exposures.

Consumer Inhalation Daily-Dose and MOE

EPA recognizes the potential for inhalation exposure to nanosilver during laundry drying of textiles incorporating Nanosilva. However, EPA lacks information on the release rate of nanosilver from textiles incorporating Nanosilva during laundry drying. While exposure may occur during laundry drying, EPA believes that when compared to exposure through dermal and oral contact with textiles incorporating Nanosilva, exposure during laundry drying will likely be of lower significance. To support this, an estimate of the inhalation dose that might occur from exposure to textiles incorporating Nanosilva during laundry drying was calculated using the following:

Inhalation Daily Dose = Amount of Nanosilva in clothing handled per day × Unit Exposure

Where:

- Amount of Nanosilva in clothing handled per day assumed that one t-shirt containin Nanosilva was laundered where the amount of nanosilver in a t-shirt is equal to 150 g × 30 mg/kg × kg ÷ 1000 g = 4.5 mg.
- The unit exposure for inhalation of wettable powder is 7.8 µg/m^3/lb when no respirator is worn (U.S. EPA, 2011a).

Table 13 – Inhalation MOE for Drying Textiles Incorporating Nanosilva

Scenario	Amount of Nanosilver Laundered per day[A]	Unit Exposure[B] (µg/m^3/lb AI)	Daily Dose[C] (µg/m^3)	MOE[D]
Unload Clothes Dryer **Without Respirator**	4.5 mg	7.8	0.000077	640,000

A. Amount of AI laundered per day = Amount of Nanosilva in t-shirt × Mass of t-shirt
B. PHED unit exposure value converted to air concentration units based on mean 8 hour TWA
C. Dose = Amount Nanosilver Handled × Unit Exposure
D. MOE = POD / Daily Dose (rounded to two significant digits)

The MOE shown in Table 13 was calculated by dividing the inhalation dose by the POD of 49 µg/m^3, which is the NOAEL from a 90-day inhalation toxicity study for nanosilver (Song et al., 2012). This assumes that Nanosilva released from textiles during drying behaves like nanosilver after inhalation. This analysis further assumes that all of the nanosilver from the textile incorporating Nanosilva becomes airborne during a single laundry drying event and this exposure would be similar to mixing and loading of wettable powders (U.S. EPA, 2011a). The MOE of 640,000 indicates that the risk for short- and intermediate-term exposure to laundering one textile incorporating Nanosilva per day is not of concern. Up to 640 t-shirts incorporating Nanosilva could be laundered per day and the calculated MOE would be greater than the target

MOE of 1,000, indicating that the risk for short- and intermediate-term exposure to drying textiles incorporating Nanosilva is not of concern.

Consumer Dermal Daily-Dose and MOEs

The dermal exposure to textiles incorporating Nanosilva was calculated using the following:

Dermal Exposure = Amount of Nanosilva in Textile × Cloth Density × Surface Area Exposed × Transfer Efficiency

Where:

- The textile incorporating Nanosilva contains 30 mg/kg nanosilver.
- The cloth density is 10 mg/cm^2 based on the density of mixed cotton and synthetics. This value is a standard assumption used in OPP risk assessments and was taken from the HERA Guidance Document Methodology (AISE/CEFIC, 2005).
- The total surface area for a 6 to < 12 month old is 4,500 cm^2/day, for a 1 to < 2 year old is 5,300 cm^2/day, and for a 2 to < 3 year old is 6,100 cm^2/day (U.S. EPA, 2011b).
- The cloth-to-skin transfer efficiency was based on the amount of silver released during the leaching study which was 0.9% (see Table 9).

Table 14 – Dermal Exposure to Textiles Incorporating Nanosilva

Age Range of Child	Application Rate (mg/kg)	Cloth Density (mg/cm^2)	Surface Area Exposed (cm^2/day)	Cloth-to-Skin Transfer Efficiency	Exposure[A] (mg/day)
6 to <12 months	30	10	4,500	0.9%	0.0122
1 to < 2 years	30	10	5,300	0.9%	0.0143
2 to < 3 years	30	10	6,100	0.9%	0.0165

A. Exposure = Application Rate × Cloth Density × Surface Area Exposed × Cloth-to-Skin Transfer Efficiency × Dermal Absorption Factor

The dermal dose was calculated from the dermal exposure using the following:

Dermal Daily Dose = Exposure × Dermal Absorption Factor / Body Weight

Where:

- Exposure is determined in the calculation above.

- The DAF is 6.7% (see Section 4.2.3).
- The body weight of a child with age between 6 and < 12 months is 9.2 kg, between 1 and < 2 years is 11.4 kg, between 2 and < 3 years is 13.8 (U.S. EPA, 2011b).

Table 15 – Dermal MOEs for Children Exposed to Textiles Incorporating Nanosilva

Age Range of Child	Body Weight of Child (kg)	Exposure (mg/day)	Daily Dose[A] (mg/kg/day)	MOE[B]
6 to <12 months	9.2	0.012	8.8×10^{-5}	340,000
1 to < 2 years	11.4	0.014	8.4×10^{-5}	360,000
2 to < 3 years	13.8	0.016	8.0×10^{-5}	370,000

A. Dose = [Exposure (mg/day)] × DAF / Body Weight (kg)
B. MOE = POD / Daily Dose (rounded to two significant figures)

The MOEs for dermal exposures were calculated from the dermal dose using the POD of 30 mg/kg/day, which is the NOAEL from a 28-day oral toxicity study (Kim et al., 2008) and the LOAEL from a 90 day oral toxicity study (Kim et al., 2010). The MOEs for dermal exposures are 340,000 for 6 to < 12 month old, 360,000 for 1 to < 2 year old children, and 370,000 for 2 to < 3 year old children, which are greater than the target MOE of 3,000 indicating that the risk for short- and intermediate-term exposure to children from wearing textiles incorporating Nanosilva is not of concern.

Consumer Incidental Oral Daily-Dose and MOEs

Incidental oral exposures were calculated using the following:

Incidental Oral Exposure = Amount of Nanosilva in Textile × Cloth Density × Surface Area Mouthed × Saliva Extraction Efficiency

Where:

- The textile incorporating Nanosilva contains 30 mg/kg nanosilver.
- The cloth density is 10 mg/cm^2 based on the density of mixed cotton and synthetics. This value is a standard assumption used in OPP risk assessments and was taken from the HERA Guidance Document Methodology (AISE/CEFIC, 2005)
- The surface area of fabric that is mouthed by a toddler per day is assumed to be 100 cm^2 (~16 in.^{2}) which represents an estimate for the area of blanket or shirt sleeve.

- The nanosilver saliva extraction efficiencies for mouthing fabric are based on the results of the leaching study which was 0.9% (see Table 9).

Table 16 – Incidental Oral Exposure to Textiles Incorporating Nanosilva

Application Rate (mg/kg)	Cloth Density (mg/cm^2)	Surface Area Mouthed (cm^2/day)	Saliva Extraction Efficiency	Exposure[A] (mg/day)
30	10	100	0.9%	0.00027

A. Exposure = Application Rate × Cloth Density × Surface Area Exposed × Saliva Extraction Efficiency

The incidental oral daily-dose was calculated from the incidental oral exposure using the following:

Incidental Oral Daily-Dose = Exposure / Body Weight

Where:

- Exposure is determined in the calculation above.
- The body weight of a child with age between 6 and < 12 months is 9.2 kg, between 1 and < 2 years is 11.4 kg, between 2 and < 3 years is 13.8, which is for children who mouth textiles (U.S. EPA, 2011b).

Table 17 – Incidental Oral MOEs for Children Exposed to Textiles Incorporating Nanosilva

Age Range of Child	Body Weight of Child	Exposure (mg/day)	Daily Dose[A] (mg/kg/day)	MOE[B]
6 to <12 months	9.2	0.00027	2.9×10^{-5}	>1,000,000
1 to < 2 years	11.4	0.00027	2.4×10^{-5}	>1,000,000
2 to < 3 years	13.8	0.00027	2.0×10^{-5}	>1,000,000

A. Dose = [Exposure (mg/day)] / Body Weight (kg)
B. MOE = POD / Daily Dose (rounded to two significant digits)

The MOEs in Table 17 are for incidental oral exposures were calculated from the incidental oral dose using the POD of 30 mg/kg/day, which is the NOAEL from a 28-day oral toxicity study (Kim et al., 2008) and the LOAEL from a 90 day oral toxicity study (Kim et al., 2010). The MOEs for incidental oral exposures are greater than 1,000,000 for 6 to < 12 month old, 1 to < 2

year old, and 2 to < 3 year old children, which are greater than the target MOE of 3,000 indicating that the risk for short- and intermediate-term exposure to children who mouth textiles incorporating Nanosilva is not of concern.

5.2.4 Consumer Aggregate Margins of Exposure to Plastics and Textiles Incorporating Nanosilver

In the Federal Food, Drug, and Cosmetic Act (FFDCA), Congress specified that, to establish an acceptable level of a given pesticide's chemical residue that could be found in or on food products, EPA must determine that "there is a reasonable certainty that no harm will result from aggregate exposures to the pesticide chemical residue, including all anticipated dietary exposures and all other exposures for which there is reliable information." Furthermore, when enacting this provision of the FFDCA, Congress also amended FIFRA's definition of unreasonable adverse effects. Specifically, Congress redefined unreasonable adverse effects to include "a human dietary risk from residues that result from a use of a pesticide in or on any food inconsistent with the standard under" the FFDCA. In other words, Congress explicitly required the consideration of aggregate exposures for registration decisions under FIFRA for food-use pesticides, but chose not to similarly alter the statutory requirements for non food-use pesticides.

For transparency, the following analysis is for children who are simultaneously exposed to nanosilver via the incidental oral and dermal routes of exposure while mouthing toys, contacting flooring, and mouthing textiles incorporating Nanosilva. Inhalation exposure to children during laundry drying of textiles incorporating Nanosilva is not anticipated and is not considered as part of the aggregate exposure. The aggregate daily dose was calculated by adding the daily oral and dermal doses using the following:

> **Aggregate Daily Dose** = Incidental Oral Dose to Toys Incorporating Nanosilva + Dermal Dose to Flooring Incorporating Nanosilva + Incidental Oral Dose to Flooring Incorporating Nanosilva + Dermal Dose to Textiles Incorporating Nanosilva + Incidental Oral Dose to Textiles Incorporating Nanoslva

Where:

- Incidental Oral Daily Dose to Toys Incorporating Nanosilva is from Table 10
- Dermal Daily Dose to Flooring Incorporating Nanosilva is from Table 11
- Incidental Oral Daily Dose to Flooring Incorporating Nanosilva is from Table 12
- Dermal Daily Dose to Textiles Incorporating Nanosilva is from Table 15
- Incidental Oral Daily Dose to Textiles Incorporating Nanosilva is from Table 17

The oral and dermal daily doses can be summed because they are evaluated using the same POD of 30 mg/kg/day. The aggregate MOE in Table 18 for 6 and < 12 month old children is 110,000,

is 140,000 for children who are 1 and < 2 years, and 170,000 for 2 to <3 year old children, which indicates that the risk for short- and intermediate-term exposure to children is not of concern.

Table 18 – Aggregate MOEs for Children Exposed to Plastics and Textiles Incorporating Nanosilva

Age Range of Child	Incidental Oral Dose to Toys	Flooring		Textiles		Aggregate Dose	Aggregate MOE[A]
		Dermal Dose	Incidental Oral Dose	Dermal Dose	Incidental Oral Dose		
6 to < 12 months	9.7×10^{-5}	3.7×10^{-5}	1.9×10^{-5}	8.8×10^{-5}	2.9×10^{-5}	2.7×10^{-4}	110,000
1 to < 2 years	5.5×10^{-5}	3.5×10^{-5}	2.1×10^{-5}	8.4×10^{-5}	2.4×10^{-5}	2.2×10^{-4}	140,000
2 to < 3 years	3.2×10^{-5}	3.3×10^{-5}	0.95×10^{-5}	8.0×10^{-5}	2.0×10^{-5}	1.7×10^{-4}	170,000

A. Aggregate MOE = POD / Daily Dose (rounded to two significant digits)

The assessment above is for the concurrent exposure to the nanosilver in Nanosilva via the incidental oral and dermal routes of exposure while mouthing toys and textiles, and contacting flooring incorporating Nanosilva. Aggregate assessments can also include other sources of exposure such as to Nanosilva in food and drinking water, and to other nanosilvers in the market place that are identical to the nanosilver in Nanosilver. There are no anticipated food exposures to Nanosilva since Nanosilva LLC has not supplied the necessary information to support a food contact application and the pesticide label for Nanosilva states that it is only for non-food contact use. Neither the nanosilver that might break away from Nanosilva or Nanosilva is anticipated to enter drinking water because these particulates will be removed by gravitational sedimentation and adsorption (see Section 5.1.1).

EPA has determined that the only other pesticide which was knowingly registered as containing nanosilver is HeiQ AGS-20 (U.S. EPA, 2011a); although, the nanosilver in HeiQ AGS-20 does not have the exact same size range and surface coatings as the nanosilver in Nanosilva. The incidental oral and dermal MOEs for HeiQ AGS-20 were all greater than 1,100 and the incidental oral and dermal MOEs for Nanosilva are all greater than 310,000. None of the MOEs for HeiQ AGS-20 were less than the target MOEs for that assessment, which was based on conservative estimates expected to significantly overestimate exposures to the nanosilver in HeiQ AGS-20. Although EPA has not determined whether it's appropriate to consider concurrent exposures to the nanosilver in Nanosilva and HeiQ AGS-20, the agency has concluded that even if exposures to these two active ingredients were to occur concurrently, they would not result in MOEs that would be of concern. In addition, EPA is currently revising the HeiQ AGS-20 risk assessment based on newly submitted leaching data and to be consistent with the risk assessment framework proposed in this decision document. EPA anticipates that the

calculated MOEs for incidental oral and dermal exposure to textiles containing HeiQ AGS-20 will increase based on newly submitted data.

As to exposure to other nanosilvers in the market place, an aggregate risk assessment involves the analysis of exposure to a single chemical by multiple pathways and routes of exposure that share a common toxic effect. For EPA to conduct such an assessment requires that the Agency possess sufficient information such as:

- identification of toxicological endpoints for each exposure route and duration;
- identification of potential exposures for each pathway (food, water, and/or residential);
- reconciliation of durations and pathways of exposure with durations and pathways of health effects;
- determination of which possible residential exposure scenarios are likely to occur together within a given time frame (co-occurrence);
- determination of magnitude and duration of exposure for all exposure combinations;
- determination of the appropriate technique (deterministic or probabilistic) for exposure assessment;
- and determination of the appropriate risk metric to estimate aggregate risk.

When EPA has the above information, or as in the case of triclosan where population-based biological monitoring data were available to assess the co-occurrence of uses, EPA develops an aggregate exposure assessment for residential uses of a single chemical. However, in the case of nanosilver, with the exception of the HeiQ products, EPA does not yet have adequate information on the composition of the nanosilver or potential nanosilver in other silver based pesticides to determine if all nanosilvers should be treated as a single chemical.

5.2.5 Conclusions for Consumer Risk

The MOEs calculated for the incidental oral exposure to nanosilver in plastic toys incorporating Nanosilva are 310,000 for 6 to < 12 month old children, 550,000 for 1 to < 2 year old children, and 940,000 for 2 to <3 year old children, which are greater than the target MOE of 3,000 indicating that there is no risk concern for short- or intermediate-term exposure to children who mouth toys incorporating Nanosilva. The MOEs calculated for the dermal exposure to nanosilver in flooring incorporating Nanosilva are 820,000 for 6 to < 12 month old children, 860,000 for 1 to < 2 year old children, and 910,000 for 2 to <3 year old children, which are greater than the target MOE of 3,000 indicating that there is no risk concern for short- or intermediate-term exposure to children who crawl on flooring incorporating Nanosilva. The MOEs calculated for the incidental oral exposure to nanosilver in flooring incorporating Nanosilva are greater than 1,000,000 for 6 to < 12 month old children, 1 to < 2 year old children, and 2 to <3 year old children, which are greater than the target MOE of 3,000 indicating that there is no risk concern for short- and intermediate-term exposure to children who mouth flooring incorporating

Nanosilva. The MOEs calculated for the dermal exposure to nanosilver in textiles incorporating Nanosilva are 340,000 for 6 to < 12 month old children, 360,000 for 1 to < 2 year old children, and 370,000 for 2 to <3 year old children, which are greater than the target MOE of 3,000 indicating that there is no risk concern for short- and intermediate-term exposure to children who wear textiles incorporating Nanosilva. The MOEs calculated for incidental oral exposures to nanosilver in textiles incorporating Nanosilva are greater than 1,000,000 for 6 to < 12 month old children, 1 to < 2 year old children, and 2 to < 3 year old children, which are greater than the target MOE of 3,000 indicating that there was no risk concern for short- and intermediate-term exposure to children who mouth and chew textiles incorporating Nanosilva.

The aggregate MOEs for children who are, within the same short time frame, mouthing a toy, flooring, and a textile while wearing a textile and crawling on flooring all incorporating Nanosilva are greater than 100,000 for all lifestages, which indicates that the risk for short- and intermediate-term simultaneous exposure to plastics and textiles incorporating Nanosilva is not a concern.

Although children's short- and intermediate-term exposure durations to plastic toys and textiles treated with Nanosilva are expected, children are not anticipated to play with Nanosilva treated toys or contact Nanosilva treated textiles every day for a period of greater than six months, thus long-term exposures are unlikely. Nanosilva is proposed to be used in flooring meaning that long-term dermal and incidental oral exposures to children contacting flooring are possible. However, EPA does not believe that children will have continuous and daily exposure to flooring for a period of greater than 6 months. Even if these unlikely long-term exposures were to occur, the calculated MOEs for children's dermal and incidental oral exposure to plastics and textiles incorporating Nanosilva are greater than 100,000 indicating that daily exposure to plastics and textiles incorporating Nanosilva for greater than 6 months are not likely to be of concern.

There are several uncertainties in the consumer risk assessment. Because Nanosilva LLC completed leaching tests using plastic coupons containing nanosilver at concentrations of 5, 10, and 20 mg/kg, EPA assumed that non-detectable concentrations of silver would also be found if a leaching test was conducted with coupons containing nanosilver at 30 mg/kg. This assumption was reasonable because the concentration of silver in the textile leaching studies, which were conducted on textiles incorporating nanosilver at 30 mg/kg, was also below the analytical detection limit, making it unlikely that Nanosilver incorporated into plastic at 30 mg/kg will release nanosilver at detectable concentrations. The PODs used were based on inhalation and oral toxicity studies completed using nanosilver, not Nanosilva or the nanosilver that might break away from Nanosilva, and thus these PODs may not equal PODs determined from testing conducted with Nanosilva or the nanosilver found in Nanosilva. Because the nanosilver in the toxicity database is different from the nanosilver in Nanosilva and because the database is incomplete with respect to reproductive and developmental effects, the Agency used a maximum

10-fold database uncertainty factor when evaluating the risk from exposure to Nanosilva and the nanosilver that might be released from products incorporating Nanosilva. There are also uncertainties in extrapolating from effects observed after feeding test-animals nanosilver (i.e., oral route) to the effects that might be observed after applying nanosilver to the skin of test animals (i.e., dermal route).

EPA is able to determine that for the period of conditional registration, there is a low probability of adverse risk to children from plastics and textiles incorporating Nanosilva. Thus, the Agency concludes that use of Nanosilva will not cause unreasonable adverse effects on the environment during the period when newly required data are being developed.

5.2.6 Consumer Exposure and Health Data Requirements

EPA has determined that an additional leaching study is required for plastics incorporating Nanosilva using biological fluids and physiological temperatures, which includes methods to determine the form of silver released. EPA has determined that the textile leaching study submitted by Nanosilva LLC is acceptable and no additional studies on textiles incorporating Nanosilva are required.

EPA is proposing to require that Nanosilva LLC conduct plastic leaching tests as part of Tier I studies because the available study was completed using a protocol that was not reviewed by EPA prior to conducting the study, the coupons tested has less than the maximum nanosilver concentration of 30 mg/kg, and the study did not use physiological fluids. The newly required leaching study should determine the nature and quantity of silver released from plastics incorporating Nanosilva under conditions of use. EPA will evaluate the results from the plastic leaching test along with results from Tier I toxicity studies discussed in Section 5.1.4 using a weight of evidence approach based on physical and chemical properties, toxicity, and exposure. If the Agency identifies a concern, then the Agency may require additional toxicity data to assess potential adverse health outcomes in young children resulting from incidental oral exposure to Nanosilva-treated plastic toys (*i.e.,* children chewing on toys). If additional toxicity data are needed, the study should evaluate peri- and post-natal exposure to Nanosilva. A more detailed description of these data requirements is provided in Appendix B.

VI. ENVIRONMENTAL RISK ASSESSMENT

EPA anticipates the following substances could enter the environment through leaching of plastics and textiles incorporating Nanosilva:

1) Silver ions released from Nanosilva;
2) Nanosilva complex; and/or
3) Nanosilver that might break away from the Nanosilva complex.

There are no studies available to characterize the environmental fate or ecotoxicity of Nanosilva. However, there are studies available in the scientific literature for nanosilver. Since nanosilver may be released from Nanosilva, the Agency has considered the scientific literature studies on nanosilver fate and ecotoxicity relevant to Nanosilva. The following sections cover the environmental fate of nanosilver, the environmental hazards posed by silver and nanosilver, and the potential risk to aquatic species from nanosilver. As part of these discussions, additional data that EPA is proposing to require in order to confirm its assessment of the environmental risks of Nanosilva are also identified.

6.1 Environmental Fate

Nanosilva LLC has not conducted any studies to characterize the environmental fate of Nanosilva or the other particles that could be released during leaching or disposal of plastics and textiles incorporating Nanosilva. In lieu of this information, the Agency is relying on studies available in the scientific literature, discussed in the following section, as the basis for determining the fate of nanosilver in the environment.

6.1.1 Nanosilver

The rate at which nanosilver transforms into ionic silver determines the length of time that these particles will reside in the environment. Although there are studies reporting that nanosilver will completely transform into ionic silver within six days after being dispersed into deionized water (Liu and Hurt, 2010), these results are only for one form of nanosilver and under conditions which are not representative of the environment. In the environment, nanosilver is likely to complex with naturally occurring anions such as chloride and sulfide or natural organic matter such as humic acids, which will significantly delay the rate at which nanosilver transforms into ionic silver. For example, Choi et al., (2009) provided spectroscopic evidence showing that nanosilver reacts with sulfide to produce stable silver-sulfide complexes, which were shown by Liu et al. (2010) and Levard et al. (2011) to have undetectable rates of nanosilver to ionic silver transformation. These stabilized nanosilver complexes are likely to partition to sediments, rather than remain suspended in water, due to gravitational settling and coagulation processes (see Page 19 FIFRA SAP, 2009). Likewise, nanosilver is anticipated to partition to biosolids during

wastewater treatment but may also be released in the effluent. Thus, there is the potential for nanosilver to reside or persist in the environment for a significant period of time where these particles are most likely to be associated with sediments.

6.1.2 Silver Ions

Ionic silver typically has low concentrations in natural waters, in the nanogram per liter range, due to its reactivity with chloride, sulfides, and natural organic matter (Andren and Armstrong, 1999). As with nanosilver, ionic silver is found in sediments and associated with biosolids in wastewater treatment plants.

6.1.3 Impacts to Wastewater Treatment/Septic Systems

There is the potential for nanosilver that might be released from plastics and textiles incorporating Nanosilva to reach publicly owned wastewater treatment and privately owned septic systems where they will most likely complex with sulfide and partition to biosolids (Kaegi et al., 2011). Once entrained in the biosolids, the nanosilver could serve as a long term source of ionic silver and could potentially adversely affect microorganisms that are vital to the wastewater treatment process. There are contradictory reports in the scientific literature regarding the impact of nanosilver on wastewater treatment systems. For example, nanosilver was reported to inhibit nitrification in the range of 50% (Choi and Hu, 2009a) to 84% (Choi and Hu, 2009b) based on a reduction in oxygen uptake rate in simulated wastewater sludge. However, Burkhardt et al. (2010) found no impact to nitrification at nanosilver dosages of 1 mg/L, the same dosage that Choi and Hu (2009a and 2009b) reported as inhibitory in municipal wastewater sludge. These two research groups are reporting different findings with the Burkhardt group suggesting little impact of nanosilver to nitrification and the Hu group suggesting that an impact to wastewater treatment systems from nanosilver is expected. A third group independently determined that nanosilver at concentrations from 0.5 to 1.5 mg/L had no detectable effect on the ability of the wastewater bacteria to biodegrade organic material, as measured by chemical oxygen demand (COD) (Wang et al., 2012a). More recent work by the Hu group reported that nanosilver at concentrations of up to 40 mg/L had negligible impact on anaerobic digestion and methanogenic organisms (Yang et al., 2012).

While there are reports suggesting the potential for nanosilver to impact wastewater treatment operations, the Agency does not anticipate that registering Nanosilva will lead to negative impacts to wastewater treatment systems. This conclusion is based on the limited amount of silver released from plastics and textiles incorporating Nanosilva (see Section 5.2.1) and the small volume of nanosilver (i.e., < 1,123 kg/yr as estimated in Section 6.3.1) expected to be introduced into commerce from plastics and textiles incorporating Nanosilva.

6.2 Environmental Toxicity

Nanosilva LLC has not conducted any studies to characterize the ecotoxicity of Nanosilva or the other particles that could be released during leaching or disposal of plastics incorporating Nanosilva. In lieu of this information, the Agency is relying on studies available in the scientific literature, discussed in the following section, as the basis for determining the ecotoxicity of nanosilver.

6.2.1 Silver Ions

EPA has considerable data on the environmental hazards posed by the release of silver ions from conventional silver-based products. The precious metal silver is a trace element found in the Earth's crust and is generally naturally present in surface waters in relatively low concentrations as compared to metals such as copper and zinc. However, it may become toxic to aquatic life at elevated concentrations. Thus, silver concentrations in natural environments, and its biological availability, are important. Naturally occurring concentrations of silver have been reported from about 0.0002 to just over 1 μg/L in freshwater systems (Campbell et al., 2002). Elevated concentrations of silver in surface waters have generally been associated with wastewater treatment plant effluent discharges (Bell and Kramer, 1999). Based on the most recent Agency assessment of silver, EPA does not expect unreasonable adverse effects to the environment from registered uses, including the materials preservative use pattern (U.S. EPA, 1993).

6.2.2 Aquatic Toxicity of Nanosilver

Although no tests with Nanosilva and aquatic organisms were submitted, there are studies in the scientific literature covering the toxicity of nanosilver to aquatic organisms (Table 19). These studies indicate to the Agency that, if sufficient quantities of nanosilver break away from Nanosilva and reach surface water, and if such nanoparticles display toxicity similar to the nanosilver used in these studies, then exposure of Nanosilva derived nanosilver may result in adverse effects to aquatic species. These results also indicate that, of the organisms tested, *Daphnia magna* is the most acutely sensitive to nanosilver. As with the toxicity for silver ions, the toxicity of nanosilver may depend on the ligands or counter ions (e.g., Ca^{2+}) present in the test media. For example, the LC_{50} reported by Laban et al. (2010) was up to 27 times greater than the LC_{50} reported by Kennedy et al. (2010) for the same organism where this difference may have been caused by the greater amount of calcium carbonate present in the work by Laban et al. (2010).

Table 19 – Nanosilver Toxicity to Aquatic Species

Aquatic Organisms (Study Citation)	Toxicity	Silver Nitrate	Nanosilver		
		Total Silver (µg/L)	Total Silver (µg/L)	Dissolved Silver (µg/L)	Water Characteristics
Freshwater Algae *Raphidocelis subcapitata* (Wang et al., 2012b)	4.5-hr EC_{50}[A]	31.3	929-120,000	Not reported	Dutch Standard Water (demineralized water with sodium bicarbonate, potassium bicarbonate, calcium chloride, and magnesium sulfate and then aerated for 24 h at 27 °C)
Newborn Neonates of Freshwater Zooplankton *Chydorus sphaericus* (Wang et al., 2012b)	48-hr EC_{50}	4.3	36.7-128.4	Not reported	
Freshwater Fish Embryos *Danio rerio* (Wang et al., 2012b)	96-hr EC_{50}	78.8	88.4-210.3	Not reported	
Freshwater Flea *D. magna* (Kennedy et al., 2010)	96-hr LC_{50}[B]	1.2±0.5	1.8 to 97.0[C]	0.3 to 1.9[C,D]	Moderately hard reconstituted water (MHRW), 80 mg/L as calcium carbonate
Freshwater Fish Larval *Pimephales promelas* (Kennedy et al., 2010)	96-hr LC_{50}	6.1±0.6	9.0 to 125.6[C]	1.5 to 5.6[C,D]	
Freshwater Flea *D. magna* (Hoheisel et al., 2012)	48-hr LC_{50}	0.98	4.3 to 30.4[E]	Not reported	Moderate ionic strength Lake Superior water
Freshwater Flea *D. magna* (Asghari et al., 2012)	48-hr LC_{50}	0.23	2 to 187[E]	Not reported	OECD 202 Media
Freshwater Flea *D. magna* (Hoheisel et al., 2012)	24-hr LC_{50}	0.81	4.3 to 10.5	3.5 to 8.1	Moderately hard reconstituted water (MHRW), 96 mg/L as sodium bicarbonate
Natural Bacterioplankton (Das et al., 2012)	48-hr EC_{50}	Not reported	15 to 276	No reported	EC_{50} values did not correlate with any of the basic limnological parameters
Freshwater Fish Embryo *Pimephales promelas* (Laban et al., 2010)	96-hr LC_{50}	15	9,400 to 10,600	40	215 to 240 mg/L as calcium carbonate; pH 8.3 to 8.5
Freshwater Fish Embryo *Danio rerio* (Wang et al., 2012b)	96-hr LC_{50}	77 to 82	72 to 223[G]	0.7 to 22.3[H]	Not reported

A. EC_{50} (medium effect concentration): Concentration at which the response is halfway between the baseline and maximum after the specified exposure time.
B. LC_{50} (median lethal concentration): Concentration required to kill half the members of a tested population after the specified exposure.
C. Range for eight different nanosilver formulations.
D. Expressed as the fraction of suspended nanosilver.
E. For nanosilvers with diameters from 10 to 50 nm.
F. Three nanosilver formulations
G. Rage of three different nanosilver formulations
H. Estimated

Based on the studies listed in Table 19, the most sensitive aquatic species to nanosilver is *Daphnia magna*. The lowest 96-hr EC_{50} was 1.8 µg/L (Kennedy et al., 2010), which is 1.5 µg/L after subtracting the fraction of ionic silver present. This test was conducted in moderately hard reconstituted water (MHRW), which is a test medium representative of surface water in the United States.

Although these studies are useful for indicating the concentration of nanosilver that might lead to effects in aquatic species, and to identify for this assessment an effect level for evaluating the ecotoxicity of nanosilver that could be released from plastics incorporating Nanosilva, the data represent short-term (e.g., 96 hr) acute exposures and do not characterize the effects from longer-term exposure to nanosilver. There is one long-term study on the effects of nanosilver on a freshwater fish (*Oryzias latipes*) that reported a no observed effect concentration (NOEC) of 100 µg/L based on the lethal effect observed at the dose of 250 µg/L during a 14-day exposure period (Wu and Zhou, 2013). Non-lethal effects were reported for all nanosilver concentration tested (50 to 500 µg/L) including histological lesions in the fish tissues along with silver accumulation in the liver.

6.3 Aquatic Risk Assessment

EPA expects that Nanosilva, silver ions released from Nanosilva, and nanosilver that might break away from Nanosilva could enter the environment through the following scenarios:

1. Leaching of plastics and textiles incorporating Nanosilva
2. Application of wastewater treatment biosolids to agricultural fields

Leaching of plastics and textiles incorporating Nanosilva is anticipated to be the primary route by which Nanosilva, silver ions, and nanosilver reach the environment. The silver released during leaching of plastics and textiles incorporating Nanosilva could be discharged to the sanitary sewer system leading to publically owned wastewater treatment and privately owned septic systems, also known as the down-the-drain discharge scenario. Once Nanosilva, silver ions, or nanosilver reach wastewater treatment and septic systems they will most likely complex with sulfide and partition to biosolids. However, some fraction of the silver compounds will

reach surface water and may potentially impact aquatic organisms. Leaching of building material such as plastic siding and decks, and outdoor furniture and carpeting incorporating Nanosilva will be discharged directly into the environment.

As stated in Section 5.2.1, silver was not found above the analytical detection limit leaching from plastic coupons and shirts incorporating Nanosilva. EPA does not expect unreasonable adverse effects to the environment from registered uses of silver ions, including the materials preservative use pattern (U.S. EPA, 1993). To evaluate the impacts on surface water from leaching of Nanosilva from plastics and textiles, EPA is assuming that nanosilver as found in Nanosilva is the only silver compound released.

6.3.1 Aquatic Risk Quotient

EPA uses a Risk Quotient (RQ) approach to assess impacts to surface water, which is similar to the MOE used for the human health risk assessment. The RQ is used to compare toxicity from environmental exposure by dividing a point estimate of exposure by a point estimate of effects. This ratio is a simple, screening-level estimate that identifies high- or low-risk situations. In this method, the estimated environmental concentration is compared to an effect level, such as an LC_{50}. After the RQ is calculated, it is compared to the Agency's Level of Concern (LOC). An LOC is a policy tool that the Agency uses to interpret the RQ and to analyze potential risk to non-target organisms and the need to consider regulatory action (U.S. EPA, 2011c).

Indoor Use

- Mass of silver release per year: 1123 kg/year. The amount of silver released from textiles incorporating Nanosilva was derived assuming that 300 million people (U.S. population) purchase one t-shirt incorporating Nanosilva each year. Each t-shirt weighs 150 grams and contains 0.003% nanosilver by weight silver and releases 1.6% silver per wash (see Section 5.2.1).
- Release Weeks: Each t-shirt is washed once per week for 52 weeks/yr.
- Wastewater Removal Efficiency: Ranged from 85 to 99% based on the range provided by Blaser et al. (2008). Wang et al. (2012a) reported nanosilver removal of 88% with biomass present.

Outdoor use

- Mass of silver release per year: 570 kg/year. The amount of silver released from plastics incorporating Nanosilva was derived assuming that 1% of the yearly plastic lumber production contains Nanosilva at 0.003%. Yearly plastic lumber production for 2006 was projected to be approximately 1900 million kg/yr (Stutz et al., 2003). This assumes that all the nanosilver in plastic incorporating Nanosilva is released during a year even though

results from the plastic leaching study indicate that little silver is released from plastic incorporating Nanosilva.

- Release Days: 365 days per year. This assumes that each plastic incorporating Nanosilva releases all its silver as nanosilver over the course of one year.
- Wastewater Removal Efficiency: 0% to represent direct release into aquatic environments from outdoor use of plastics incorporating Nanosilva (i.e., no removal of nanosilver in the run-off due to adsorption to soil).

Down the Drain Model

The concentration of nanosilver in surface water resulting from the use of Nanosilva in plastics was calculated using the Down the Drain Module of the Exposure and Fate Assessment Screening Tool (E-FAST model, version 2). The following input values were used:

- Stream Dilution Factor: 1.0 or 20.1. These values are the 10th and 50th percentile values for the dilution that occurs during one day of lowest stream flow over a ten year period (1Q10) (U.S. EPA, 2007b).
- The toxicity value for nanosilver: 1.5 µg/L based on the 96-hr EC_{50} value for *Daphnia magna*.
- Level of Concern for the RQ: The presumptive level of concern (LOC) is 0.05 for listed (i.e. endangered or threatened) aquatic organisms and 0.5 for non-listed organisms.

Table 20 – Risk Quotients for Nanosilver in Surface Water

Use Scenario	Wastewater Treatment Removal[A]	Stream Dilution Factor	Surface Water Silver Concentration (µg/L)	Acute Risk Quotient[D]	Acute RQ Exceeds LOC?*
Indoor Use (t-shirts)	85%	1.0[B]	0.004	0.003	No
		20.1[C]	0.0002	0.0001	No
Outdoor Use (plastic lumber)	0%[E]	1.0	0.014	0.009	No
		20.1	0.001	0.0007	No

*The LOC is 0.05 (listed species) and 0.5 (non-listed species). RQs that exceed the LOC are of concern

A. Silver removed from wastewater during treatment before discharge to a water body (e.g. lake, river etc.).

B. 10th Percentile dilution factor for 1Q10 stream flow.

C. 50th Percentile dilution factor for 1Q10 stream flow.

D. Acute RQ = Surface Water Concentration / LC_{50} for *Daphnia magna* (1.0 µg/liter)

E. Assumes direct discharge to surface water with no retention in soil or sediment.

The down-the-drain modeling results are shown in Table 20 and these results were divided by the 96-hr EC_{50} of 1.5 µg/L for *Daphnia magna* to obtain acute risk quotients (RQs). The effect level used to calculate the acute RQs (i.e., 1.5 ug/L) was chosen to represent the most sensitive aquatic organism Daphnia magna and to represent conditions that are representative of surface water in the United States. The acute RQ of 0.009 at the worst case stream dilution factor of 1.0 indicates that it is unlikely that the registration of Nanosilva as a plastics and textiles preservative will lead to adverse effects for listed or non-listed aquatic organisms during the four year period of conditional registration. However, this assessment does not consider long-term exposures or exposure to estuarine and marine species.

6.3.2 Conclusions for Aquatic Risk

The acute RQs for *Daphnia magna* exposed to nanosilver ranged from 0.0001 to 0.009, which are not of concern for listed or non-listed organisms because they were less than the acute LOC of 0.5 and 0.05, respectively. The effect level used to calculate the acute RQs (i.e., 1.5 µg/L) was chosen to represent the most sensitive aquatic organism *Daphnia magna* and to represent conditions of moderately hard reconstituted water (MHRW), which is a test medium representative of surface water in the United States. This analysis assumes that 1% of the projected yearly production of plastic lumber in the U.S. would contain Nanosilva at 0.003% resulting in a nanosilver production-volume of 570 kg/year. This may be an overestimate given that the total mass of silver distributed as a material preservative in the U.S. during 2009 was less than 6,800 kg, based on EPA confidential records. This analysis also assumes that all the nanosilver in plastic lumber incorporating Nanosilva would be released into the environment during a one year period when in fact the leaching study for plastic coupons did not find silver at concentrations above the analytical detection limit implying that significantly less silver would be released from plastic lumber incorporating Nanosilva (see Section 5.2.1).

There are several uncertainties regarding the exposure portion of this evaluation. The production volume of Nanosilva is unknown since this product is not currently available in the U.S. Because of this, EPA made the assumption that 1% of all plastic lumber produced every year in the U.S. would contain 0.003% Nanosilva. The nanosilver that could be released from the lumber incorporating Nanosilva was assumed to directly enter the aquatic environment without any removal in soil or sediments. The rate at which nanosilver entered the sanitary sewer systems was assumed to be constant in the down-the-drain model, which does not account for variability in the distribution of plastic products incorporating Nanosilva among other factors. The ecotoxicity values shown in Table 19 are for acute effects and mortality, and there is only one study on the long-term exposure to nanosilver. Finally, this assessment only considers silver that could be released by Nanosilva and does not include other sources of silver which will contribute to the environmental loading of silver.

Given that all calculated RQs were below the presumptive LOC for listed species and the conservative assumptions used in calculating these RQs, EPA concludes, with a level of confidence acceptable for the period of conditional registration, that there is a low probability of adverse risk to the environment from plastics and textiles incorporating Nanosilva.

6.4 Ecological Effects and Environmental Fate Data Requirements.

Even though EPA does not anticipate adverse ecological effects from plastics and textiles incorporating Nanosilva during the period of conditional registration, EPA is requesting additional testing to confirm its conclusions. The testing is based on a tiered approach. Tier I studies will determine the nature and quantity of silver released from plastics incorporating Nanosilva and confirm that the nanosilver in Nanosilva is consistent with the risk from Table 19. The Tier I environmental fate testing categories include:

- Product Characterization and Testing
- Silver Release from Plastics: Form, Rate and Characteristics

Completion of these Tier I studies will provide Nanosilva product characteristics and determine the substance or substances released during leaching of plastics incorporating Nanosilva. Information from these studies will be used to substantiate Nanosilva's claim that no nanosilver is released from plastics incorporating Nanosilva during use. However, if nanosilver is found to be released during Tier I studies, and the RQ calculated using this release rate results in a risk concern or is within one order of magnitude of the LOC, then Tier II studies will be required. If necessary, the Tier II studies will provide quantitative toxicity data on the amounts and forms of silver released, which will then be used by EPA to re-run the environmental risk assessment for Nanosilva. The Tier II environmental fate and ecological effects testing categories include:

- Product Characterization
- Sorption/Desorption Characteristics
- Bioaccumulation Characteristics
- Impacts to Wastewater Treatment
- Wastewater Treatment Removal Efficiency
- Aquatic Plant testing
- Acute Effects to Freshwater Fish
- Chronic Effects to Freshwater Fish and Invertebrates
- Acute and Chronic Effects to Estuarine/Marine Animals
- Chronic Effects to Sediment Dwelling Organisms
- Terrestrial Plant Toxicity

A more detailed description of these data requirements is provided in Appendix B.

VII. REGULATORY ACTION

A registration for a new active ingredient may be granted under either section 3(c)(5) or section 3(c)(7)(C) of FIFRA. A FIFRA section 3(c)(7)(C) registration is appropriate where, as here, the data for registration of the new active ingredient are newly required or identified. As discussed more thoroughly below, because EPA has not reached a final decision with regard to which types of data would be further required, the data requirements for this registration are considered newly required.

In addition, we believe granting the proposed conditional registration with the terms and conditions identified in Appendix B is particularly appropriate given EPA suspects that some already-registered like-situated products on the market contain nanosilver as the active ingredient. While EPA approved these registrations without knowledge that these products may contain nanoscale silver and without specifically assessing any potential risks that might be associated with any nanosilver contained in those products, they are nonetheless on the market. To avoid disparate treatment and consistent with the analytic decision framework in this document, EPA intends to seek similar data along with comparable terms on already registered products that are identified to contain nanosilver. As part of this effort, EPA opened the Nanosilver Registration Review (docket EPA-HQ-OPP-2011-0370) on July 6, 2012, which is the first step towards issuing a Data Call-In for products containing nanosilver.

7.1 Legal Framework

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) section 3(c)(7)(C) provides that:

> The Administrator may conditionally register a pesticide containing an active ingredient not contained in any currently registered pesticide for a period reasonably sufficient for the generation and submission of required data (which are lacking because a period reasonably sufficient for generation of the data has not elapsed since the Administrator first imposed the data requirement) on the condition that by the end of such period the Administrator receives such data and the data do not meet or exceed risk criteria enumerated in regulations issued under this Act, and on such other conditions as the Administrator may prescribe. A conditional registration under this subparagraph shall be granted only if the Administrator determines that use of the pesticide during such period will not cause any unreasonable adverse effect on the environment and that use of the pesticide is in the public interest.

Therefore, EPA must make four findings in order to grant a section 3(c)(7)(C) conditional registration for a pesticide product containing a new active ingredient:

1) Nanosilva contains an active ingredient, silver nanoparticles also known as nanosilver, that is not an active ingredient in any currently registered pesticide (i.e., a "new" active ingredient);
2) Use of Nanosilva will not cause unreasonable adverse effects on the environment during the period when newly required data are being developed;
3) Insufficient time has elapsed for Nanosilva LLC to generate and submit the newly required data; and
4) Use of Nanosilva is in the public interest.

7.2 Findings and Registration Decision

The Agency proposing to issue a conditional, four year registration for Nanosilva in accordance with FIFRA section 3(c)(7)(C) and is proposing to require as a condition of registration specified use conditions as well as proposing that the company provide data from a number of studies that will allow the Agency to confirm its current assessment of the risks. Specific data requirements are outlined in Appendix B. Although these data requirements are specific to Nanosilva, they form a starting point for identifying the types of data the Agency will require for other nanomaterial-based products on a case-by-case basis.

Consistent with Nanosilva LLC's application, label language is required to reduce potential exposures. The pesticide label must include the following:

1. The application rate must be less than 30 ppm (mg/kg) or 0.003% silver on a weight basis for plastics and textiles which incorporate Nanosilva.
2. Nanosilva may only be incorporated into linear low-density polyethylene (LLDPE) plastic and polyethylene terephthalate (PET) based textiles consistent with the materials used in the leaching studies.
3. Nanosilva may only be used to preserve plastics and textiles and may not be used for drinking water or food contact uses.
4. It is required that the following mitigation measures be employed when mixing and loading Nanosilva during treatment of articles:
   - Closed system loading of Nanosilva containing suspension.
   - NIOSH certified full-face respirators with P100 or equivalent filter cartridges immediately available for use in an emergency.
   - Gloves which are chemically resistant to all of the components of the Nanosilva liquid suspension.
   - A long-sleeve shirt, long pants, shoes plus socks.

5. The label must have an environmental hazard statement consisting of the following:

"This pesticide is toxic to fish and aquatic invertebrates, and birds, and can't be used for irrigation purposes."

"Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans, or other waters unless in accordance with the requirements of a National Pollutant Discharge Elimination System (NPDES) permit and the permitting authority has been notified in writing prior to discharge. Do not discharge effluent containing this product to sewer systems without previously notifying the local sewage treatment plant authority. For guidance contact your State Water Board or Regional Office of the EPA".

7.3 Basis for Conditional Registration

The Agency's basis for the proposed conditional registration is as follows:

7.3.1 Data Generation

Nanosilva LLC submitted its registration application thinking its product was a new pesticide ingredient not contained in any currently registered silver-based pesticide product and should be given a registration under FIFRA section 3(c)(5). At the time of application for a new chemical, Nanosilva LLC was required to submit all the applicable information and studies under 40 CFR 161. Although Nanosilva LLC submitted some of the required studies using guidelines not modified to be applicable for testing nanosilver, they also submitted a request to waive some of the data requirements. In addition, Nanosilva LLC completed a leaching study using a protocol that was modified to be applicable for nanosilver. Although Nanosilva LLC provided EPA with some data in an effort to address Agency questions and concerns, EPA had not reached a final decision with regard to which types of data would be further required for Nanosilva. This was due in large part to the need to understand and apply the advice provided in the report from the consultation with the FIFRA Scientific Advisory Panel. As a result, EPA has determined that insufficient time has elapsed from the point at which EPA determined and informed Nanosilva LLC of the data requirements needed to assess Nanosilva LLC's application for Nanosilva LLC to have generated the data.

EPA is proposing to require the tests listed below to confirm the adequacy of the 10 fold database uncertainty factor, to reduce the uncertainties related to differences in the physical properties of the nanosilver, and because there are currently no acceptable studies on the reproductive and developmental toxicity for nanosilver. Because this list of data requirements are only being proposed with today's action, Nanosilva LLC has not yet been able to conduct these studies. Therefore, the Agency is proposing to require these studies as a condition of registration, allowing sufficient time for the study protocols to be agreed upon, the studies to be conducted, and for the Agency to review them. The required studies include:

- 90-Day Inhalation Toxicity (Rat) (OCSPP 870.3465) modified to include *in vivo* bone marrow assay and functional observational battery, motor activity and detailed neuropathology
- Reproduction/Developmental Toxicity Screening Test (Modified OCSPP 870.3550/ OECD TG 421)
- Product Characterization and Testing
- Silver Release from Plastics: Form, Rate and Characteristics

EPA will evaluate the results from the above studies using a weight of evidence approach based on physical and chemical properties, toxicity, and exposure. If the Agency identifies a concern, then one or a combination of the following tests will be triggered:

- Peri- and post-natal exposure to Nanosilva
- Product Characterization
- Sorption/Desorption Characteristics
- Bioaccumulation Characteristics
- Impacts to Wastewater Treatment
- Wastewater Treatment Removal Efficiency
- Aquatic Plant testing
- Acute Effects to Freshwater Fish
- Chronic Effects to Freshwater Fish and Invertebrates
- Acute and Chronic Effects to Estuarine/Marine Animals
- Chronic Effects to Sediment Dwelling Organisms
- Terrestrial Plant Toxicity

As discussed above, a listing of the studies that are proposed to be required as terms and conditions of this registration of Nanosilva are in Appendix B. If Nanosilva LLC does not meet the conditions set forth in this Decision Document, EPA will issue a notice of intent to cancel Nanosilva LLC's registration under section 6(e). In addition, Nanosilva LLC's conditional registration for Nanosilva will automatically expire four years after being issued. Nanosilva LLC should request an amendment to remove the expiration date once they have submitted the required data if they wish to continue to sell and distribute Nanosilva in the United States.

7.3.2 Public Interest

As required under FIFRA section 3(c)(7)(C), for the reasons summarized below, the Agency believes granting a conditional registration with the terms and conditions specified is in the public interest. EPA believes that use of Nanosilva offers the potential for conservation of the environment and consumer benefits which contribute to the public interest. These points are discussed in more detail below:

Conservation of the Environment

EPA has already registered a number of silver-based pesticide products for use as materials preservatives. All silver-based pesticide products act via the release of a low concentration of silver ions that then interact with bacteria. Commonly, regardless of the silver additive type, upon contact with moisture, silver ions are released from the object incorporating the additive. The antimicrobial potency of a silver additive is therefore directly related to the potential for releasing silver ions. The release potential differs between various silver materials. As the size of silver particles decreases (e.g., from micro-size silver to nano-size silver), the potential for releasing silver ions increases, due to the increasing unit of surface area (i.e., availability of ions for release) per unit mass of silver.

Specifically, most silver-based pesticide products currently contain a silver salt, [e.g., AgCl or $AgNO_3$]. Compared to the amount of silver in Nanosilva LLC's product, most currently registered silver-based materials preservatives incorporate larger amounts of silver. Therefore, the overall potential environmental loading of silver resulting from the lower-volume use of the Nanosilva product should be smaller than from a comparable use of currently registered silver-based pesticides.

Consumer Benefits

A nanosilver materials preservative should maintain its ability to reduce the number of odor and stain causing bacteria longer than other silver active ingredients due to the expected gradual and controlled release of silver ions from nanosilver as opposed to the rapid release of silver ions from a zeolite structure or the immediate dissolution of the silver salt. In the case of Nanosilva, the release of silver ions from plastics and textiles incorporating Nanosilva are at concentrations below the analytical detection limit while these same plastics and textiles are reported to retain the ability to reduce the number of odor and stain causing bacteria. While it may be that other silver active ingredients are present at greater silver concentrations, it is expected that their effects on odor and stain causing bacteria are only short-lived due to the rapid release of silver ions. Thus, plastics or textiles incorporating nanosilver may have longer-term ability to reduce the number of odor and stain causing bacteria as compared to other similar products on the market that contain conventional silvers.

Innovation

EPA sees the emergence of nanotechnology as offering potential benefits for society in many different fields, including pest control products. The use of nanotechnology in pesticide products may allow for more effective targeting of pests and use of smaller quantities of pesticide. These

could contribute to improved human and environmental safety and could lower pest control costs. Therefore, EPA seeks to encourage innovative work to realize these benefits.

In the case of the Nanosilva LLC's application, EPA's proposed conditional registration of Nanosilva is in the public interest in that it will allow an innovative product to reach the market.

7.3.3 No Unreasonable Adverse Effects

In assessing the potential risks to human health and the environment associated with the distribution and use of Nanosilva as a materials preservative, EPA relied on data submitted by Nanosilva LLC and data in the public literature on nanosilver, and use of uncertainty factors and conservative assumptions. As a result of this analysis and taking into account the terms and conditions on this conditional registration, EPA believes that the likely risks from the use of Nanosilva during the period of the conditional registration are small. Moreover, EPA expects the overall quantity of silver used in plastics and textiles as a result of this conditional registration will be lower than the overall quantity of silver used in other materials preservative products containing conventional silver resulting in expected reductions in environmental loadings of silver and to humans. EPA concludes that the registration would not cause unreasonable adverse effects on the environment during the conditional period. This conclusion is based on the findings discussed in the following sections.

Risks to Human Health

As discussed above, humans could be exposed to silver ions, to Nanosilva, and to nanosilver. With respect to silver ions, the Agency completed a risk assessment (U.S. EPA, 1993) for silver salts and determined that the risks from registered uses, including the materials preservative use pattern, were acceptable. The Agency concludes that human exposure to silver ions from the use of Nanosilva and consumer exposures to plastics and textiles incorporating Nanosilva is not unreasonable.

With respect to Nanosilva, the acute toxicity of Nanosilva is low by all routes of exposure. Because there are no subchronic or chronic oral or dermal toxicity studies available for Nanosilva or on the nanosilver that might break away from products incorporating Nanosilva, the subchronic or chronic toxicities were estimated using studies on nanosilvers reported in the literature. The Agency relied on the results from studies submitted by Nanosilva LLC where the liquids in contact with plastic and shirts incorporating Nanosilva were analyzed for silver content to estimate exposure the amount of Nanosilva and nanosilver that might be released from plastics and textiles. The Agency recognized the uncertainties and incorporated multiple uncertainty factors and conservative assumptions when calculating the Margin of Exposures (MOEs) for estimating the risk to children from plastics and textiles incorporating Nanosilva.

Based on all of the foregoing, EPA concludes, with a level of confidence acceptable for the period of conditional registration, that there is a low probability of adverse risk to consumers from short- and intermediate-term exposure to toys, flooring, and textiles incorporating Nanosilva. Thus, the Agency concludes that use of Nanosilva will not cause unreasonable adverse effects on the environment during the period when newly required data are being developed.

Risks to the Environment

The environment could be exposed to silver ions, to Nanosilva, and to nanosilver. With respect to silver ions, the Agency completed a risk assessment (U.S. EPA, 1993) for silver salts and determined that the risks from registered uses, including the materials preservative use pattern, were acceptable. The Agency concludes that environmental exposure to silver ions from the use of Nanosilva and plastics and textiles incorporating Nanosilva is acceptable.

With respect to the fate and ecotoxicity of Nanosilva, because there are no fate or ecotoxicity studies available for Nanosilva or the nanosilver that might break away from plastics or textiles incorporating Nanosilva, the fate and ecotoxicity were estimated using studies on nanosilvers reported in the scientific literature. Direct release from leaching of plastics and laundering of textiles incorporating Nanosilva were anticipated to be the primary route by which Nanosilva, silver ions, and nanosilver reach the environment. The Agency assessed impacts to surface waters assuming that nanosilver was the only compound released. Environmental exposure from indoor use was assessed assuming that 300 million people (U.S. population) each purchased one t-shirt containing 0.003% nanosilver where 1.6% of the silver in the t-shirt incorporating Nanosilva was released during each weekly washing over a one year period. Environmental exposure from outdoor use was assessed assuming that 1% of the projected yearly production of plastic lumber in the U.S. would contain Nanosilva at 0.003% where all the silver would be released into the environment as nanosilver during a one year period even though the leaching study for plastic coupons incorporating Nanosilva did not find silver at concentrations above the analytical detection limit. EPA estimates, derived from down-the-drain modeling, of the concentrations of nanosilver resulting from the use of Nanosilva do not exceed the Agency's estimate of the highest concentration of nanosilver in surface water to which an aquatic community can be exposed without resulting in an unacceptable effect. Thus, the Agency concludes that use of Nanosilva will not cause unreasonable adverse effects on the environment during the period when newly required data are being developed.

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Appendix A –
Evaluation of Nanosilva LLC's Data Waiver Requests

August 27, 2013

I. Introduction

On August 10, 2009, Nanosilva LLC submitted an application to register Nanosilva as a new active ingredient (PRIA A420). In 2009, as an applicant for registration of a new active ingredient, Nanosilva LLC was required to submit all the applicable information and studies under 40 CFR part 161. EPA published a Notice of Proposed Rulemaking on October 8, 2008 proposing to revise and update the existing data requirements under 40 CFR part 161 for antimicrobial pesticides. These data requirements were made final as 40 CFR part 185W on May 8, 2013 (78 FR 26936). In 2009, the Nanosilva LLC application included a request to waive the requirement to generate data for Nanosilva using the following test guidelines:

1. Hydrolysis (OPPTS 835.2120)
2. Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids (OPPTS 850.1010)
3. Fish Acute Toxicity Test, Freshwater and Marine (OPPTS 850.1075)
4. Avian Acute Oral Toxicity Test (OPPTS 850.2100)
5. 90-Day Dermal Toxicity (OPPTS 870.3250)
6. Prenatal Developmental Toxicity Study (OPPTS 870.3700)
7. Bacterial Reverse Mutation Test (OPPTS 870.5100)
8. Detection of Gene Mutations in Somatic Cells in Culture (OPPTS 870.5300)
9. In Vitro Mammalian Cytogenetics (OPPTS 870.5375)
10. Mammalian Bone Marrow Chromosome Aberration Test (OPPTS 870.5385)
11. Immunotoxicity (OPPTS 870.7800)

The test guidelines in items 1 through 4 are cited in 40 CFR part 161 and were required at the time Nanosilva applied to registered Nanosilva. Guidelines in items 5 through 11 were proposed in 2008 to be included in 40 CFR part 158W, which was made final in 2013 and therefore, the test guidelines in items 5 through 11 were not required at the time Nanosilva LLC applied to register Nanosilva. Even though these guidelines were not required at the time of application, EPA evaluated the request to waive each of these data requirements.

Waivers for the above studies were requested because of the lack of acute toxicity noted in the six acute toxicity studies (MRID 47828918 through 47828923), because Nanosilva has a specific gravity greater than water, because Nanosilva has low solubility in water as determined by the water column generator method (MRID 47828917), and due to the non-leaching characteristic of Nanosilva because silver at concentrations greater than the analytical detection limit were not detected in leaching studies conducted with a plastic incorporating Nanosilva (MRID 47828925).

The data requirements listed above in items 1 through 11 are either waived or satisfied. In general, the data requirement is met or satisfied because:

- certain guideline studies are not appropriate for nanosilver and where appropriate are being modified and required as terms and conditions on the registration (i.e., Reproduction/Developmental Toxicity Screening Test)

- certain environmental data are satisfied because the environmental hazard labeling statement was determined using literature studies completed with nanosilver
- Dermal guideline studies are not appropriate given there was no risk concern
- *in vitro* mutagenicity study requirements are satisfied and have shown that nanosilver is possibly mutagenic
- the immunotoxicity study is initially waived and only triggered after the potential immune toxicity of nanosilver is determined during the 90-day inhalation toxicity study required as a term and condition on the registration

Each of the above studies is addressed in the Environmental Fate, Wildlife and Aquatic Organism, and Toxicology data requirements listed in 40 CFR part 161 for antimicrobial pesticides. The following sections contain an evaluation of each waiver request.

A. Environmental Fate Data Requirements

The following test for environmental fate is required for antimicrobials under 40 CFR 161.202 to assess the exposure to pesticides after application:

1. Hydrolysis, OPPTS 835.2120

This test is waived because nanosilver undergoes dissolution with the generation of ionic silver rather than undergoing hydrolytic transformation.

B. Wildlife and Aquatic Organism Data Requirements

The following tests are required for solid-formulation manufacturing use antimicrobials under 40 CFR 161.440:

2. Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids (OPPTS 850.1010)
3. Fish Acute Toxicity Test, Freshwater and Marine (OPPTS 850.1075)
4. Avian Acute Oral Toxicity Test (OPPTS 850.2100)

The acute ecotoxicity testing waiver request was based on the non-leaching characteristic of Nanosilva. However, the results of these tests are required for use in determining the appropriate precautionary labeling on packages containing the Nanosilva liquid suspension prior to incorporating into a product. Therefore, the non-leaching characteristic is not an appropriate basis for waiving these tests. As summarized in Table 19 of the Decision Document, EPA has information on the acute toxicity of nanosilver for Daphnids and Freshwater Fish. Because the EC_{50} values were less than 1 mg/L, as indicated in Chapter 8 of the Pesticide Label Review Manual, the following environmental hazard statement is required on the Nanosilva label:

This pesticide is toxic to fish and aquatic invertebrates.

Given that there are no test results for Avian species available for nanosilver, EPA requires that the environmental hazard statement for the Nanosilva label be expanded to include:

This pesticide is toxic to fish and aquatic invertebrates, and birds.

EPA is requiring that Nanosilva LLC place the above environmental hazard statement on the pesticide label for Nanosilva. However, if Nanosilva LLC wishes to remove birds from this statement then they can submit results from an Avian Acute Oral Toxicity Test (OPPTS 850.2100) showing that the nanosilver in Nanosilva is non-toxic to birds with an LD_{50} of greater than 100 mg/kg.

In addition, Nanosilva LLC is required to place the following statement on the label for Nanosilver liquid suspension:

> "Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans, or other waters unless in accordance with the requirements of a National Pollutant Discharge Elimination System (NPDES) permit and the permitting authority has been notified in writing prior to discharge. Do not discharge effluent containing this product to sewer systems without previously notifying the local sewage treatment plant authority. For guidance contact your State Water Board or Regional Office of the EPA".

Thus, the data requirements in items 2 through 4 are satisfied by data in the public literature for nanosilver and required labeling.

C. Toxicology Data Requirements

The following tests are required for antimicrobials under 40 CFR part 158.2230 (i.e., part 158W):

5. 90-Day Dermal Toxicity (OPPTS 870.3250
6. Prenatal Developmental Toxicity Study (OPPTS 870.3700)

The requirement for the 90-Day Dermal Toxicity study is waived because the calculated Margin of Exposures (MOEs) for dermal and incidental exposure to products incorporating Nanosilva where greater than 1,000,000 as compared to the highest target MOE of 3,000. However, if nanosilver is determined to be released during the leaching test of plastics incorporating Nanosilva in amounts well above those found in the plastics leaching study already submitted such that the MOE calculated using the newly determined nanosilver release rate results in a risk

concern, then the following route-specific subchronic tests is proposed to be required to confirm the risk assessment for the nanosilver in Nanosilva.

- Dermal penetration study in rats (OSCPP 870.7600)

The requirement for the Prenatal Developmental Toxicity Study is waived because the following study has been determined to be more appropriate for Nanosilva:

- Modified 870.3550/ OECD TG 421: Reproduction/Developmental Toxicity Screening Test

The following tests are required for pesticides under 40 CFR part 158.2230 (e.g., 158W):

7. Bacterial Reverse Mutation Test (OPPTS 870.5100)
8. Detection of Gene Mutations in Somatic Cells in Culture (OPPTS 870.5300)
9. *In Vitro* Mammalian Cytogenetics (OPPTS 870.5375)
10. Mammalian Bone Marrow Chromosome Aberration Test (OPPTS 870.5385)
11. Immunotoxicity (OPPTS 870.7800)

The mutagenicity studies [Bacterial Reverse Mutation Test (OPPTS 870.5100), Detection of Gene Mutations in Somatic Cells in Culture (OPPTS 870.5300), In Vitro Mammalian Cytogenetics (OPPTS 870.5375), Mammalian Bone Marrow Chromosome Aberration Test (OPPTS 870.5385)] are satisfied because there are *in vitro* studies available for nanosilver in the scientific literature which address/satisfy these data needs and show that nanosilver is possibly mutagenic. Thus, the Agency is requiring as a term and condition on the registration an *in-vivo* bone marrow assay to clarify the mutagenic properties of the nanosilver in Nanosilva.

The immunotoxicity (OPPTS 870.7800) is waived at this time because OPP has published a document (U.S. EPA, 2013) recommending that immune related endpoints be evaluated during the inhalation toxicity test, required as a term and condition on the registration, before determining if the immunotoxicity test is required. The currently available oral toxicity studies indicate that nanosilver causes liver and kidney toxicity in laboratory animals where silver is distributed to all organs and tissues with accumulation of silver in the brain and male animal testes. Inhalation toxicity studies also identified liver toxicity as well as lung effects including chronic alveolar inflammation. The toxicology database for nanosilver does not reveal any evidence of treatment-related effects on the immune system, which suggests that nanosilver does not directly target the immune system.

U.S. EPA, 2013. Part 158 Toxicology Data Requirements: Guidance for Neurotoxicity Battery, Subchronic Inhalation, Subchronic Dermal and Immunotoxicity Studies. May 1st. Available at: http://www.epa.gov/pesticides/regulating/part158-tox-data-requirement.pdf

Appendix B –
Data Requirements, Enforceable Schedule, and Conditions of the Proposed Conditional Registration for Nanosilva

August 27, 2013

I. Introduction

Nanosilva is a liquid suspension containing 1% nanosilver by weight where the nanosilver active-ingredient is covalently bonded to crystalline silica via a thiolate bond. The diameter of the spherical silica core is 320 nm on average (Lee et al., 2006) and the nanosilver particles that are attached to the silica core have mean diameters between 6.9 and 10.6 nm (Lee et al., 2007). The complex is formed by reacting spherical silica particles modified with thiol groups with silver nitrate and polyvinylpyrrolidone (PVP) in ethanol (Lee et al. 2007). Thus, the resulting liquid suspension contains 1% nanosilver by weight where the nanosilver surface is coated with sulfur and PVP, and is attached to silica. Reference to the nanosilver in Nanosilva (whether when part of the composite or when broken away from the complex) is to the "new active ingredient" and reference to "Nanosilva" or the Nanosilva "complex" is to the end-use product.

II. Exposure Pathways

The human and environmental exposures resulting from the use of Nanosilva, and use and disposal of plastics and textiles incorporating Nanosilva will largely be a function of what materials are available for inhalation or dermal exposure during treatment of plastics and textiles or what materials leach or break away from the plastics and textiles incorporating Nanosilva during use and disposal. EPA anticipates that humans and the environmental will potentially be exposed to the following materials:

1. Silver ions released from Nanosilva;
2. Nanosilva complex; and/or
3. Nanosilver that might break away from the Nanosilva complex.

EPA expects that occupational inhalation and dermal exposures to Nanosilva and the nanoparticles that might break away from Nanosilva are likely to occur during the following use scenarios:

1. Mixing and loading of Nanosilver during preparation of a master batch
2. Mixing, loading, and applying the Nanosilva containing master batch during treatment of plastics and textiles
3. Handling plastics and textiles incorporating Nanosilva

EPA expects consumer exposures to Nanosilva, the silver ions derived from the Nanosilva complex, and the nanoparticles that break away from the Nanosilva complex could potentially occur during the following use scenarios:

1. Incidental oral exposure to plastics and textiles incorporating Nanosilva
2. Dermal exposure to plastics and textiles incorporating Nanosilva

Leaching of plastics and textiles incorporating Nanosilva is anticipated to be the primary route by which Nanosilva, silver ions, and nanosilver reach the environment. The silver released

during leaching of plastics and textiles incorporating Nanosilva could be discharged to the sanitary sewer system leading to publically owned wastewater treatment and privately owned septic systems, also known as the down-the-drain discharge scenario. Leaching of building material such as plastic siding and decks and outdoor furniture incorporating Nanosilva will be discharged directly into the environment. Once Nanosilva, silver ions, or nanosilver reach wastewater treatment and septic systems they will most likely complex with sulfide and partition to biosolids. However, some fraction of the silver compounds will reach surface water and may potentially impact aquatic organisms.

III. Data Needed to Confirm the Estimates for Risks of Exposure to Nanosilva

As a condition of the registration, EPA is requiring the Nanosilva LLC to conduct a number of studies, based on a tiered approach, which will allow the Agency to confirm the findings of the risk assessment completed for the proposed conditional registration and discussed in the decision document. These tests include Tier I studies to determine the nature and quantity of silver released from plastics incorporating Nanosilva under conditions of use, product characterization testing, and silver release characteristics from the Nanosilva complex. If nanosilver is determined to be released from plastics during the leaching tests as part of Tier I testing and the weight of evidence based on physical and chemical properties, toxicity, and exposure result in a risk concern then route-specific toxicity and environmental fate tests as part of Tier II studies to confirm the nanosilver risk assessment will be triggered. Data must be submitted within four years and according to the schedule provided in Table 3B to avoid cancellation of the conditional registration.

The duration of four years was chosen to allow time for protocol reviews prior to initiation of the studies, completion of the studies, and Agency review of the studies following completion. The Agency will evaluate these data as they are submitted during the period of the conditional registration to confirm the Agency's determination that the product is not expected to cause unreasonable adverse effects to human health and the environment. If EPA determines that Nanosilva LLC has failed to take appropriate steps to initiate the required studies, or failed to submit the protocols or studies, as required pursuant to this Appendix , EPA will issue a notice of intent to cancel Nanosilva's registration under FIFRA section 6(e).

The following factors were considered in developing the data requirements for Nanosilva:

- Submitted as a new active ingredient application, the Nanosilva nanosilver was subject to the data requirements for the registration of antimicrobial pesticides that are detailed in 40 CFR Part 161. These requirements include studies on physical and chemical characteristics, residue chemistry, environmental fate, toxicology, reentry protection, spray drift, wildlife and aquatic organisms, plant protection, nontarget insects, and product performance.
- Although some studies, such as those dealing with physical and chemical characteristics, are required for all use patterns, many of the data requirements are conditional based on the use pattern. Information provided by Nanosilva LLC and information from the literature was used to tailor the data requirements to the proposed use pattern.
- Additional studies in the area of physical and chemical characterization that are not

specifically included in 40 CFR Part 161 are needed because Nanosilva contains nanosilver. These studies are needed because nanosized materials may have unique and new characteristics which are not present in the bulk or conventional materials. These characteristics have been recognized in the FIFRA SAP Report (FIFRA SAP, 2009) and by the MINChar Initiative (2008).

In addition, the following recommendations from the FIFRA SAP report were considered in the development of the data requirements as terms and conditions on the proposed conditional registration of Nanosilva:

- Both nano-sized particles of silver (Ag) as well as ionic silver (Ag^{+}) can contribute to toxic effects of nanosilver. The rate of silver ion production, as well as the distribution of nanosilver in tissues and the environment, may differ substantially between nanosilver and other forms of silver, as nanosilver can potentially deliver silver ions directly to specific tissues, cell membranes or inside cells – places where other forms of silver compounds cannot reach. Therefore, the hazard profile of nanosilver may differ from other forms of silver.

- Particle size can substantially impact particle properties, such as rate and concentration of silver ion release, reactivity and catalytic efficiency, plasmon resonance, and quantum effects. Smaller sized-particles are more easily taken up by organisms and are distributed more widely. Other physicochemical properties, such as shape, surface area, surface charge or coating, are also likely to impact biological response and environmental fate.

- The Panel "disagreed that nanosilver applied to a substrate will permanently bind with the substrate." It is "especially challenging to determine that there is no release of nanomaterials from a substrate" under current state of science and available measurement standards. The Panel suggested that the Agency require tests that simulate realistic use of products and potential nanosilver release along with quantitative life-cycle analysis and risk assessment.

A listing of the studies that are needed for the registration of Nanosilva is included in Tables 1B and 2B. The studies included in Table 1B are considered to be Tier I, meaning that their need is not based on the results of other studies. The studies listed in Table 2B are considered to be Tier II, because they may or may not be required depending upon the results of the Tier I studies. The Nanosilva complex and plastics/textiles incorporating Nanosilva will be the test material during Tier I studies and the test material for Tier II studies will depend on the results of the Tier I leaching and dissolution studies. Figure 1B contains a conceptual diagram outlining the Tier I and Tier II testing approach.

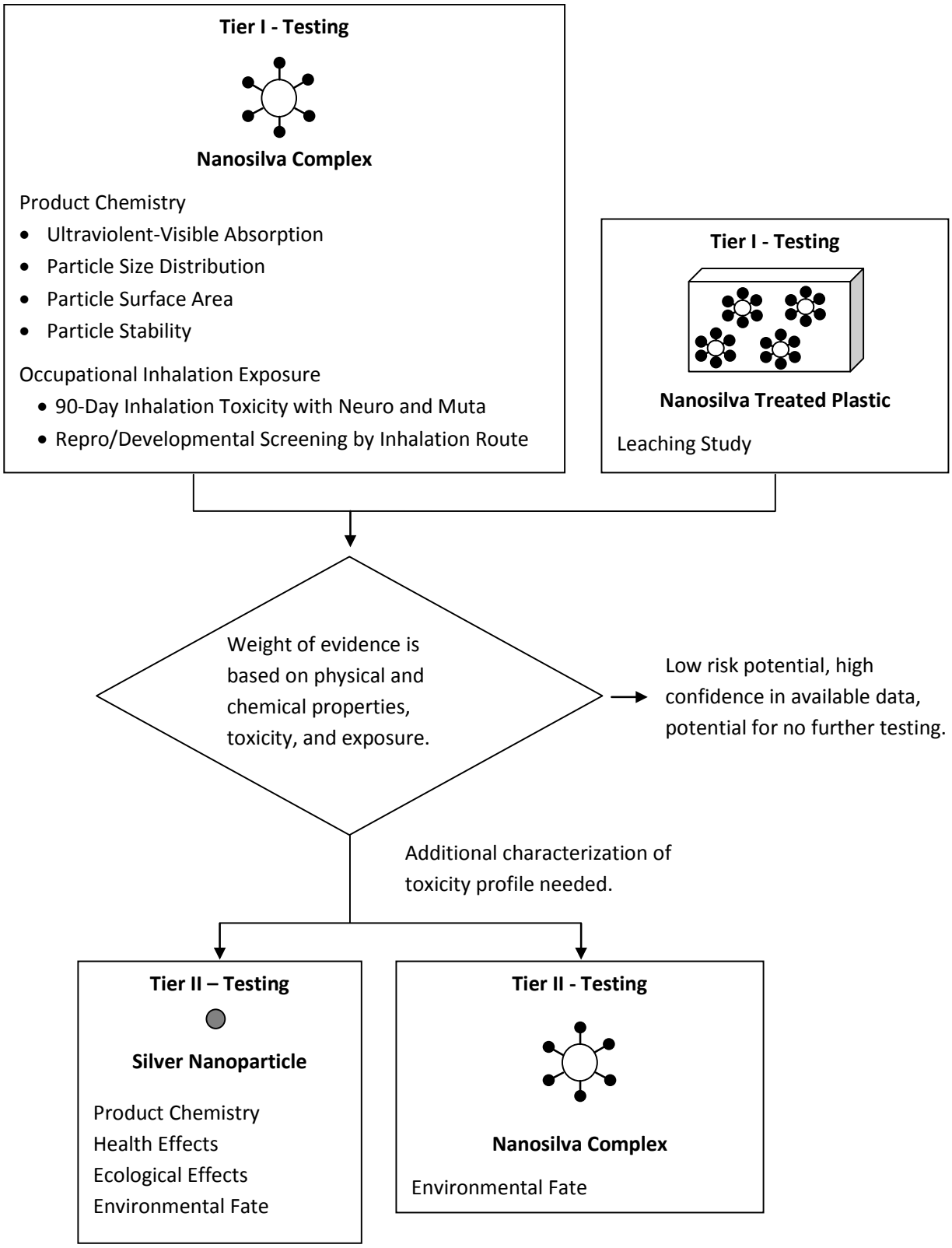

Figure 1B - Test material and tiered approach for Nanosilva and plastic incorporating Nanosilva. See Tables 1B and 2B for the specific tests required for each material.

IV. Non Data-Related Terms and Conditions of Registration

Based on Nanosilva LLC's original application, amendments to the proposed label language are required. The pesticide label must include the following:

1. The application rate must be stated as being less than 30 ppm (mg/kg) or 0.003% silver on a weight basis for plastics incorporating Nanosilva.
2. Nanosilva may only be incorporated into linear low-density polyethylene (LLDPE) plastic and polyethylene terephthalate (PET) based textiles consistent with the materials used in the leaching studies.
3. Nanosilva may only be used to preserve plastics and textiles and may not be used for drinking water or food contact uses.
4. It is required that the following mitigation measures be employed when mixing and loading Nanosilva during treatment of articles:
    - Closed system loading of Nanosilva containing suspension.
    - NIOSH certified full-face respirators with P100 or equivalent filter cartridges immediately available for use in an emergency.
    - Gloves which are chemically resistant to all of the components of the Nanosilva liquid suspension.
    - A long-sleeve shirt, long pants, shoes plus socks.
5. The label must have an environmental hazard statement consisting of the following:

    "This pesticide is toxic to fish and aquatic invertebrates, and birds, and can't be used for irrigation purposes."

    "Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans, or other waters unless in accordance with the requirements of a National Pollutant Discharge Elimination System (NPDES) permit and the permitting authority has been notified in writing prior to discharge. Do not discharge effluent containing this product to sewer systems without previously notifying the local sewage treatment plant authority. For guidance contact your State Water Board or Regional Office of the EPA".

V. Enforceable Schedule

Before EPA will issue a final determination on this application, Nanosilva LLC must provide information to satisfy the outstanding guideline items listed in Table 1B under Product Chemistry. EPA will not issue a registration until all Product Chemistry information is submitted by Nanosilva LLC and accepted by the EPA.

EPA has prepared an enforceable schedule that is presented in Table 3B. This schedule is an estimation of the time required for developing and submitting protocols for review, conducting the studies, and submitting the resulting data, as well as EPA's review of the submitted data. However, unforeseen technical issues may arise due to the unique nature of Nanosilva (a difficult-to-test substance), which may cause a delay in testing. If such a case arises, Nanosilva LLC shall submit a written request justifying the nature of the delay. In addition to technical delays, there may be delays by EPA in reviewing protocols and data submitted by Nanosilva LLC. In this case, the EPA shall submit a written statement to Nanosilva LLC outlining the nature of the delay. In either case, if EPA determines a delay in the enforceable schedule is appropriate, it will amend the terms and conditions on the conditional registration.

Notwithstanding technical delays or delays in reviewing data, if Nanosilva LLC fails to take appropriate steps to initiate the required studies, or fails to submit the protocols or data, as required pursuant to this Appendix, EPA will issue a notice of intent to cancel Nanosilva LLC's registration under FIFRA section 6(e). Specifically, Nanosilva LLC shall:

1. Submit protocols modified for Nanosilva LLC to EPA's satisfaction for each of the data requirements listed in Table 1B.

2. Perform each test and submit the results from each test as described in Table 1B and 2B.

These items shall be submitted according to the schedule provided in Table 3B. If EPA determines that Nanosilva LLC has failed to initiate or to submit the required studies by the dates indicated in Table 3B, then EPA will issue notice of intent to cancel Nanosilva LLC's registration under FIFRA section 6(e). In addition, Nanosilva LLC's conditional registration for Nanosilva will automatically expire four years after being issued. Nanosilva LLC will have to submit an application for an unconditional registration if they wish to continue to sell and distribute Nanosilva in the United States.

EPA will use this data to confirm EPA's determination that the conditional registration of Nanosilva will not cause unreasonable adverse effects on the environment, taking into account the terms and conditions on the registration.

All tests shall be conducted using laboratories that comply with the Good Laboratory Practice (GLP) standards of 40 CFR Part 160. In accordance with those regulations, the pesticide testing facility and the sponsor must meet GLPs in the following areas:

1. Organization and personnel
2. Facilities
3. Equipment
4. Testing facilities operation
5. Test, control, and reference substances
6. Protocol for and conduct of a study
7. Records and reports

Thus, the EPA expects that all studies performed to support, among other things, the registration or continued registration of a pesticide will address those requirements. However, studies deviating from those requirements may be considered if, as set forth in 40 CFR 160, a statement describing in detail all differences between the practices used in the study and those required by 40 CFR Part 160 is provided [see 40 CFR Part 160.12(b)]. As part of its review of the subject study, EPA will evaluate any such differences in determining if the study is acceptable for use in supporting, among other things, product registration or continued registration.

VI. Time-Limited Conditional Registration

The conditional registration for Nanosilva will automatically expire four years after being issued. Nanosilva LLC should request an amendment to remove the expiration date once they have submitted the required data if they wish to continue to sell and distribute Nanosilva in the United States.

References:

FIFRA SAP, 2009. FIFRA Scientific Advisory Panel Meeting held November 3 - 5, 2009 on the Evaluation of Hazard and Exposure Associated with Nanosilver and Other Nanometal Pesticide Products *Available at:*
http://www.epa.gov/scipoly/sap/meetings/2009/november/110309ameetingminutes.pdf

Lee, J.M., Kim, D.W., Jun, Y.D., Oh, S.G. 2006. Preparation of silica-silver heterogeneous nanocomposite particles by one-pot preparation strategy using polyol process: Size-controlled immobilization of silver nanoparticles. *Materials Research Bulletin* 41:1407-1416.(MRID 48379901 and 48379903)

Lee, J.M., Kim, D.W., Kim, T.H., Oh, S.G. 2007. Facile route for preparation of silica-silver heterogeneous nanocomposite particles using alcohol reduction method. *Materials Letters* 61:558-1562. (MRID 48379902 and 48379904)

The Minimum Information for Nanomaterial Characterization (MINChar) Initiative, 2008. *Available at*: http://characterizationmatters.files.wordpress.com/2008/11/minchar-parameters-list.pdf

Table 1B - Summary of Tier I Required Data for Nanosilva			
OSCPP Data Requirement (Note 1) Guideline Number: Study Title	**Reason for Study**	**Test Material**	**Comments**
Product Chemistry			
830.1620: Production Process	Required for antimicrobial pesticides per 40 CFR part 158.	Nanosilva	Completely describe quantities of reactants, reaction conditions, and reaction time.
830.1650: Formulation Process			Completely describe details regarding the formulation of the master batch.
830.1670: Formation of Impurities			Describe all possible reaction by-product impurities.
830.1700: Preliminary Analysis			Distinguish between the amount of silver and silver ions present.
830.1800: Analytical Method			ICP-AES only provides amount of silver present, also need method to quantify the amount of nanosilver present.
830.1900: Submittal of Samples			Nanosilva LLC must provide samples of Nanosilva for EPA use in verifying analysis and identity of Nanosilva.
830.6314: Oxidation/Reduction; Chemical Incompatibility			MSDS states that halogen salts and chlorides should be avoided; however, Nanosilva LLC states that the Nanosilva complex is stable. Need to fully describe stability of Nanosilva to other chemicals.
830.6317: Storage Stability			Only the concentration of silver was determined. Need to describe changes in particle size and UV/Visible spectroscopy with storage time.
830.7050: UV-Visible Light Adsorption			UV/Visible spectroscopy is required to evaluate the assessment of the structural integrity of the active ingredient.
Nanosilva Characterization			
Non-Guideline: Particle Size and Diameter (size) Distribution	Required to characterize product.		Determine both the size of the silica core particle and the nanosilver particles.

Table 1B - Summary of Tier I Required Data for Nanosilva			
OSCPP Data Requirement (Note 1) Guideline Number: Study Title	**Reason for Study**	**Test Material**	**Comments**
Non-Guideline: Surface Area Determination	Required to characterize product.		Determine both the surface area of the silica core particle and the nanosilver particles.
Non-Guideline: Particulate Stability	Required to assess stability of Nanosilva.	Nanosilva	Nanosilva LLC claims that Nanosilva is a stable complex that does not release nanosilver. This test should be designed to demonstrate Nanosilva stability to mechanical stress (normal and shear) and common reactants and solvents. EPA will use this information to evaluate Nanosilva LLC claims of Nanosilva stability.
Human Exposure			
Non-Guideline: Plastic Leaching Study (Confirm Nanosilva LLC study results)	Required to confirm assessment on human and environmental exposure. This test will determine what test substance will be used for Tier II studies.	Nanosilva Treated Plastics	A leaching study is needed to determine the quantity and form of silver that is released from Nanosilva treated plastics under conditions of use. Nanosilva LLC submitted a leaching study using food simulants and determined the total silver concentration. EPA requires an additional, follow-up study using physiological fluids and methods to determine the form of silver released from Nanosilva treated plastic. The exposure data will be used in to confirm the Agency's evaluation of the risk of exposure to Nanosilva.
Health Effects			
870.3465: 90-Day Inhalation Toxicity (Rat) (Replace Song et al., 2012 study results) Modified to include *in vivo* bone marrow assay and functional observational battery, motor activity and detailed neuropathology	Required to confirm the adequacy of the 10 fold database uncertainty factor, to reduce the uncertainties related to differences in the physical properties of the nanosilver.	Nanosilva	The inhalation study is a route-specific study required to evaluate the effects of inhaling nanosilver when mixing and loading Nanosilva.
Modified 870.3550/ OECD TG 421: Reproduction/Developmental Toxicity Screening Test[1]	Required because there are currently no acceptable studies on the reproductive and developmental toxicity for nanosilver. Occupational exposure to workers of reproductive age.	Nanosilva	The combined repeated-dose toxicity study with the reproduction/developmental toxicity screening test will provide initial information on possible effects on reproduction and/or development. In addition, the study may also provide a toxicity endpoint applicable to a risk assessment for oral incidental exposure.
Ecological Effects – No Tier I Studies Required			

[1]The use of a combined study that utilizes the 2-generation reproduction study in rodents as a basic protocol for the addition of other endpoints or functional assessments in the immature animal is encouraged.

Table 2B - Summary of Tier II Required Data for Nanosilva			
OSCPP Data Requirement (Note 1) Guideline Number: Study Title	**Reason for Study**	**Test Material**	**Comments**
Product Chemistry			
830.7050: UV-Visible Light Adsorption	These studies are required to characterize the nanosilver if released during Tier I stability, dissolution or leaching studies.	Nanosilver	
Non-Guideline: Particle Size and Diameter (size) Distribution			
Non-Guideline: Surface Area Determination			
830.7840: Solubility			
Non-Guideline: Zeta Potential and Surface Charge Determination			
Human Exposure – No Tier II Studies are Required			
Health Effects			
Non-Guideline: peri- and post-natal exposure to Nanosilva	Children's exposure to nanosilver while contacting Nanosilva treated plastics and textiles. Occupational exposure to nanosilver for workers of child bearing age.	See Note 2	Based on the results of the plastics leaching study, the Agency may require additional toxicity data to assess potential adverse health outcomes in young children resulting from incidental oral exposure to Nanosilva-treated plastic toys (i.e., children chewing on toys).
Ecological Effects			
850.1850: Modified Aquatic Food Chain Transfer	Required to determine bioavailability and biomagnifications.	See Note 2	Traditionally use Fish and Oyster BCF to estimate bioaccumulation for chemicals, however, mesocosm tests more likely to yield useful information.
850.4100: Terrestrial Plant Toxicity	Required to determine effects to plants during early critical stages in their development.	See Note 2	Nanosilver is likely to partition to biosolids during wastewater treatment. If those biosolids are then used in land farming, nanosilver may impact growth of plants in farm fields.
850.4400: Aquatic Plant Toxicity, Tier 2	Required to determine the toxicity to freshwater and aquatic plants.	See Note 2	Aquatic plants are the primary source of cellular carbon and chemical energy for aquatic environments. Impacts to these primary producers would have broad implications for the aquatic food chain.
850.5400: Algal Toxicity, Tier 2	Required to determine the phytotoxicity to freshwater and marine algae.	See Note 2	Algae are the primary source of cellular carbon and chemical energy for aquatic environments. Impacts to these primary producers would have broad implications for the aquatic food chain.
Non-Guideline: Measuring the Chronic Effects of Freshwater Sediment-Associated Contaminants on *Chironomus dilutes*	Required to determine chronic impact to freshwater sediment dwelling organisms.	See Note 2	Silver nanoparticles if released into aquatic environments are likely to partition to sediment. Chronic tests on a freshwater benthic emergent insect (*Chironomus dilutes*, formerly *Chironomus tentans*) with

Table 2B - Summary of Tier II Required Data for Nanosilva			
OSCPP Data Requirement (Note 1) Guideline Number: Study Title	**Reason for Study**	**Test Material**	**Comments**
			epibenthic ecological niche will be used to estimate potential risks to freshwater benthic organisms.
Non-Guideline: Measuring the Chronic Effects of Freshwater Sediment-Associated Contaminants on *Hyalella azteca*	Required to determine chronic impact to freshwater sediment dwelling organisms.	See Note 2	Silver nanoparticles if released into aquatic environments are likely to partition to sediment. Chronic tests on a freshwater benthic amphipod (*Hyalella azteca*) with infaunal ecological niche will be used to estimate potential risks to freshwater benthic organisms.
Non-Guideline: Measuring the Chronic Effects of Marine and Estuarine Sediment-Associated Contaminants on *Leptocheirus plumulosus*	Required to determine chronic impact to marine sediment dwelling organisms.	See Note 2	Silver nanoparticles if released into aquatic environments are likely to partition to sediment. Chronic tests on an esuarine/marine benthic amphipod (*Leptocheirus plumulosus*) will be used to estimate potential risks to marine benthic organisms.
850.1075: Acute Toxicity Test with Freshwater and Marine Fish	Required to confirm the chronic effects of nanosilver.	See Note 2	Acute toxicity test with fish species is to help in the assessment of possible risk to similar species in natural environments, as an aid in determination of possible water quality criteria for regulatory purposes, and for use in correlation with acute testing of other species for comparative purposes.
850.1300: Daphnid Chronic Toxicity Test	Required to determine the chronic effects of nanosilver.	See Note 2	This guideline prescribes a chronic toxicity test in which daphnids are exposed to a chemical either in a static-renewal or a flow through system.
850.1400: Early-Life Stage Toxicity Test for Freshwater, Estuarine, and Marine Fish	Required to determine the chronic effects of nanosilver.	See Note 2	Tests with the early-life stages of fish are intended to define the lethal and sublethal effects of chemicals on the stages and species tested. They yield information of value for the estimation of the chronic lethal and sublethal effects of the substance on other fish species.
Environmental Fate			
Non-Guideline: Rate of Deposition	The rates of aggregation and sedimentation are required for confirming estimates on the environmental fate and potential ecological impacts of these materials.	See Note 2	Knowing the rate of aggregation and sedimentation of the products released from textiles would give primary information on the behavior of these compounds in the aquatic environment.
835.1100: Activated Sludge Sorption Isotherm	Required to determine wastewater treatment removal efficiency.	See Note 2	The EPA uses Guideline 835.1110 test results to estimate the removal efficiency of a chemical as it passes through a wastewater treatment plant.

Table 2B - Summary of Tier II Required Data for Nanosilva			
OSCPP Data Requirement (Note 1) Guideline Number: Study Title	**Reason for Study**	**Test Material**	**Comments**
835.1230: Adsorption/Desorption (Batch Equilibrium)	Required to determine partitioning to solids.	See Note 2	If the material is removed during wastewater treatment, it may be deposited on land through the deposition of sludge (i.e. land farming). If the material is not removed during wastewater treatment it may be released into aquatic environments and may bind to sediment. Adsorption/desorption equilibrium studies required to determine the mobility of Nanosilva or nanosilver in the environment.
835.1240: Leaching Studies (Soil Column Tests)	Required to determine the mobility in subsurface environment.	See Note 2	The distance that nanoparticles move in soil and groundwater is thought to depend on interaction with soil grains. Attachment of nanoparticles to soil grains depends on the physical processes of sedimentation, interception, and diffusion rather than partitioning to natural organic matter. Nanoparticle to soil grain interaction depends on nanoparticle diameter, aqueous chemistry, and the arrangement of soil grains and must be measured using soil column tests.
850.6800: Modified Activated Sludge, Respiration Inhibition Test for Sparingly Soluble Chemicals	Required to determine impact to wastewater treatment systems.	See Note 2	Silver from industrial processes (e.g., film processing) has been shown to reduce microbial activity in wastewater treatment systems. The purpose of the study is to assess the impact of Nanosilva or nanosilver on microbial activity during wastewater treatment.

Note 1: These guidelines only provide general guidance. Protocols shall be submitted prior to conducting these studies.

Note 2: The test material shall include materials that are released during the stability and plastics leaching studies. These materials include nanosilver released from the Nanosilva complex or plastics treated with Nanosilva and or the Nanosilva complex released from plastics treated with Nanosilva.

Table 3B – Enforceable Schedule

The Nanosilva complex and plastics treated with Nanosilva will be the test material during Tier I studies. EPA anticipates that data will be developed in a phased approach. Thus, the schedule is separated into phases where Phase 1 – Product Characterization and Phase 2 – Product Testing will occur prior to developing protocols for Phase 3 – Release Characteristics/Exposure, and Phase 4 – Health Effects.

Tier I: Nanosilva and Nanosilva Treated Plastics Testing

		Prepare and Submit Protocols*	Perform Study and Submit Results*
	Submittal of samples		
Guideline	**Phase 1 – Product Characterization**	**2**	**6**
Non-Guideline	Particle Size Distribution		
Non-Guideline	Surface Area		
	Phase 2 – Product Testing	**5**	**9**
Non-Guideline	Stability		
	Phase 3 – Release Characteristics/Exposure	**21**	**27**
Non-Guideline	Dissolution Kinetics		
Non-Guideline	Leaching Test of Plastic		
	Phase 4 – Health Effects	**27**	**37**
870.3250	90-Day Inhalation		
870.3550	Modified Reproduction/Developmental Toxicity Screening Test		

*Number of months after conditional registration is issued.

Table 3B – Enforceable Schedule

The test material for Tier II studies will depend on the results of the Tier I leaching and dissolution studies. EPA anticipates that data will be developed in a phased approach. Thus, the schedule is separated into phases where Phase 5 – Characterization will occur prior to developing protocols for Phase 7 – Health Effects, Phase 7 – Ecological Effects, and Phase 8 – Environmental Fate.

Tier II: Testing for Nanosilver and /or Nanosilva Released during Tier I Tests

		Prepare and Submit Protocols*	Perform Study and Submit Results*
Guideline	**Phase 5 – Characterization**	**19**[†]	**24**
830.7050	UV-Vis		
Non-Guideline	Particle Size Distribution		
Non-Guideline	Surface Area		
830.7840/7860	Solubility		
Non-Guideline	Zeta-potential		
	Phase 6 - Health Effects	**27**	**37**
Non-Guideline	peri- and post-natal exposure to Nanosilva		
	Phase 7 – Ecological Effects	**25**	**36**
850.1850	Modified Aquatic Food Chain Transfer		
850.4100	Terrestrial Plant Toxicity, Seedling Emergence		
850.4400	Aquatic Plant Toxicity, Tier II		
850.5400	Algal Toxicity, Tier II		
Non-Guideline	Measuring the Chronic Effects of Freshwater Sediment-Associated Contaminants on *Chironomus dilutes*		
Non-Guideline	Measuring the Chronic Effects of Freshwater Sediment-Associated Contaminants on *Hyalella azteca*		
Non-Guideline	Measuring the Chronic Effects of Marine and Estuarine Sediment-Associated Contaminants on *Leptocheirus plumulosus*		
850.1075	Acute Toxicity Test with Freshwater and Marine Fish		
850.1300	Daphnid Chronic Toxicity Test		
850.1400	Early-Life Stage Toxicity Test for Freshwater, Estuarine, and Marine Fish		
	Phase 8 – Environmental Fate	**25**	**36**
Non-Guideline	Rate of Deposition		
850.1100	Activated Sludge Sorption Isotherm		
835.1230	Adsorption/Desorption		
835.1240	Leaching Studies (Soil Column Tests)		
850.6800	Modified Activated Sludge, Respiration Inhibition Test for Sparingly Soluble Chemicals		

* Number of months after conditional registration is issued.