

**Decision Document**

**Conditional Registration of HeiQ AGS-20 as a Materials  
Preservative in Textiles**

**December 1, 2011**

Environmental Protection Agency  
Office of Pesticide Programs  
Antimicrobials Division

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### **Appendix A: Data Requirements for the Registration of HeiQ AGS-20**

## EXECUTIVE SUMMARY

EPA is granting a conditional registration for the HeiQ Materials AG product named “HeiQ AGS-20” (hereafter referred to as “AGS-20”) 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:

- AGS-20 contains nanosilver as an active ingredient and the nanosilver in AGS-20 is not in any currently registered pesticide;
- Use of AGS-20 will not cause unreasonable adverse effects on the environment during the period when newly required data are being developed;
- Insufficient time has elapsed for HeiQ to generate and submit the newly required data; and
- Use of AGS-20 is in the public interest.

AGS-20 is a nanosilver-silica composite where the nanosilver active-ingredient is sintered onto amorphous silicon dioxide having typical particle diameters of one micrometer (1  $\mu\text{m}$  or 1,000 nm). AGS-20 will be incorporated into textiles to suppress the growth of bacteria, which cause textile odors, stains, and degradation, through the slow release of silver ions. HeiQ proposed that textiles would be treated by application of AGS-20 either as a surface coating (e.g., textile finishing) or by incorporation into the starting materials (e.g. fiber spinning). The final textile article will contain less than 0.01% silver by weight to impart durable antimicrobial and preserving activity. The treated textiles can be manufactured into indoor use articles such as sheets, blankets, towels, napkins, outerwear, sportswear, sleepwear, undergarments, socks and hosiery, and outdoor use articles such as sailcloth, tarps, tents and awnings.

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

- 1) Silver ions released from the AGS-20 particles;
- 2) The AGS-20 particles; and
- 3) Nanosilver that might break away from AGS-20 particles.

For the purposes of risk from exposure to silver ions, EPA relied on the existing reregistration decision for silver and concluded that the human health or ecological risk from exposure to silver ions derived from AGS-20 treated textiles is not of concern.

For purposes of risk from exposure to AGS-20, HeiQ submitted results from short-term acute animal-toxicity tests completed using high-level doses of AGS-20 showing that there were no mortalities or abnormalities in test animals after administration of AGS-20 by oral, dermal, and inhalation routes. AGS-20 caused moderate to no irritation to the skin and eyes of test animals, and was not a skin sensitizer. Based on these results, shipping containers filled with AGS-20 are required to carry a label stating “CAUTION” where contact with items treated with AGS-20 is restricted for 12 hours after application and AGS-20 does not require child-resistant packaging.

There are no intermediate- or long-term human or environmental toxicity studies available for AGS-20 or for the nanosilver that might break away from AGS-20. However, there are intermediate-term toxicity studies available in the scientific literature for analogous forms of nanosilver. In the absence of intermediate-term toxicity studies for AGS-20 or the nanosilver that

might break away from AGS-20, EPA evaluated the risk from occupational and consumer exposure using the data in the scientific literature on nanosilver. In addition, because the available exposure data are limited, EPA calculated the daily-dose to workers assuming that all the silver in AGS-20 was freely available as nanosilver. Likewise for the daily-dose to consumers, EPA assumed that all of the silver found in the wash water from color-fast testing of AGS-20 treated textiles and during drying of AGS-20 treated textiles consisted of nanosilver. Both of these assumptions overestimate the daily-dose of nanosilver that a person could potentially receive when working with AGS-20 or wearing AGS-20 treated textiles. Because the studies on which the assessment relies did not evaluate toxicity over all life stages or evaluate all potential effects, the Agency used a maximum 10-fold database uncertainty factor when evaluating the risk from exposure to the nanosilver that might break away from AGS-20.

Impact to the environment was based on ecotoxicity studies available in the scientific literature for analogous forms of nanosilver and environmental exposure was assessed assuming that 300 million people (U.S. population) each purchased one t-shirt treated with AGS-20 and that all the silver in those t-shirts was released during a year as nanosilver.

Using conservative assumptions which overestimate the dose of nanosilver that could potentially be derived from AGS-20 along with 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 children and the environment from textiles treated with AGS-20. Thus, the Agency concludes that use of AGS-20 will not cause unreasonable adverse effects on the environment during the period when newly required data are being developed. The Agency does have a risk concern for occupational exposure when handling AGS-20 powder during mixing and loading operations. As a result of this concern, HeiQ amended its application so that the pesticide label requires workers to wear personal protective equipment and use engineering controls when handling AGS-20 powder.

As a condition of registration, EPA is requiring HeiQ to conduct a number of studies during the period of conditional registration. The required tests include route-specific toxicity studies for occupational exposure scenarios as well as product characterization and stability tests to determine if nanosilver breaks away from AGS-20. If nanosilver is found to break away from AGS-20 or textiles treated with AGS-20, then additional testing will be triggered to determine the affect of AGS-20 derived nanosilver on humans and the environment. 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 that the use of AGS-20 will not cause unreasonable adverse effects to human health and the environment. If HeiQ fails to take appropriate steps to initiate the required studies, or if HeiQ fails to submit the protocols or data, EPA will issue a notice of intent to cancel HeiQ's registration under FIFRA section 6(e).

EPA believes that the use of HeiQ's product is in the public interest. Use of AGS-20 may lead to less environmental loading of silver as compared to currently registered products with the same use patterns. In addition, AGS-20 appears to offer prolonged ability to suppress the growth of

odor causing bacteria through the slow release of silver ions as compared to the rapid release of silver ions from registered products containing silver salts.

## I. REGULATORY DECISION SUMMARY

EPA is granting a conditional registration for the HeiQ Materials AG product named “HeiQ AGS-20” (hereafter referred to as “AGS-20”) 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:

- 1) AGS-20 contains an active ingredient, silver nanoparticles also known as nanosilver. The AGS-20 nanosilver is not an active ingredient in any currently registered pesticide (i.e., a “new” active ingredient);
- 2) Use of AGS-20 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 HeiQ to generate and submit the newly required data; and
- 4) Use of AGS-20 is in the public interest.

The product is the subject of an application submitted by HeiQ Materials AG (“HeiQ”) in September 2008. The product is named “HeiQ AGS-20,” referred to as AGS-20 in this document, and the nanosilver active ingredient is intended for use as a preservative in textile products. AGS-20 is a nanosilver-silica composite with typical particle diameters of one micrometer (1  $\mu\text{m}$  or 1,000 nm) where the nanosilver active-ingredient is sintered onto amorphous silicon dioxide (i.e., silica).

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 of an ingredient as an attribute relevant when 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 data on the nanoscale material to make the requisite statutory findings.

In September, 2008 HeiQ submitted an application for registration of AGS-20 as a “me-too” registration under FIFRA section 3(c)(7)(A). However, after consultation with the FIFRA Scientific Advisory Panel, which was held from November 3 through 5, 2009, EPA could not conclude that the nanoscale material in AGS-20 was an active ingredient in any currently registered pesticide. Consequently, EPA requested that HeiQ re-classify its application to register AGS-20 from “me-too” to a new active ingredient. HeiQ agreed to this request and on March 31, 2010 EPA announced that AGS-20 was re-classified as a product containing a new

active ingredient (Federal Register, Vol. 75, No. 61, Page 16110). Because the Agency had not reached a final decision with regard to which types of data would be further required for nanoscale materials, HeiQ did not have the benefit in support of an application for a new active ingredient of a reasonable period of time within which to generate and submit this data to the Agency.

With respect to the third conclusion, EPA has determined that the use of HeiQ's product 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 is unlikely to cause unreasonable adverse effects on humans or the environment. Although the available data on nanosilver are limited, EPA is able to determine that for the period of conditional registration, there is a low probability of adverse risk to children and the environment from textiles treated with AGS-20. Thus, the Agency concludes that use of AGS-20 will not cause unreasonable adverse effects on the environment during the period when newly required data are being developed. The Agency does have a risk concern for occupational exposure when handling AGS-20 powder during mixing and loading operations. As a result of this concern, HeiQ amended its application so that the pesticide label requires workers to wear personal protective equipment and use engineering controls when handling AGS-20 powder.

For a variety of reasons, the Agency has determined that granting a section 3(c)(7)(C) registration at this time is in the public interest. EPA believes that HeiQ'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, HeiQ's product appears to offer prolonged ability to reduce the number of odor causing bacteria through the slow release of silver ions as compared to the rapid release of silver ions from registered products containing silver salts. Moreover, because we can find that a section 3(c)(7)(C) registration is unlikely to cause an unreasonable adverse effect on the environment, allowing such registration to be granted before the newly-required data is generated allows the HeiQ product to compete with other like-situated products. Allowing HeiQ's product on the market pending generation of data allows HeiQ to participate in the 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.

As a condition of registration, EPA is requiring HeiQ to conduct a number of studies during the period of conditional registration. The required tests were adapted from 40 CFR Part 161 for nanoscale ingredients and include route-specific toxicity studies for occupational exposure scenarios as well as product characterization and stability tests to determine if nanosilver breaks away from AGS-20. If nanosilver is found to break away from AGS-20 or textiles treated with AGS-20 then additional testing will be triggered to determine the affect of AGS-20 derived nanosilver on humans and the environment. 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 that the use of AGS-20 will not cause unreasonable adverse effects to human health and the environment. If HeiQ fails to take appropriate steps to initiate the required studies, or if HeiQ fails to submit the protocols or data, EPA will issue a notice of intent to cancel HeiQ's registration under FIFRA section 6(e).

## II. BACKGROUND

### 2.1 Regulatory History

In September 2008, HeiQ submitted an application for registration of a new antimicrobial pesticide product named AGS-20, which is a silver-based product proposed for use as a materials preservative additive to coatings, polymers and textiles. HeiQ's application originally claimed that AGS-20 was similar to other currently registered silver-based antimicrobial pesticide products and should be given a "me-too registration" under FIFRA section 3(c)(7)(A). The company later amended its application to limit the proposed use of AGS-20 for treatment only of textiles. After consultation with the FIFRA Scientific Advisory Panel, which was held from November 3 through 5, 2009, EPA determined that the nanosilver active ingredient in AGS-20 differed from the active ingredients in currently registered silver-based antimicrobial products. Consequently, EPA requested that HeiQ re-classify its application to register AGS-20 from a "me-too" to a new active ingredient. HeiQ agreed to this request and on March 31, 2010 EPA announced that AGS-20 was re-classified as a product containing a new active ingredient (Federal Register, Vol. 75, No. 61, Page 16110). In response to discussion with HeiQ regarding potential risk concerns, which resulted from using conservative assumptions and data in the public literature on nanosilver formulations, HeiQ submitted an amended application reducing the application rate for fabric treated by surface coating from 30 ppm to 20 ppm and requiring use of personal protective equipment and engineering controls.

### 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.

### **2.3 Proposed Decision Document**

On August 12, 2010, EPA posted a draft version of this Decision Document to the Pesticides Public Regulatory Docket for public comment. EPA received comments on the draft Decision Document along with supporting information for a period of 30 days until September 13, 2010. EPA is posting responses to the 45 comments received regarding this decision in the Pesticides Docket.

The majority of comments concerned the level of testing required prior to registering AGS-20. While a few commenters believed that nanosilver is substantially similar to already-registered pesticides containing silver and therefore should not require additional testing, many commenters suggested that, because the existing data are allegedly insufficient, all EPA data requirements should be satisfied prior to registering AGS-20. EPA considered these comments carefully and believes that additional testing is necessary but that there are sufficient data for making an informed scientific evaluation regarding the conditional registration of AGS-20. Other key comments were submitted regarding potential impacts to the operation of wastewater treatment plants, potential development of widespread antibiotic resistance, and potential exposures during laundry drying of AGS-20 treated textiles. EPA considered these issues as discussed in this decision document. In addition, studies published in the scientific literature after August 2010 covering the effects of nanosilver on organisms were provided and incorporated into this document. Finally, EPA revised the data requirements being imposed in connection with this conditional registration based on EPA's consideration of the submitted public comments and new studies in the scientific literature.

## **III. PRODUCT CHARACTERIZATION AND TESTING**

### **3.1 Product Description and Proposed Use**

AGS-20 contains nanosilver sintered onto amorphous silicon dioxide (SiO<sub>2</sub>). The SiO<sub>2</sub> fine structure consists of aggregate matrix particles with an average diameter of approximately 1,000

nm or 1 micron. Each silica particle contains many nanosilver particles with a typical diameter between 1 and 10 nm (Egger et al., 2009) and some particles in the 50 nm range. Reference to the nanosilver in AGS-20 (whether when part of the composite or when broken away from the composite) is to the “new active ingredient” and reference to “AGS-20” or the AGS-20 “composite” is to the end use product.

AGS-20 is a powder and was proposed to be incorporated into textiles to suppress the growth of bacteria that cause odors, stains, and degradation. HeiQ may not make any public health claims relating to the antimicrobial activity of AGS-20. HeiQ proposed to use AGS-20 to treat textiles either as a surface coating (e.g., textile finishing) or by incorporation into the starting materials (e.g. fiber spinning) prior to textile manufacture. The final textile article was proposed to contain less than 0.01% silver by weight to impart durable antimicrobial and preserving activity to manufactured products. The types of textiles include those made from natural and synthetic fibers, which are used to manufacture indoor use articles such as sheets, blankets, towels, napkins, outerwear, sportswear, sleepwear, undergarments, socks and hosiery, and outdoor use articles such as sailcloth, tarps, tents and awnings.

### **3.2 Product Testing**

HeiQ submitted results from testing of AGS-20 to determine the product identity and composition, physical and chemical properties, and acute toxicity as summarized in Table 1. The tests were performed using standard EPA test guidelines<sup>1</sup>. These tests are listed in the U.S. Code of Federal Register Title 40 Parts 158 and 161, and identify the types of data EPA expects an applicant to provide to support an application for registration of a pesticide product. HeiQ performed these tests in anticipation of obtaining a “me-too registration” prior to notifying EPA that AGS-20 contained nanosilver. Although the test results provided useful information, 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, future applicants for products containing nanoscale materials should consult with the EPA prior to performing any tests.

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<sup>1</sup> Available at <http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm>

**Table 1 – Product Testing Submitted by HeiQ for AGS-20**

<b>Guideline Number</b>	<b>Guideline Name</b>	<b>Required under 40 CFR</b>
<b>Product Identity and Composition</b>		
830.1550	Product identity and composition	161.155
830.1600	Description of materials to produce product	161.160
830.1620	Description of production process	161.162
830.1650	Description of formulation process	161.165
830.1670	Discussion of formulation of impurities	161.167
830.1700	Preliminary analysis	161.170
830.1750	Certified limits	161.175
830.1800	Enforcement analytical method	161.180
<b>Physical and Chemical Properties</b>		
830.6302	Color	161.190
830.6303	Physical state	161.190
830.6304	Odor	161.190
830.6317	Storage stability	161.190
830.6320	Corrosion characteristics	161.190
830.7000	pH	161.190 (if dispersible with water)
830.7200	Melting point/melting range	161.190
830.7300	Density	161.190
830.7520	Particle size, fiber length, and diameter distribution	Not Required, information in Egger et al., 2009
830.7840	Water solubility	161.190
830.7950	Vapor Pressure	161.190 (waived for solids)
<b>Health Effects (Toxicology)</b>		
870.1100	Acute Oral Toxicity	161.340
870.1200	Acute Dermal Toxicity	161.340
870.1300	Acute Inhalation Toxicity	161.340
870.2400	Acute Eye Irritation	161.340
870.2500	Acute Dermal Irritation	161.340
870.2600	Skin Sensitization	161.340

In addition to the test results listed in Table 1, HeiQ subsequently submitted the following supplemental studies to support the registration of AGS-20:

Leaching Studies

- Height, M.J. 2009. Are silver particles released from textiles treated with the HeiQ AGS-20 silver additive? (MRID 477287-01)
- Height, M.J. 2009. Supplemental to MRID 47728701 (Are silver particles released from textiles treated with the HeiQ AGS-20 silver additive?): Response to EPA Letter of July 24, 2009. (MRID 478391-01)

Efficacy

- Egger, S., Lehmann, R.P., Height, M.J., Loessner, M.J., Schuppler, M. 2009. Antimicrobial Properties of a Novel Silver-Silica Nanocomposite Material. Applied and Environmental Microbiology 75:2973-2976. (MRID 477575-03)

### Occupational Exposure

- Demou, E., Peter, P., Hellweg, S. 2008. Exposure to Manufactured Nanostructured Particles in an Industrial Pilot Plant. *Annals Occupational Hygiene* 52:695-06. (MRID 477575-02)

### Impact to Wastewater Treatment

- Meyer, M., Height, M. 2008. Toxicity of HeiQ AGS-20 and HeiQ AGS-20 TF textile finish in municipal wastewater treatment. (MRID 479344-01)

## **IV. HUMAN HEALTH RISK ASSESSMENT**

Based on the proposed use pattern of AGS-20, EPA anticipates humans could be exposed to the following substances:

1. Silver ions released from AGS-20 treated textiles;
2. AGS-20 particles; and
3. Nanosilver that might break away from the AGS-20 particles.

Because the levels of silver in AGS-20 treated textiles are lower than for other currently registered products which use silver ions (U.S. EPA, 1993), EPA concluded that the risk from exposure to silver ions derived from AGS-20 treated textiles is not of concern. HeiQ 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 AGS-20. In addition, EPA has also reviewed data and information from the scientific literature. This section discusses EPA's assessment of the potential risks to human health from the use of AGS-20. The first section addresses information concerning toxicity of AGS-20 and nanosilver. The second section addresses potential levels of occupational exposure and risk to workers who handle AGS-20 powder. The final section addresses potential levels of consumer exposure and risk to children who wear AGS-20 treated textiles. As part of these discussions, additional data requirements are identified as necessary to confirm the assessment of risks from exposure to AGS-20 as are use restrictions consistent with those proposed in the original and amended applications.

### **4.1 Acute Human Toxicology of AGS-20**

HeiQ submitted results from short-term guideline acute animal-toxicity tests completed using high-level doses of AGS-20 as outlined in Table 2. There were no mortalities or abnormalities noted in test animals after administration of AGS-20 by oral, dermal, and inhalation routes at dose levels of up to 2,000 mg/kg, 5,000 mg/kg, and 1,081 mg/L, respectively; AGS-20 caused moderate to no irritation to skin or eyes at dose levels of up to 1,000 mg and 5,000 mg, 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 AGS-20 are required to carry a label stating "CAUTION" where contact with items treated with AGS-20 is restricted for 12 hours after application and AGS-20 does not require child-resistant packaging.

**Table 2 – Acute Toxicity Profile for AGS-20**

<b>Study</b>	<b>Toxicity Category</b>
Acute Oral Toxicity	Category III No mortality or abnormalities after dose of 2,000 mg/kg
Acute Dermal Toxicity	Category III No mortality or abnormalities after dose of 5,000 mg/kg
Acute Inhalation Toxicity	Category III No mortality or abnormalities after dose of 1.081 mg/L
Acute Eye Irritation	Category III Moderate to not irritating after dose of 1,000 mg
Acute Dermal Irritation	Category IV Mild or slight irritation after dose of 5,000 mg
Skin Sensitization	Not a sensitizer

## **4.2 Subchronic and Chronic Toxicity of Nanosilver**

There are no repeated-dose subchronic or chronic toxicity studies available for AGS-20. However, there are repeated-dose toxicity studies available in the scientific literature for analogous forms of nanosilver. Since nanosilver might be released from AGS-20, the Agency considers the scientific literature studies on nanosilver toxicity relevant to AGS-20.

### **4.2.1 Inhalation Toxicity of Nanosilver**

There is one literature study on the inhalation toxicity of nanosilver (Sung et al., 2009). This study 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) of rats after administration of nanosilver with average diameters between 18 to 19 nm (minimum diameter of 2 nm and maximum diameter of 65 nm) for 13 weeks (90-day) via the inhalation route at the high-dose level of  $515 \mu\text{g}/\text{m}^3$  ( $3.03 \times 10^6$  particles/ $\text{cm}^3$ ). The Agency considers these effects adverse. Also, 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. Based on the toxic effects observed, a No-Observed-Adverse-Effect-Level (NOAEL) of  $133 \mu\text{g}/\text{m}^3$  ( $1.4 \times 10^6$  particles/ $\text{cm}^3$ , the mid-dose level) was determined.

### **4.2.2 Oral Toxicity of Nanosilver**

There are three studies in the scientific literature that investigate toxicity of nanosilver via the oral route. 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 rats (Kim, et al, 2008). The effects reported 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. A dose-dependent increase in silver distribution in many tissues (liver, kidneys, stomach, brain, lungs,

testes, and blood), with a two-fold higher accumulation in the kidneys of female rats when compared with male rats across all dose groups, was also reported. A NOAEL of 30 mg/kg/day (lowest dose level), based on the observed liver effects and increase in alkaline phosphatase (ALP) and cholesterol at 300 mg/kg/day (mid dose level), was determined. The second study was performed by the same group in a different strain of rats 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 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 was performed by 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 liver or the small intestines examined. A NOAEL of 0.5 mg/kg/day was determined, 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. This study provided the most conservative NOAEL via the oral exposure route for nanosilver.

#### **4.2.3 Dermal Toxicity of Nanosilver**

There is little data on dermal toxicity resulting from dermal exposure to nanosilver. However, 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.

There are no 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). 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 are no Agency guideline or scientific literature studies conducted in animals for the *in vivo* dermal absorption of the nanosilver in the AGS-20 composite or of nanosilver available to the Agency. However, there is a human clinical study that examined silver levels in blood after application of burn wound dressings containing nanosilver (Moiemen et al., 2011). The study tracked both the absorption and elimination of silver and the EPA used this information to derive a conservative DAF of 0.1% for nanosilver. 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). EPA requires *in vivo* data on the ingredient in question to lower the DAF below the 100% default. The available human *in vivo* study indicating absorption of nanosilver is below 0.1% 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 0.1% for the nanosilver that might break away from the AGS-20 composite. This DAF will be used by the Agency until Tier I dermal toxicity data are provided by HeiQ for AGS-20, which will provide a route-specific study for use in subsequent risk assessments.

#### **4.2.4 Neurotoxicity of Nanosilver**

There are no studies in the scientific literature that investigate the neurotoxicity of nanosilver in mammals. A recent review of the scientific literature and clinical observations regarding the neurotoxicity of silver included observations for nanosilver used in burn wound bandages. In this review, it was concluded that although silver is known to be distributed throughout human tissues and organs after administration via pharmaceutical and burn wound bandage applications, there was little evidence suggesting that silver enters tissues of the central nervous system or is the cause of neurotoxic damage (Lansdown, 2007). The review suggested that silver be classified along with iron, zinc, and gold as a metal that is sequestered by tissue and not associated with pathological changes or pathophysiological consequences, including changes in the blood-cerebrospinal fluid barrier.

Arguing against this are *in vivo* studies completed with nanosilver indicating concentration/dose-related increases in the levels of silver in the olfactory bulb and/or brain after inhalation (Sung et al. 2009) and oral administration of nanosilver in rats (Kim et al. 2010, Kim et al. 2008). A caveat of these studies is that it is not possible to determine if silver identified in the olfactory bulb and/or brain was in the nanoparticulate or ionic form. However, there are reports in the literature from *in vivo* studies the nanosilver can cross the blood brain barrier in rats (Tang et al., 2009; Lankveld et al. 2010) and induce brain edema (Sharma et al., 2010), indicating that nanosilver particles can reach brain tissue. Once in the brain, there is the question of whether or not nanosilver would cause neurotoxicity. The following *in vitro* studies indicate that nanosilver can cause neuronal toxicity and depletion of the neurotransmitter dopamine (Hussain et al., 2006) and changes in inhibitory action potentials in hippocampal neurons (Liu et al., 2009), which suggests the potential for nanosilver to cause neurotoxicity by altered neurotransmission and direct cytotoxicity. However, it is unknown if the doses used in the *in vitro* studies would approximate *in vivo* levels.

#### **4.2.5 Chronic Toxicity of Nanosilver**

There are no studies in the scientific literature that investigate the chronic toxicity or carcinogenicity of nanosilver.

#### **4.2.6 Reproductive and Developmental Toxicity of Nanosilver**

There are no studies in the scientific literature that investigate the reproductive or developmental toxicity of nanosilver in mammals. However, one study does show significant, dose-dependent increases in the concentration of silver in the testes of rats after ingesting nanosilver via gavage for 28 days at doses ranging from 30 to 1,000 mg/kg/day (Kim et al., 2008). No increases in silver were reported in the testes or reproductive organs of rats after inhaling nanosilver for 13 weeks at concentrations up to 515  $\mu\text{g}/\text{m}^3$  (Sung et al., 2009). The technique used to detect silver in these studies did not distinguish between nanosilver and silver ions. Thus, these animal data indicate that silver ions released from nanosilver or nanosilver can accumulate in the testes after oral, but not inhalation exposure. The only study that the Agency is aware of is an *in vitro* study investigating the toxicity of 15 nm nanosilver on spermatogonia 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  $\mu\text{g}/\text{ml}$ ; however, the effects from nanosilver were greater than observed for silver ions. It is unclear what the extrapolated dose to a whole mammal would be since there are no pharmacokinetic data on nanosilver. The conservative interpretation of this data is that nanosilver which reach the testes may be able to cause decreased fertility due to toxicity to spermatogonia. This effect would be more severe for nanosilver than for silver ion.

#### **4.2.7 Mutagenicity of Nanosilver**

Genotoxic potential has been investigated *in vivo* by measuring micronucleated polychromatic erythrocytes (MN PCEs) 28 days after oral administration of carboxymethyl cellulose-coated nanosilver with average diameter of 60 nm (minimum diameter of 53 nm and maximum diameter of 71 nm) (Kim, et al, 2008). No statistically significant treatment-related increase of MN PCEs in the male and female rats when compared to study rats not treated with nanosilver (i.e., negative control). This study indicates that nanosilver is not genotoxic *in vivo*, although a limitation of this study is that no measurements were performed to determine if nanosilver reached the bone marrow.

#### **4.2.8 Silver Ions**

Humans may also be exposed to silver ions that would be released by AGS-20. Conventional silver, and the silver ions it releases, are pesticides. 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 notes that safe exposure levels for silver have been established by several regulatory agencies, including OSHA and EPA. These safe exposure levels are based on the common endpoint argyria, a blue-gray discoloration of the skin. Silver and compounds, excluding nanosilver, are currently being re-evaluated through the Agency's Registration Review program.

#### **4.2.9 Conclusions from Toxicity Studies**

Together, these studies indicate to the Agency that, if sufficient quantities of nanosilver break away from AGS-20, and if such nanosilver displays toxicity similar to the nanosilver used in these studies, then route-specific exposure to AGS-20 derived nanosilver may result in adverse health effects. The inhalation NOAEL of 133  $\mu\text{g}/\text{m}^3$  was determined after inhalation of nanosilver in rats over 90 days and was based on effects observed in the liver and lungs, and systemic accumulation of silver in tissues. The oral NOAEL of 0.5 mg/kg/day was determined after oral administration of nanosilver to mice over 28 days and was based on the elevated blood enzymes and effects observed in kidney tissues. These NOAELs were deemed to represent the lowest inhalation and oral daily-doses for nanosilver that does not cause an effect during short-term (<30 days) and intermediate-term (1 to 6 months) exposures.

The nanosilvers used in the 90-day inhalation toxicity and 28-day oral toxicity studies have physical properties similar to the nanosilver present in AGS-20. The nanosilver sizes in AGS-20 typically range from 1 to 10 nm (Egger et al., 2009) and some particles in the 50 nm range. The nanosilver used in the inhalation toxicity study (Sung et al., 2009) had average diameters between 18 to 19 nm (minimum diameter of 2 nm and maximum diameter of 65 nm) and the nanosilver used in the 28-day mouse oral toxicity study (Park, et al., 2010) had an average diameter of 42 nm (minimum diameter of 25 nm and maximum diameter of 55 nm). Thus, the size of nanosilvers in these studies overlaps the size range for nanosilver present in AGS-20. In addition, these studies involved uncoated nanosilver similar to the nanosilver present in AGS-20.

The Agency believes the inhalation and oral toxicity studies described above are sufficient for an assessment of the risks from use of the nanosilver in the AGS-20 composite during generation of the requisite data because the data establish dose-response target endpoints in female and male animals, without animal mortality, for relevant routes of exposure over a duration sufficient to evaluate intermediate-term exposures (US EPA, 2002). Because the studies do not establish toxicity over all life stages, nor do they evaluate all potential effects, appropriate and relevant uncertainty factors are applied as discussed further below.

#### **4.3 Antimicrobial Resistance**

Silver is currently used as a broad spectrum antibiotic in wound dressings. There is a concern that increasing use of silver, such as nanosilver for preserving textiles, 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 AGS-20 is possible, the likelihood is low that the levels used in AGS-20 treated textiles will lead to the development of widespread bacterial resistance to silver.

#### **4.4 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.

##### **4.4.1 Calculation of MOE**

The MOE is calculated by dividing the toxicological point of departure (POD) by the estimated daily dose to which humans will be exposed as expressed by the following:

$$\text{MOE} = \text{POD} / \text{Daily Dose}$$

The POD is the lower confidence bound on the lowest experimental dose that showed an effect in a dose-response study. 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 effects were observed in laboratory animals.

After calculating a MOE from the POD and daily dose, EPA evaluates the risk from exposure to a chemical by comparing the calculated MOE to a target MOE. If the calculated MOE is greater than the target MOE, then EPA does not have a risk concern. If the calculated MOE is less than the target MOE, EPA does have a risk concern. In this case, mitigation measures such as engineering controls and/or personal protective equipment (PPE) are employed until the calculated MOE exceeds the target MOE. These relationships are summarized below:

- If calculated MOE > target MOE: risk is not of concern and mitigation is not required
- If calculated MOE ≤ target MOE: risk is of concern and mitigation is required

##### **4.4.2 Point of Departures for AGS-20**

Inhalation, oral, or dermal subchronic and chronic toxicology studies are not available for AGS-20 or the nanosilver that might break away from textiles treated with AGS-20. In place of these studies, the Agency is using results from subchronic inhalation and oral toxicity studies found in the scientific literature for nanosilvers that have physical properties similar to the nanosilver present in AGS-20. The NOAEL from the 90-day inhalation toxicity study by Sung et al. (2009) was used to determine the POD for inhalation exposures to the nanosilver that might break away from AGS-20 (Table 3). The NOAEL from the 28-day oral toxicity study by Park et al. (2010) was used as the POD for oral and dermal exposures to the nanosilver that might break away from textiles treated with AGS-20 (Table 3).

**Table 3 – Points of Departures**

<b>Exposure Route</b>	<b>POD</b>	<b>Basis</b>
Inhalation	133 $\mu\text{g}/\text{m}^3$	NOAEL of 133 $\mu\text{g}/\text{m}^3$ based on the observed effects in rats after inhaling nanosilver for 13 weeks (Sung et al., 2009)
Incidental Oral	0.5 mg/kg/day	NOAEL of 0.5 mg/kg/day based on the observed effects in mice after ingesting nanosilver for 28 days (Park et al., 2010).
Dermal	0.5 mg/kg/day	No subchronic studies are available for dermal toxicity of nanosilver. Because of this, a POD was chosen by route-to-route extrapolation from the oral toxicity study with a NOAEL of 0.5 mg/kg/day (Park et al., 2010).

The Agency’s MOE approach 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 nanoparticle risks and therefore acknowledges the potential for limitations of mass-based risk estimates.

#### 4.4.3 Target MOEs

The target MOE for continuous, daily inhalation, oral, and dermal exposures to AGS-20 for short-term (< 30 days) and intermediate-term (1 to 6 months) durations is 1,000 and is 3,000 for long-term (> 6 months) duration exposures (Table 4).

**Table 4 – Target Margins of Exposures**

<b>Continuous and Daily Exposure Duration</b>	<b>Target MOE</b>		
	<b>Inhalation</b>	<b>Oral</b>	<b>Dermal</b>
Short-Term (<30 days)	1,000	1,000	1,000
Intermediate-Term (1 to 6 months)	1,000	1,000	1,000
Long-Term (> 6 months)	3,000	3,000	3,000

Target MOEs are 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 there is sufficient inhalation and oral toxicity data for nanosilver which can be used to determine the potential health effects caused by nanosilver released from AGS-20 (Table

3). However, the database is incomplete with respect to reproductive and developmental toxicity, neurotoxicity, immunotoxicity, and mutagenicity. Therefore, the Agency is using the maximum 10 fold database uncertainty factor to extend the inhalation and oral toxicity data to cover the missing information on developmental toxicity, neurotoxicity, immunotoxicity, and mutagenicity (US EPA, 2002). The Agency is requiring studies investigating these effects in order to complete the toxicity database for AGS-20 and any material that leaches from treated textiles. A new risk assessment will be conducted when these data are available. In the interim, the Agency believes that the 10 fold database uncertainty factor is health protective. Thus, the target MOE for short- and intermediate-term duration exposures is:

$$\text{Target MOE short- and intermediate-term} = 10 (UF_A) \times 10 (UF_H) \times 10 (UF_D) = 1,000$$

Because there are no repeated-dose chronic toxicity studies available for evaluating the long-term exposures to nanosilver that might break away from AGS-20, a duration adjustment was necessary to extrapolate the available nanosilver subchronic toxicity study results to chronic results. This extrapolation was accomplished by using a 3 fold uncertainty factor to account for the increase in liver toxicity and bioaccumulation observed for nanosilver between the 28-day (Kim, et al, 2008) and 90-day (Kim, et al, 2010) rat studies. Thus, the target MOE for long-term duration exposures is:

$$\text{Target MOE long-term} = 10 (UF_A) \times 10 (UF_H) \times 10 (UF_D) \times 3 = 3,000$$

## **4.5 Occupational Risk Assessment**

EPA expects occupational inhalation and dermal exposures to AGS-20 and the nanoparticles that might break away from AGS-20 are likely to occur during the following use scenarios:

1. Mixing, loading, and applying AGS-20 powder during the treatment of textiles;
2. Preparing textile articles from AGS-20 treated textiles; and
3. Laundering AGS-20 treated textiles.

EPA evaluates the risk of occupational exposures from mixing, loading, and applying or handling pesticide products. Although exposure to AGS-20 during subsequent work activities involving AGS-20 treated textiles are occupational (items 2 and 3), these scenarios are addressed under the residential risk assessment (see Section 4.5).

### **4.5.1 Occupational Exposure when Handling AGS-20 Powder**

HeiQ submitted an article published in a peer reviewed journal (Demou et al., 2008) where the authors measured the occupational inhalation exposures during post-production handling and processing of AGS-20 powder. The process monitored was a small-scale test version of the full-scale process that will be used for the production of AGS-20. Airborne particulate concentrations were measured using non-specific direct reading instruments such as Condensation Particle Counters (CPC), a Dust Trak<sup>TM</sup> aerosol monitor and a Scanning Mobility Particle Sizer (SMPS), which counted the number of particles with diameters between 6 and 673 nm. Although these instruments cannot determine the composition of the particles detected, they are often used to identify and characterize emissions sources in workplaces where nanoparticles might be present.

In this study, temporal and spatial analysis of particle concentrations and sizes were performed during AGS-20 production, maintenance, and handling and packaging. The results indicated that the highest particle concentrations of 50,000 particles per cubic centimeter occurred during production and that these concentrations were significantly greater than the background particle concentrations of 9,000 particles per cubic centimeter on average. (Note - heating system combustion byproducts, vehicle exhaust, and electric motors are background sources of nanoparticles.) It was reported by the authors that the rate of AGS-20 production was related to both the profile and magnitude of the airborne particle concentration. It was determined that particle re-suspension was not relevant because airborne particle concentrations did not increase when equipment operation and production was not occurring. Manual cleaning of the reactor with a vacuum cleaner also resulted in a significant increase in the number of particles in the 6 to 673 nm range. Transfer of AGS-20 powder during post-production handling and processing caused maximum particle concentrations of 15,000 particles per cubic centimetre compared to average background particle concentrations of approximately 9,000 particles per cubic centimeter.

Workers may be exposed to AGS-20 particles and nanosilver particles that break away from the composite particle. If AGS-20 particles remain intact during mixing, loading, and applying (i.e., handling), workers would be exposed to the 1,000 nm sized AGS-20 particles (Egger et al, 2009). Although not conclusive, the submitted literature study suggests that this might be the case because the number of particles in the 6 to 673 nm range did not greatly increase above background levels during the handling of AGS-20 powder, which would be expected if nanosilver were breaking away from AGS-20. However, this study was completed for the AGS-20 production process and may not represent conditions when AGS-20 powder is being mixed, loaded, and applied during the treatment of textiles. Because of this shortcoming, the Agency is using the standard occupational handler unit exposure values for mixing and loading of wettable powders, as shown in Table 5, which are provided in the Pesticide Handlers Exposure Database (PHED) (U.S. EPA, 2011a). Wettable powders were chosen because these powders have a high proportion of particles with diameters less than 5,000 nm (Matthews, 2000), which is similar to AGS-20 particles with average diameters of 1,000 nm.

**Table 5 – Unit Exposures for Mixing and Loading of Wettable Powders (Matthews, 2000)**

<b>Personal Protective Equipment (PPE) Level</b>	<b>Exposure Route</b>	<b>Unit Exposure</b>
No gloves, use of long-sleeve shirt, long pants, shoes plus socks	Dermal	3.7 (mg/lb)
Gloves, long-sleeve shirt, long pants, shoes plus socks	Dermal	0.17 (mg/lb)
No respirator	Inhalation	7.8 ( $\mu\text{g}/\text{m}^3/\text{lb}$ )

The values in Table 5 indicate, for example, that if 100 lb of AGS-20 were handled during a work day, 370 mg of AGS-20 would reach the skin of a worker who was not wearing gloves. If gloves were worn then 17 mg of AGS-20 would reach the skin.

#### 4.5.2 Particle Penetration through Respirator Filters when Handling AGS-20 Powder

The penetration of particles through four types of respirator filters was determined for particles generated during the production of AGS-20 (Demou et al., 2008). Three of these respirator filters were certified to the European Standard EN 143:2000 P3, which indicates that they provide the highest level of protection while one filter was certified to the EN 149:2001 P2 standard which indicates a lower level of protection. Particle laden air from the AGS-20 production process was drawn through each respirator filter and particle concentration measurements were taken on each side of the filter using Condensation Particle Counters. The initial chamber flow rate was set at one of two levels (1.0 m<sup>3</sup>/hr and 2.15 m<sup>3</sup>/hr) to represent the inhalation rates of adults engaged in light and moderate activities. These breathing rates correspond to 6.7 liters per minute (LPM) for light activities and 36 LPM for moderate activities. The results indicated that at least 99.89% of the incoming particles were retained in the P3 respirator filters and 96.66% retained in the P2 respirator filters. These results are consistent with other studies cited in the National Institute for Occupational Safety and Health Document Approaches to Safe Nanotechnology (NIOSH, 2009) which reported very high retention rates for respirator filters challenged with nanoparticles.

#### 4.5.3 Conclusions from the Occupational Exposure Study

The submitted study discussed above provides some useful information regarding occupational exposure during post-production handling and processing of AGS-20 powder, but it does not provide quantitative data for purposes of estimating exposures from mixing, loading, and applying (i.e., handling) AGS-20 powder. EPA is using the PHED values for mixing and loading of wettable powders to evaluate the exposure to AGS-20 powder during transfer to shipping packages and application to textiles.

#### 4.5.4 Occupational Margins of Exposure

EPA expects that workers will be exposed to AGS-20 powder through the routes of inhalation and dermal contact. EPA recognizes the potential for inhalation and dermal exposure to nanosilver during laundry drying of AGS-20 treated textiles; however, EPA lacks information on the release rate of nanosilver from AGS-20 treated textiles during laundry drying and is therefore requiring HeiQ to perform an attrition study to determine this information. While exposure may occur during laundry drying, EPA estimates that when compared to exposure through inhalation during mixing, loading, and applying (i.e., handling) of AGS-20 powder to textiles, exposure during laundry drying is of lower significance (see Section 4.6.4). As detailed in the following sections, EPA evaluated the risk to workers who mix, load, and apply AGS-20 powder to textiles.

#### Occupational Inhalation Daily-Dose and MOEs

The inhalation dose from exposure to AGS-20 powder was calculated using the following:

**Inhalation Daily Dose** = Amount of AGS-20 handled per day × Unit Exposure

Where:

- Amount of AGS-20 handled per day: OPP standard assumption is that 20,000 lb of textiles are treated per day at each textile treatment facility and the maximum amount of

nanosilver in AGS-20 that can be applied to textiles is 0.01% silver by weight, thus the amount of nanosilver in AGS-20 that is handled per day is 20,000 lb/day × 0.01% ÷ 100% = 2 lb/day.

- The unit exposure for inhalation is 7.8 µg/m<sup>3</sup>/lb when no respirator is worn (see Table 5).
- The unit exposure for inhalation is 0.16 µg/m<sup>3</sup>/lb when a full-face respirator is worn. This value was calculated by dividing 7.8 µg/m<sup>3</sup>/lb by a protection factor of 50 which was recommended in Approaches to Safe Nanotechnology (NIOSH, 2009).
- The unit exposure for inhalation is 0.016 µg/m<sup>3</sup>/lb when a full-face respirator is worn and engineering controls are used. This value was calculated by dividing 0.16 µg/m<sup>3</sup>/lb by a protection factor of 10. The efficacy of the engineering controls such as local exhaust ventilation is dependent upon their specific design characteristic, but local exhaust without enclosure can be assumed to result in a 3 to 10 fold reduction in exposure (AIHA, 2006; Marquart, 2008; Burgess, 1995) while local exhaust with enclosure can be assumed to result in a 30 to 100 fold reduction in exposure (Marquart, 2008; AIHA, 2006).

**Table 6 – Inhalation MOEs for AGS-20 Occupational Handlers**

Scenario	Amount of Textile Treated per day <sup>A</sup>	Application Rate <sup>B</sup>	Amount of AI handled per day	Unit Exposure <sup>C</sup> (µg/m <sup>3</sup> /lb AI)	Daily Dose <sup>F</sup> (µg/m <sup>3</sup> )	MOE <sup>G</sup>
Mix/Load AGS-20 Powder <b>Without Respirator</b>	20,000 lb	0.01% silver by weight	2 lb	7.8	16	10
Mix/Load AGS-20 Powder <b>With Full-Face Respirator</b>	20,000 lb	0.01% silver by weight	2 lb	0.16 <sup>D</sup>	0.32	420
Mix/Load AGS-20 Powder <b>With Engineering Controls</b>	20,000 lb	0.01% silver by weight	2 lb	0.016 <sup>E</sup>	0.032	4,200

A. Standard OPP assumption for textile treatment per manufacturing facility

B. Maximum amount of nanosilver in AGS-20 for treatment of textiles

C. PHED unit exposure value converted to air concentration units based on mean 8 hour TWA

D. PHED unit exposure value divided by 50 for full-face respirator

E. PHED unit exposure value divided by 50 for full-face respirator and divided by 10 for engineering controls

F. Dose = Amount AI Handled × Unit Exposure

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

The MOEs shown in Table 6 were calculated by dividing the inhalation dose by the POD of 133 µg/m<sup>3</sup>, which is the NOAEL from a 90-day inhalation toxicity study for nanosilver (Sung et al., 2009). This assumes that AGS-20 behaves like nanosilver after inhalation. When no respirator is worn, the MOE is 10 which is less than the target MOE of 1,000 and indicates a risk concern. The MOE increased to 420 assuming that full-face respirators are worn, which is also less than

the target MOE of 1,000 and indicates a risk concern. HeiQ recommended that its workers wear full-face respirators during manufacture of AGS-20 (MRID 479344-02). If local exhaust ventilation engineering controls, which provide a protection factor of greater than 10, in addition to the full-face respirator are used, the MOE increases to 4,200 which is greater than the target MOE of 1,000 indicating the risk from short- and intermediate-term exposures is not of concern.

### Occupational Dermal Daily-Dose and MOEs

The dermal dose from exposure to AGS-20 powder was calculated using the following:

$$\text{Dermal Daily Dose} = (\text{Amount of AGS-20 handled per day} \times \text{Unit Exposure} \times \text{Dermal Absorption Factor}) / \text{Body Weight}$$

Where:

- Amount of AGS-20 handled per day: OPP standard assumption is that 20,000 lb of textiles are treated per day at each textile treatment facility and the maximum amount of nanosilver in AGS-20 that can be applied to textiles is 0.01% silver by weight, thus the amount of nanosilver in AGS-20 that is handled per day is  $20,000 \text{ lb/day} \times 0.01\% \div 100\% = 2 \text{ lb/day}$ .
- The unit exposures for dermal are 3.7 mg/lb when no gloves are worn and 0.17 mg/lb when gloves are worn (Table 5).
- The dermal absorption factor is 0.1% (see Section 4.2.3).
- The body weight of an adult is 70 kg which is a standard assumption from the Exposure Factors Handbook (U.S. EPA, 1997).

**Table 7 – Dermal MOEs for AGS-20 Occupational Handlers**

Scenario	Amount of AI added or handled <sup>A</sup>	Unit Exposure <sup>B</sup> (mg/lb AI)	Exposure <sup>C</sup> (mg/day)	Daily Dose <sup>D</sup> (mg/kg/day)	MOE <sup>E</sup>
Mix/Load AGS-20 Powder During Textile Treatment <b>Without gloves</b>	2 lb	3.7	7.4	0.00011	4,500
Mix/Load AGS-20 Powder During Textile Treatment <b>With gloves</b>	2 lb	0.17	0.34	0.000049	100,000

A. Based on the same assumptions as those used for the inhalation MOE (see Table 6)

B. PHED unit exposure data for wettable powder (see Table 5).

C. Dermal Exposure = Amount AI Handled × Dermal Unit Exposure

D. Dermal Dose = [Dermal Exposure × Dermal Absorption Factor (0.1%)] / Body Weight (70 kg)

E. Dermal MOE = POD / Daily Dose (rounded to two significant digits)

The MOEs shown in Table 7 were calculated by dividing the dermal dose by the POD of 0.5 mg/kg/day, which is the NOAEL from a 28 day oral toxicity study (Park et al., 2010). This

assumes that AGS-20 behaves like nanosilver after dermal absorption. The MOE for dermal exposures is 4,500 when no gloves are worn and is 100,000 when gloves are worn, which are both greater than the target MOE of 1,000 indicating that the risk from short- and intermediate-term occupational dermal exposures are not of concern.

#### **4.5.5 Conclusions for Occupational Risk**

The MOE for occupational inhalation exposure to AGS-20 was 10 assuming that AGS-20 behaves like nanosilver after inhalation. The MOE increased to 420 assuming that full-face respirators are worn and 4,200 when engineering controls in addition to full-face respirator are utilized. These MOEs indicate that the risk concern for inhalation exposures to AGS-20 powder during handling is mitigated when workers wear full-face respirators and utilize engineering controls which provide a protection factor of 10 or greater. The MOE for occupational dermal exposure to AGS-20 for a worker who wears a long-sleeve shirt, long pants, and shoes plus socks was 4,500 without the use of gloves and 100,000 assuming that gloves were worn. These MOEs indicate that the risk concern for dermal exposure to AGS-20 powder is not of concern when workers wear long-sleeve shirts, long pants, and shoes plus socks.

There are several uncertainties in the occupational risk assessment. AGS-20 exposure values may be different from the PHED unit exposure values for wettable powders since AGS-20 particle size is approximately 5 times smaller than the particle size for wettable powders. The PODs used were based on inhalation and oral toxicity studies completed using nanosilver, not AGS-20 or the nanosilver that might break away from AGS-20, and thus these PODs may not represent the AGS-20 nanosilver-silica composite or the nanosilver found in AGS-20. Due to deficiencies in the toxicity studies available for nanosilver, the Agency used an additional 10 fold database uncertainty factor resulting in a target MOE of 1,000. 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) for purposes of using the oral NOAEL to calculate a POD for dermal toxicity. However, the Agency believes that the 0.1% DAF used in calculating a dermal dose is conservative because the available human *in vivo* study indicates that absorption of nanosilver is below 0.1% and the *in vitro* data indicate absorption of nanosilver from intact and abraded human skin is substantially below 0.1% (see Section 4.2.3).

Occupational inhalation exposures during mixing, loading, and applying (i.e., handling) AGS-20 powder can be minimized through the use of engineering controls such as closed system loading or local exhaust ventilation. The use of engineering controls as a primary method of reducing worker exposure to nanoparticles is discussed in Approaches to Safe Nanotechnology (NIOSH, 2009). In addition, a secondary method of inhalation exposure control, which was proposed by HeiQ, requires workers to wear full-face respirators with high-efficiency filter cartridges (i.e. P100) and, assuming that they are properly fitted, these respirators will provide a protection factor of 50. Occupational dermal exposures to AGS-20 powder can be minimized by requiring workers to wear long-sleeve shirts, long pants, and shoes plus socks. However, because the particle size of AGS-20 powder is smaller than the particle size for the wettable powder which was used to estimate exposures to AGS-20, gloves and overalls or a Tyvek suit are recommended as an additional level of protection when handling AGS-20 powder .

If engineering controls and personal protective equipment are used when handling AGS-20 powder, the Agency anticipates that the inhalation and dermal MOEs will be greater than the target MOE of 1,000 for short- and intermediate-term exposures, indicating that the occupational risk from AGS-20 will not be of concern during the four year period of conditional registration. EPA does not typically consider long-term occupational exposures to antimicrobial preservatives used to treat textiles because application of these chemicals does not typically occur on a daily basis for more than 6 months. However, if continuous daily production of AGS-20 treated textiles occurs for more than 6 months, the MOEs for inhalation and dermal exposures to AGS-20 powder are greater than the target MOE of 3,000, indicating that any potential risk from long-term inhalation and dermal exposure to AGS-20 powder would not be of concern.

#### **4.5.6 Occupational Exposure and Health Data Requirements**

EPA is requiring that HeiQ conduct an indoor applicator study to quantify the unit exposure values during mixing, loading, and applying AGS-20 powder. EPA will use these values to confirm the occupational risk assessment summarized above.

Based on the potential occupational exposures to AGS-20, the Agency has determined that route-specific subchronic tests are required as Tier I studies to confirm the Agency's determination that the risks of this product to workers are not unreasonable. The Tier I health effects testing categories include:

- Route-specific subchronic inhalation study in rats with *in vivo* bone marrow assay and functional observational battery, motor activity and detailed neuropathology
- Route-specific dermal toxicity study in rats
- Reproduction and developmental toxicity screening tests in rats
- An *in-vitro* micronucleus assay for mutations in genetic material

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

#### **4.6 Consumer Risk Assessment**

EPA expects consumer exposures to AGS-20, the silver ions derived from AGS-20, and the nanoparticles that might break away from AGS-20 could potentially occur during the following use scenarios:

1. Inhalation exposure during laundry drying of AGS-20 treated fabrics;
2. Incidental oral exposure to AGS-20 treated fabrics; and
3. Dermal exposure while wearing AGS-20 treated fabrics.

To determine the amount and form of silver that consumers will be exposed to when wearing and chewing on AGS-20 treated textiles would require a leaching study. These studies typically involve immersing textiles in biological fluids such as simulated sweat and saliva solutions for extended periods of time at physiological temperatures (i.e., 98.6 °F or 37 °C) and measuring the amount and form of silver released to those fluids. Such "leaching studies" using biological

fluids were not submitted by HeiQ for AGS-20 treated textiles. However, HeiQ submitted two leaching studies involving AGS-20 treated textiles. One was completed using ultrapure water and the other involved alkaline water with detergents and simulated laundering conditions.

#### **4.6.1 Ultrapure Water Textile Leaching Study**

A textile leaching study (MRID 477287-01) was submitted by HeiQ and involved agitating AGS-20 treated fabric samples immersed in room temperature (i.e., 68 °F) ultrapure water for 24 hours. The wash water was analyzed for silver ions using an ion specific electrode (ISE) and for nanosilver using scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDX). According to HeiQ, no ionic silver was detected using ISE and no nanosilver was detected using SEM/EDX. There were other particles detected, but they were confirmed to be non-silver using EDX.

EPA does not believe that this study is sufficient to support HeiQ's conclusion that no ionic silver and no nanosilver are released from AGS-20 treated textile during use. The ISE method that was used to detect silver ions had a detection limit of either 160 µg/L or 800 µg/L, which are much higher than other available methods such as inductively coupled plasma mass spectrometry (ICP-MS) with a silver detection limit of 0.2 µg/L. The magnification used for SEM, which was at the 1,000 nm scale, was insufficient to detect the possible presence of nanosilver particles that are 1-10 nm in diameter. At best, this study supports the limited conclusions that no 1,000 nm sized AGS-20 composite particles were released and that the release of ionic silver did not exceed 160 µg/L.

#### **4.6.2 Alkaline Water and Simulated Laundering Textile Leaching Study**

In addition to the ultrapure water textile leaching study mentioned above, HeiQ submitted a literature study that examined textiles treated with AGS-20 (Geranio et al., 2009). During this study, 9 textiles treated with silver, including two treated with AGS-20, were 1) immersed in water at pH 10 with detergent followed by agitation or 2) machine washed using an International Standards Organization (ISO) test for color fastness of textiles during laundering to determine the effect of pH, surfactants, and bleaching agents on the rate of silver release. The HeiQ samples were polyester fabrics treated with AGS-20 by either a surface coating (NP-PES-SURF) or by incorporation into the polyester fiber during manufacturing (NP-PES). Samples of the wash water were analyzed for silver ions using ISE before and after filtration using 450 nm filters and 30 kDa membranes (~5 nm) to separate large and small particulate silver from silver ions. The ISE used in the Geranio et al. (2009) study reported a detection limit of 9 µg/L. The amount of silver retained by the filters was determined using inductively coupled plasma optical emission spectroscopy (ICP-OES).

After immersing the surface coated textile (NP-PES-SURF) in pH 10 water with detergent for 150 min (dissolution), approximately 15% of the nanosilver was released with 80% as ionic silver and 20% retained on a 450 nm filter (Table 8). The amount of silver released during the initial ISO color-fastness test (1<sup>st</sup> washing) of the surface coated fabric was 35% with 80% of the released silver retained on a 450 nm filter, 15% had diameter less than 450 nm, and 5% was composed of ionic silver. Thus, the nanosilver in textiles surface treated with AGS-20 will likely transform into ionic silver after exposure to alkaline conditions while coarse silver-containing

particles will likely be released during mechanical washing of these textiles. Geranio et al. (2009) did not perform a second or third washing of the surface coated textile.

For the textile containing AGS-20 incorporated into the polyester fiber (NP-PES), no ionic silver was found above the 8 µg/L detection limit during the pH 10 water dissolution test or the ISO color-fastness test. Instead, the 1.3 to 1.5% of silver released was retained on a 450 nm filter.

**Table 8 – Silver Released During Washing of AGS-20 Treated Textile**

Designation in Geranio et al. 2009	Designation in MRID 477287-01	Treatment Type	Silver Content µg/g*	Silver Released (%)	
				Dissolution	1 <sup>st</sup> Washing
NP-PES-SURF	Sample #2	Surface Coating	29	15 <sup>A</sup>	35 <sup>B</sup>
NP-PES	Sample #3	Incorporation	99	1.5 <sup>C</sup>	1.3 <sup>C</sup>

\* Units are in µg silver / g of textile

A. 80% as ionic silver and 20% retained on 450 nm filter

B. 80% retained on 450 nm filter, 15% with diameter < 450 nm, and 5% as ionic silver

C. 100% retained on 450 nm filter

#### 4.6.3 Conclusions Regarding the Consumer Exposures

EPA has concluded that the AGS-20 treated textiles, particularly when surface coated, release silver in the form of ionic silver and coarse particulates, but it is not known if these particulates consist of nanosilver in fibers or coating fragments, aggregates of nanosilver, or precipitates of ionic silver. Although Geranio et al. (2009) only reported results for the initial washing of the surface coated textile; EPA anticipates that less silver would be released during subsequent washings meaning that the initial washing probably released the greatest amount of silver and that subsequent washings would release less silver.

The leaching study performed by Geranio et al. (2009) did not use biological fluids and does not directly simulate the release of silver in saliva or perspiration. However, the ISO color-fastness test involved the use of detergent, alkaline conditions, steel balls, and physiologic temperature (40 °C) to determine the persistence of color in textiles during laundering. EPA concludes that while this ISO color-fastness test does not exactly simulate wearing or chewing on textiles, because it involved aggressive conditions potentially resulting in greater release than might otherwise occur during chewing on or wearing textiles, it does provide a reasonable first estimate for the amount silver transferred to the mouth while chewing on and transferred to skin while wearing textiles treated with AGS-20.

EPA has determined that an additional leaching study is required using biological fluids and physiological temperatures and includes electron microscopy to characterize any particulate that have been released. This study is needed to refine EPA understanding regarding the amount and form of silver released from AGS-20 treated textiles.

#### 4.6.4 Consumer Margins of Exposure

EPA expects that consumers will be exposed to AGS-20 by the routes of inhalation, dermal, and incidental oral exposures. As detailed in the following sections, EPA evaluated the risk to children, as the likely most vulnerable subpopulation, from chewing on and wearing textiles treated with AGS-20. Because the ratio of dose and skin surface area to the body weight are greater for children than for adults, evaluating the risk to children is also protective for adults including workers who process AGS-20 treated textiles.

##### Consumer Inhalation Daily-Dose and MOEs

EPA recognizes the potential for inhalation exposure to nanosilver during laundry drying of AGS-20 treated textiles. However, EPA lacks information on the release rate of nanosilver from AGS-20 treated textiles during laundry drying and is therefore requiring HeiQ to perform an attrition study to determine this information. While exposure may occur during laundry drying, EPA believes that when compared to exposure through dermal and oral contact with AGS-20 treated textiles, 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 AGS-20 treated textiles during laundry drying was calculated using the following:

**Inhalation Daily Dose** = Amount of AGS-20 in clothing handled per day × Unit Exposure

Where:

- Amount of AGS-20 in clothing handled per day assumed that one 150 g t-shirt treated with 100 ppm of AGS-20 as silver was laundered, thus the amount of nanosilver in AGS-20 that is laundered per day is  $150 \text{ g} \times 100 \text{ mg/kg} \times \text{kg}/1000 \text{ g} = 15 \text{ mg}$ .
- The unit exposure for inhalation is  $7.8 \text{ } \mu\text{g}/\text{m}^3/\text{lb}$  when no respirator is worn (see Table 5).

**Table 9 – Inhalation MOEs for Drying AGS-20 Treated Textiles**

Scenario	Amount of AI Laundered per day <sup>A</sup>	Unit Exposure <sup>B</sup> ( $\mu\text{g}/\text{m}^3/\text{lb AI}$ )	Daily Dose <sup>C</sup> ( $\mu\text{g}/\text{m}^3$ )	MOE <sup>D</sup>
Unload Clothes Dryer Without Respirator	15 mg	7.8	0.00026	51,000

A. Amount of AI laundered per day = Amount of AGS-20 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 AI Handled × Unit Exposure

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

The MOEs shown in Table 9 were calculated by dividing the inhalation dose by the POD of  $133 \text{ } \mu\text{g}/\text{m}^3$ , which is the NOAEL from a 90-day inhalation toxicity study for nanosilver (Sung et al., 2009). This assumes that AGS-20 released from textiles during drying behaves like nanosilver after inhalation. This analysis further assumes that all of the nanosilver from the AGS-20 treated textile becomes airborne during a single laundry drying event and that exposure would be similar to mixing and loading of wettable powders (U.S. EPA, 2011a). The MOE of 51,000 indicates that the risk for short- and intermediate-term exposure to laundering one AGS-20 treated textile per day is not of concern. Up to 510 t-shirts treated with AGS-20 could be laundered per day

without exceeding the target MOE of 1,000 for risk from short- or intermediate-term exposure to AGS-20.

#### Consumer Incidental Oral Daily-Dose and MOEs

Incidental oral exposures were calculated using the following:

**Incidental Oral Exposure** = Pesticide Content × Cloth Density × Surface Area Mouthed × Saliva Extraction Efficiency

Where:

- The textile contains 100 ppm silver when AGS-20 is incorporated during fiber production and 20 ppm silver when AGS-20 is applied as a coating, as proposed in the AGS-20 application.
- The cloth density is 10 mg/cm<sup>2</sup> 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<sup>2</sup> (~16 in.<sup>2</sup>) 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 Geranio et al. (2009) leaching study.

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 toddler (3 years old) is 15 kg which is a standard assumption from the Exposure Factors Handbook (U.S. EPA, 1997).

The MOEs for incidental oral exposures were calculated from the incidental oral dose using the NOAEL of 0.5 mg/kg/day from Park et al. (2010) as the POD. The MOEs for incidental oral exposures are listed in Table 10 and range from 1,100 to 5,700 depending upon the application rate and saliva extraction factor used. All of these MOEs are greater than the target MOE of 1,000 indicating that the risk for short- and intermediate-term exposure to toddlers who chew on AGS-20 treated textiles is not of concern.

**Table 10 – Incidental Oral MOEs for Toddlers Exposed to AGS-20 Treated Textiles**

Application Rate (mg/kg) Treatment Type	Cloth Density (mg/cm <sup>2</sup> )	Surface Area Mouthed (cm <sup>2</sup> /day)	Saliva Extraction Efficiency (See Table 8)	Exposure <sup>A</sup> (mg/day)	Daily Dose <sup>B</sup> (mg/kg/day)	MOE <sup>C</sup>
20 Surface Coated	10	100	15% (Dissolution)	0.0030	0.00020	2,500
			35% (1 <sup>st</sup> Washing)	0.007	0.00047	1,100
100 Incorporated in Fibers	10	100	1.5% (Dissolution)	0.0015	0.00010	5,000
			1.3% (1 <sup>st</sup> Washing)	0.0013	0.000087	5,700

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

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

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

### Consumer Dermal Daily-Dose and MOEs

The dermal exposure was calculated using the following:

**Dermal Exposure** = Pesticide Content × Cloth Density × Surface Area Exposed × Transfer Efficiency

Where:

- The textile contains 100 ppm silver when AGS-20 is incorporated during fiber production and 20 ppm silver when AGS-20 is applied as a coating.
- The cloth density is 10 mg/cm<sup>2</sup> 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 exposed is 5,700 cm<sup>2</sup>/day, which is the median surface area of clothing contacting the skin of a 3-year-old toddler. This value is a standard assumption used in OPP risk assessments and was derived from the Child Specific Exposure Factors Handbook (U.S. EPA, 2008).
- The cloth-to-skin transfer efficiency was based on the amount of silver released during the Geranio et al. (2009) leaching study.

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 dermal absorption factor is 0.1%
- The body weight of a toddler (3 years old) is 15 kg which is a standard assumption from

the Exposure Factors Handbook (U.S. EPA, 1997).

The MOEs for dermal exposures were calculated from the dermal dose using the NOAEL of 0.5 mg/kg/day from Park et al. (2010) as the POD. These MOEs are listed in Table 11 and range from 8,800 to 45,000 depending upon the application rate and cloth-to-skin transfer efficiency used. These dermal MOEs exceed the target MOE of 1,000 indicating that the risk for short- and intermediate-term exposure to toddlers from wearing AGS-20 treated textiles is not of concern. Although these MOEs were calculated for children, they are protective of adults wearing and working with clothing treated with AGS-20, since ratio of skin surface area to the body weight is greater for children than for adults.

**Table 11 – Dermal MOEs for Toddlers Exposed to AGS-20 Treated Textiles**

Application Rate (mg/kg) Treatment Type	Cloth Density (mg/cm <sup>2</sup> )	Surface Area Exposed (cm <sup>2</sup> /day)	Cloth-to-Skin Transfer Efficiency (See Table 8)	Exposure <sup>A</sup> (mg/day)	Daily Dose <sup>B</sup> (mg/kg/day)	MOE <sup>C</sup>
20 Surface Coated	10	5,700	15% (Dissolution)	0.17	0.000011	45,000
			35% (1 <sup>st</sup> Washing)	0.40	0.000027	18,500
100 Incorporated in Fibers	10	5,700	1.5% (Dissolution)	0.085	0.000057	8,800
			1.3% (1 <sup>st</sup> Washing)	0.074	0.000049	10,000

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

B. Dose = [Exposure (mg/day) × Dermal Absorption Factor (0.1%)] / Body Weight (kg)

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

### Consumer Aggregate Daily Dose and MOEs

The possibility that toddlers could be simultaneously exposed to AGS-20 via the dermal and incidental oral routes of exposure while wearing and chewing on AGS-20 treated clothing comprises the aggregate assessment.

The aggregate dose was calculated by adding the daily oral and dermal doses using the following:

$$\text{Aggregate Daily Dose} = \text{Daily Oral Dose} + \text{Daily Dermal Dose}$$

Where:

- Incidental Daily Oral Dose is from Table 10
- Dermal Daily Dose is from Table 11

The oral and dermal daily doses can be added because they are both evaluated using the NOAEL of 0.5 mg/kg/day from Park et al. (2010) as the POD and the target MOE of 1,000. Aggregate assessments can also include other sources of exposure such as food and drinking water, and other nanosilvers in the market place. There are no anticipated food exposures since AGS-20 is not used in food contact applications. Neither AGS-20 nor the nanosilver that might break away are anticipated to enter drinking water because these particulates will be removed by gravitational sedimentation and adsorption (see Section 5.1.1). Finally, there is insufficient information to aggregate exposures to other nanosilvers currently in the market place because the Agency does not have information on the composition of the other nanosilvers, their distribution in the market place, or use by consumers to determine which exposure scenarios are likely to occur together.

The MOEs for aggregate exposures were calculated from the aggregate daily dose using the NOAEL of 0.5 mg/kg/day from Park et al. (2010) as the POD. These MOEs are listed in Table 12 and range from 1,000 to 3,600 depending upon the application rate, treatment type and cloth to skin transfer efficiency. All of these MOEs are greater than the target MOE of 1,000 indicating that the risk for short- and intermediate-term exposure to toddlers from wearing AGS-20 treated textiles is not of concern.

**Table 12 – Aggregate MOEs for Toddlers Exposed to AGS-20 Treated Textiles**

Application Rate (mg/kg) Treatment Type	Cloth-to-Skin Transfer Efficiency (See Table 3)	Incidental Oral Dose <sup>A</sup> (mg/kg/day)	Dermal Dose <sup>B</sup> (mg/kg/day)	Aggregate Dose <sup>C</sup> (mg/kg/day)	Aggregate MOE <sup>D</sup>
20 Surface Coated	15% (Dissolution)	0.00020	0.000011	0.00021	2,400
	35% (1 <sup>st</sup> Washing)	0.00047	0.000027	0.00050	1,000
100 Incorporated in Fibers	1.5% (Dissolution)	0.00010	0.000057	0.00016	3,100
	1.3% (1 <sup>st</sup> Washing)	0.000087	0.000049	0.00014	3,600

A. Incidental Oral Dose is from Table 10

B. Dermal Dose is from Table 11

C. Aggregate Dose = Incidental Oral Dose + Dermal Dose

D. MOE = POD/Dose where the POD is the NOAEL of 0.5 mg/kg/day from Park et al. (2010)

#### 4.6.5 Conclusions for Risk to Consumers

The MOE for inhalation exposure to nanosilver during laundry drying of a textile treated with AGS-20 was estimated to be 51,000, which is not of concern because it exceeds the target MOE of 1,000. The MOEs for dermal exposures ranged from 8,800 to 45,000 and the MOEs for incidental oral exposure ranged from 1,100 to 5,700, all of these MOEs are greater than the target MOE of 1,000 indicating that the risk for short- and intermediate-term exposure to toddlers who wear and chew on AGS-20 treated textiles is not of concern. Aggregating the

dermal and oral daily doses for children yielded MOEs ranging from 1,000 to 3,600, which is not of concern because they exceed the target MOE of 1,000.

The lowest MOEs, including the 1,100 value for oral exposure and the 1,000 for aggregate exposure, were based on the amount of silver released after the initial washing of AGS-20 treated textiles in the ISO color-fastness test. EPA anticipates that lesser amounts of silver would be released during subsequent washings as other similarly silver-treated textiles showed less silver release during subsequent washings (Geranio et al., 2009). While the ISO color-fastness test does not exactly simulate wearing or chewing on textiles, it does provide a reasonable estimate for the amount silver transferred to the mouth while chewing on and transferred to skin while wearing textiles treated with AGS-20. In using this study for the exposure assessment, EPA made the conservative assumption that all silver found in this study was in the form of nanosilver whereas the study showed the release of coarse silver-containing particles of unknown composition. Therefore, using this study to estimate the amount of daily nanosilver release may have overestimated the potential for incidental and oral exposures to nanosilver by toddlers and children.

The estimate for the inhalation dose during drying of AGS-20 treated textiles was based on a unit exposure value that was derived from pouring kg quantities of wettable powders into mix tanks and does not represent conditions likely to be encountered during drying of AGS-20 treated textiles. EPA anticipates that exposure to AGS-20 during drying of AGS-20 treated textiles is likely to be less than a comparable exposure to AGS-20 powder. This is because AGS-20 in treated textiles is likely to be associated with textile fibers and these fibers would be vented or caught in the lint trap during clothes drying thereby reducing the airborne concentration of AGS-20 in comparison to AGS-20 powder.

There are several uncertainties regarding the consumer risk assessment. The POD used was based on an oral toxicity study completed using nanosilver, not AGS-20 or nanosilver that might break away from AGS-20, and thus the POD may not represent AGS-20 or the nanosilver found in AGS-20. The oral toxicity study did not establish toxicity of nanosilver over all life-stages or evaluate all potential effects, for this reason the Agency used a maximum 10-fold database uncertainty factor when calculating the target MOE, resulting in a target MOE of 1,000. 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) for purposes of using the oral NOAEL to calculate a POD for dermal toxicity.

In the absence of information to the contrary, EPA typically does not consider long-term consumer exposures for textiles treated with an antimicrobial preservative because 1) the portion of the antimicrobial that is not bound to the textile is available for transfer and is expected to be depleted or washed out of the textile in less than 6 months (the remaining antimicrobial is bound to the textile and not available for transfer) and 2) because there is a low probability that an individual will be exposed to the same antimicrobial on a continuous, daily basis for 6 months (the scenario classified as long-term). However, EPA has received comments from the public regarding long-term exposure to AGS-20 treated textiles. There are limited use scenarios where the potential for long-term exposure to AGS-20 treated textiles could be expected. For example,

consumers could seek out textiles treated with AGS-20 because of its unique properties. Another example would be use of AGS-20 treated textiles by military personnel where there is a probability that they would be exposed to AGS-20 on a daily basis for more than 6 months. In the case of the surface applied treatment, given that 35% of the silver leached out after the color-fast test, EPA anticipates that the silver in this product will be depleted or washed out of the textile in less than 6 months (the remaining antimicrobial is bound to the textile and not available for transfer). In the case of AGS-20 incorporated into fibers, the fact that only 1.5% of the silver was removed during the color-fast test indicates that the silver in these textiles could be expected to remain in the textile for greater than 6 months. Regardless, the risk from long-term inhalation, incidental oral, and dermal exposure to AGS-20 treated textiles would not be of concern as the MOEs are greater than the target MOE of 3,000 (Table 4) for textiles treated with AGS-20 (Tables 9, 10, 11, and 12).

Although the available data on nanosilver are limited, EPA is able to determine that for the period of conditional registration, there is a low probability of adverse risk to children and the environment from textiles treated with AGS-20. Thus, the Agency concludes that use of AGS-20 will not cause unreasonable adverse effects on the environment during the period when newly required data are being developed.

#### **4.6.6 Consumer Exposure and Health Data Requirements**

EPA is requiring that HeiQ conduct textile leaching and laundry drying tests as part of Tier I studies to determine the nature and quantity of silver released from AGS-20 treated textiles under conditions of use. EPA will use these results to confirm the consumer risk assessment for the conditional registration of AGS-20. The Tier I health effects testing categories include:

- Route-specific subchronic inhalation study in rats with *in vivo* bone marrow assay and functional observational battery, motor activity and detailed neuropathology
- Route-specific dermal toxicity study in rats
- Reproduction and developmental toxicity screening tests in rats
- An *in-vitro* micronucleus assay for mutations in genetic material

The results of these studies will be used to confirm the findings of the risk assessment performed using scientific literature data for nanosilver. A more detailed description of these data requirements is provided in Appendix A.

If nanosilver is determined to be released during the textile leaching or drying tests as part of Tier I testing then the Agency will require route-specific subchronic tests as part of Tier II studies to confirm the nanosilver risk assessment. The Tier II health effects testing categories include:

- Route-specific subchronic oral study in rats with *in vivo* bone marrow assay and functional observational battery, motor activity and detailed neuropathology
- Route-specific dermal toxicity study in rats
- Reproduction and developmental toxicity screening tests in rats
- An *in-vitro* micronucleus assay for mutations in genetic material

## **V. ENVIRONMENTAL RISK ASSESSMENT**

Based on the proposed use pattern of AGS-20, EPA anticipates the following substances could enter the environment through washing or disposal processes:

1. Silver ions released from AGS-20 treated textiles;
2. AGS-20 particles; and
3. Nanosilver that might break away from AGS-20 particles.

There are no studies available to characterize the environmental fate or ecotoxicity of AGS-20. However, there are studies available in the scientific literature for analogous forms of nanosilver. Since nanosilver may be released from AGS-20, the Agency has considered the scientific literature studies on nanosilver fate and ecotoxicity relevant to AGS-20.

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 requiring in order to confirm its assessment of the environmental risks of AGS-20 are also identified.

### **5.1 Environmental Fate**

HeiQ has not conducted any studies to characterize the environmental fate of AGS-20 or the other particles that could be released during machine washing or disposal of AGS-20 treated textiles. 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.

#### **5.1.1 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.

### **5.1.2 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.

### **5.1.3 Impacts to Wastewater Treatment/Septic Systems**

There is the potential for nanosilver that might be released from AGS-20 treated textiles to reach publically 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. To evaluate this potential, HeiQ submitted test results on the impact of AGS-20 to wastewater treatment bacteria (MRID 479344-01). This test involved introducing AGS-20 powder and an AGS-20 liquid formulation used to surface treat textiles (AGS-20 TF) into bottles containing biosolids obtained from a wastewater treatment plant. Based on the similarity between the biological oxygen demand (BOD) caused by introducing glucose into bottles with AGS-20 and those without, there was no impact to the microorganisms in these bottles for AGS-20 loadings from 3.8 to 188 mg/L (0.76 to 37.6 mg/L as silver) or the AGS-20 TF liquid at loadings from 1 to 50 g/L over the 26 day test period. Thus, AGS-20 is not expected to adversely impact wastewater treatment or septic systems. However, this study was not designed to assess the impacts to wastewater treatment from nanosilver that might be released during the washing of textiles treated with AGS-20.

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.

While there are reports suggesting the potential for nanosilver to impact wastewater treatment operations, the Agency does not anticipate that registering AGS-20 will lead to negative impacts to wastewater treatment systems. This conclusion is based on the small volume of nanosilver (i.e., < 4,500 kg/yr as estimated in Section 5.3) expected to be introduced into commerce from AGS-20 treated textiles. However, if nanosilver is found to be released from AGS-20 treated textiles during leaching studies then EPA will require HeiQ to determine the impact to wastewater treatment processes.

## **5.2 Environmental Toxicity**

HeiQ has not conducted any studies to characterize the ecotoxicity of AGS-20 or the other particles that could be released during machine washing or disposal of AGS-20 treated textiles.

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.

### 5.2.1 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). Silver and compounds, excluding nanosilver, are currently being re-evaluated through the Agency's Registration Review program.

### 5.2.2 Nanosilver

In contrast, the ecotoxicity information available to assess the potential hazards of nanosilver to aquatic life is limited. HeiQ submitted a study on the antimicrobial activity of AGS-20 as compared to silver nitrate, which readily dissociates to form silver ions in solution. Compared to silver ions, AGS-20 was 10 times less effective at inhibiting the growth of a wide variety of gram-negative and -positive microorganisms (Egger et al., 2009). Thus, AGS-20 appears to be less toxic to microorganisms than silver nitrate. Although no tests with AGS-20 and aquatic organisms were submitted, there are three studies in the scientific literature covering the toxicity of nanosilver to aquatic organisms (Table 13). The first study involved determining the concentration of silver nitrate and carbonate-coated nanosilver with average diameter of 40 nm (minimum diameter of 10 nm and maximum of 200 nm) that affected the photosynthetic activity of the freshwater algae *Chlamydomonas reinhardtii* (Navarro et al., 2008). Although nanosilver had lower toxicity than silver nitrate on a silver mass basis, nanosilver had greater toxicity on a silver ion basis suggesting that the presence of nanosilver enhanced the toxicity relative to silver nitrate. Through further experimentation the authors concluded that the observed effect of nanosilver on photosynthetic activity of algae was due to silver ions originating from nanosilver during the exposure period. The second study involved determining the acute toxicity of silver nitrate and eight different nanosilver formulations on the freshwater flea *Daphnia magna* and the larval-stage freshwater fish *Pimephales promelas* (Kennedy et al. 2010). Like the first study, nanosilver was less acutely toxic than silver nitrate on a total silver content basis. However, after accounting for the fraction of nanosilver suspended in solution, nanosilver toxicity was similar to that of silver nitrate (i.e., silver ions). For example, the nanosilver obtained from the ASAP formulation had an average diameter of 228 nm (minimum of 171 nm and maximum of 310 nm) in moderately hard reconstituted water (MHRW); however, after fractionation, the particles with average diameter of 1.5 nm (minimum of 1.3 nm and maximum of 1.7 nm) were more predictive of acute toxicity. The third study also involved the freshwater fish *Pimephales promelas*, however, in the embryonic rather than the larval stage (Laban et al., 2010). Compared to silver

nitrate, nanosilver were significantly less toxic on a total silver basis but similar in toxicity on a silver ion basis.

**Table 13 – Nanosilver Toxicity to Aquatic Species**

Aquatic Organisms	Toxicity	Silver Nitrate	Nanosilver		
		Total Silver (µg/L)	Total Silver (µg/L)	Dissolved Silver (µg/L)	Water Characteristics
Freshwater Algae (Navarro et al., 2008)	1-hr EC <sub>50</sub> <sup>A</sup>	20±7	356±62	3.6±0.5	10 mM morpholine propanesulfonic acid (MOPS), buffered at pH 7.52
	2-hr EC <sub>50</sub>	20±9	113±43	1.1±0.4	
Freshwater Flea <i>D. magna</i> (Kennedy et al., 2010)	96-hr LC <sub>50</sub> <sup>B</sup>	1.2±0.5	1.8 to 97.0 <sup>C</sup>	0.3 to 1.9 <sup>C,D</sup>	Moderately hard reconstituted water (MHRW), 80 mg/L as calcium carbonate
Freshwater Fish Larval <i>Pimephales promelas</i> (Kennedy et al., 2010)	96-hr LC <sub>50</sub>	6.1±0.6	9.0 to 125.6 <sup>C</sup>	1.5 to 5.6 <sup>C,D</sup>	
Freshwater Fish Embryo <i>Pimephales promelas</i> (Laban et al., 2010)	96-hr LC <sub>50</sub>	15	9,400 to 10,600	40	215 to 240 mg/L as calcium carbonate; pH 8.3 to 8.5

A. Concentration at which the response is halfway between the baseline and maximum after some specified exposure time.

B. Concentration required to kill half the members of a tested population at 96 hrs.

C. Range for eight different silver nanoparticle formulations.

D. Expressed as the fraction of suspended nanosilver.

These studies indicate to the Agency that, if sufficient quantities of nanosilver break away from AGS-20 and reach surface water, and if such nanoparticles display toxicity similar to the nanosilver used in these studies, then exposure of AGS-20 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<sup>2+</sup>) present in the test media. For example, the LC<sub>50</sub> reported by Laban et al. (2010) was up to 27 times greater than the LC<sub>50</sub> 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).

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 AGS-20 treated textiles, the data represent short-term (e.g., 96 hr) acute exposures and do not characterize the effects from longer-term exposure to nanosilver. Given this limitation, as a condition of this registration, the Agency is requiring additional testing on the long-term or chronic ecotoxicity effects of AGS-20 and/or nanosilver found to be released from AGS-20 during leaching studies.

## 5.3 Aquatic Risk Assessment

EPA expects that AGS-20, silver ions released from AGS-20, and nanosilver that might break away from AGS-20 could enter the environment through the following scenarios:

1. Accidental release during transport
2. Laundering AGS-20 treated textiles
3. Application of wastewater treatment biosolids to agricultural fields
4. After disposing of textiles in landfills or during incineration

Laundering AGS-20 treated textiles is anticipated to be the primary route by which AGS-20, silver ions, and nanosilver reach the environment. The silver released during washing of AGS-20 treated textiles will 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 AGS-20, silver ions, or nanosilver reach the 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.

As stated in Section 4.6.3, there is no adequate study available to determine the form of silver released from AGS-20 treated textiles during clothes washing. However, 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 laundering AGS-20 treated textiles, EPA is assuming that nanosilver is the only silver compound released.

### 5.3.1 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<sub>50</sub>. 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, 2011b).

The concentration of nanosilver in surface water resulting from the use of AGS-20 in textiles 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:

- Mass of silver release per year: 4,500 kg/year. The value was derived assuming that 300 million people (U.S. population) purchase one t-shirt treated with HeiQ AGS-20 each year. Each t-shirt weighs 150 grams and contains 100 ppm by weight silver.
- Release Days: 365 days per year. This assumes that each t-shirt treated with HeiQ AGS-20 releases all its silver as nanosilver over the course of one year.

- Wastewater Removal Efficiency: Ranged from 85 to 99%. These values are based on the range provided by Blaser et al. (2008).
- Stream Dilution Factor: 1.0 or 20.1. These values are the 10<sup>th</sup> and 50<sup>th</sup> percentile values for the dilution that occurs during one day of lowest stream flow over a ten year period (1Q10).
- The toxicity value for nanosilver: 1 µg/L based on the LC<sub>50</sub> values 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.

The down-the-drain modeling results are shown in Table 14 and these results were divided by the LC<sub>50</sub> of 1.0 µg/L for *Daphnia magna* to obtain risk quotients (RQs). The RQ of 0.016 at the worst case stream dilution factor of 1.0 and lowest wastewater removal efficiency indicates that it is unlikely that the registration of AGS-20 as a textile preservative will lead to adverse effects for listed or non-listed aquatic organisms.

**Table 14 – Risk Quotients for Nanosilver in Surface Water**

Wastewater Treatment Removal <sup>A</sup>	Stream Dilution Factor	Surface Water Silver Concentration (µg/liter)	Risk Quotient <sup>D</sup>	RQ Exceeds LOC?*
85%	1.0 <sup>B</sup>	0.016	0.016	No
	20.1 <sup>C</sup>	0.0007	0.0007	No
99%	1.0 <sup>B</sup>	0.0011	0.0011	No
	20.1 <sup>C</sup>	0.000045	0.00005	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. 10<sup>th</sup> Percentile dilution factor for 1Q10 stream flow.  
C. 50<sup>th</sup> Percentile dilution factor for 1Q10 stream flow.  
D. RQ = Surface Water Concentration / LC<sub>50</sub> for *Daphnia magna* (1.0 µg/liter)

### 5.3.2 Conclusions for Aquatic Risk

The RQs for *Daphnia magna* exposed to nanosilver ranged from 0.00005 to 0.016, which are not of concern for listed or non-listed organisms because they were less than the LOC of 0.5 and 0.05, respectively. The effect level used to calculate the RQs (i.e., 1.0 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 level has an inherent safety factor ranging from 6 to 50 times because researchers found that only a fraction of the nanosilver added to cultures produced the toxic effect (Table 13). This analysis assumes that every person in the U.S. would purchase a t-shirt treated with AGS-20 resulting in a nanosilver production-volume of 4,500 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. It was also assumed that all the silver contained in these t-shirts would be released as nanosilver even though Geranio et al. (2009) found that silver-containing particles with diameters greater than 450 nm will likely be released during mechanical washing of AGS-20 treated textiles.

There are several uncertainties regarding the exposure portion of this evaluation. The production volume of AGS-20 is unknown since this product is not currently available in the U.S. Because of this, EPA made the conservative assumption that every person in the U.S. would purchase one AGS-20 treated t-shirt. The nanosilver that could be released from AGS-20 treated textiles were assumed to enter the environment through the sanitary sewer system, no evaluation of nanosilver in landfills or other routes were evaluated. The rate at which nanosilver entered the sanitary sewer systems was assumed to be constant in the down-the-drain model at  $4.23 \times 10^{-5}$  g/person/day, which does not account for variability in clothes washing patterns among other factors. The ecotoxicity values shown in Table 13 are for acute effects and mortality, and do not evaluate the long-term exposure to nanosilver. Finally, this assessment only considers silver that could be released by AGS-20 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 textiles treated with AGS-20.

### **5.3.3 Ecological Effects and Environmental Fate Data Requirements**

Even though EPA does not anticipate adverse ecological effects from AGS-20 treated textiles 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 AGS-20 powder and AGS-20 treated textiles. The Tier I environmental fate testing categories include:

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

In addition, EPA requires acute avian, fish, and aquatic invertebrate data for labeling packages which contain AGS-20 for shipment.

Completion of these Tier I studies will provide AGS-20 product characteristics and determine the substance or substances released during laundering of AGS-20 treated textiles. Information from these studies will be used to substantiate HeiQ's claim that no nanosilver is released from AGS-20 treated textiles during laundering. However, if nanosilver is found to be released during Tier I studies, 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 AGS-20. 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
- Chronic Effects to Sediment Dwelling Organisms
- Terrestrial Plant Toxicity

## VI. 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 had 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 a conditional registration with the terms and conditions identified in Appendix A is particularly appropriate given EPA suspects that some already-registered like-situated products on the market contain nanosilver as the active ingredient. However, 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, EPA intends to seek similar data along with comparable terms on already registered products that are identified to contain nanosilver.

### 6.1 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. AGS-20 contains an active ingredient, silver nanoparticles also known as nanosilver. The AGS-20 silver nanoparticles are not active ingredients in any currently registered pesticide (i.e., a “new” active ingredient);
2. Use of AGS-20 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 HeiQ to generate and submit the newly required data; and
4. Use of AGS-20 is in the public interest.

## 6.2 Findings and Registration Decision

The Agency is issuing a conditional, four year registration for HeiQ AGS-20 in accordance with FIFRA section 3(c)(7)(C) and requiring as a condition of registration specified use conditions as well as requiring 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 A. Although these data requirements are specific to HeiQ AGS-20, 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 HeiQ's original and amended applications, label language is required to reduce potential exposures. The pesticide label will include the following:

1. The application rates must be less than 100 ppm (mg/kg) or 0.01% silver on a weight basis for textile fibers which incorporate AGS-20 and less than 20 ppm (mg/kg) or 0.002% for textiles surface coated with AGS-20.
2. AGS-20 powder shall be applied in facilities equipped with engineering controls such as closed system loading or local exhaust ventilation systems that provide, at the least, a 10 fold reduction in the concentration of airborne AGS-20 as compared to the AGS-20 concentration generated without engineering controls. AGS-20 powder cannot be applied using open pouring methods.
3. The following PPE must be worn when handling AGS-20 powder.
  - Gloves that are chemically resistant to all of the components of the textile fiber master batch or coating formulations to which the AGS-20 powder is added.
  - A long-sleeve shirt, long pants, shoes plus socks, and overalls or a Tyvek® suit that cover the arms, legs and torso.
  - NIOSH certified full-face respirators with P100 or equivalent filter cartridges.

## 6.3 Basis for Conditional Registration

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

### 6.3.1 Data Generation

HeiQ submitted its registration application thinking its product was similar to currently registered silver-based antimicrobial pesticide products and provided all of the required product-specific data for this type of application. EPA typically does not require generation of any additional generic data to support such an application; applicants fulfill the applicable generic data requirements by citing previously submitted data. Over a year after HeiQ submitted its application the Agency determined that the nanosilver particles in AGS-20 are a new active ingredient. Throughout the application review process, EPA and HeiQ have discussed what data might be necessary to register a new active, nanoscale ingredient. Although HeiQ provided EPA with some data in an effort to address Agency questions and concerns, until today, EPA had not reached a final decision with regard to which types of data would be further required. 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 HeiQ of the data requirements needed to assess HeiQ's application for HeiQ to have generated the data.

EPA conducted an assessment of risks to human health and the environment associated with the use of AGS-20 as a materials preservative. However, the majority of the studies used for the risk assessment were not conducted with AGS-20 or the nanosilver that might break away from AGS-20; rather the studies were conducted using other silver nanomaterials. As indicated in the 2009 SAP report, nanomaterials such as AGS-20 can be physically and chemically different from single molecules or bulk materials or even nanosized versions of the same substance, and these differences may impart properties that ultimately affect the potential risks of the nanoscale material. Therefore, EPA determined that more extensive product chemistry, toxicology, exposure, and environmental data are necessary to confirm EPA's assessment of the risks from the proposed use of AGS-20. Because this list of data requirements are only being finalized with today's action, HeiQ has not yet been able to conduct these studies. Therefore, the Agency is requiring 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.

As discussed above, a listing of the studies that are needed for this section 3(c)(7)(C) registration of HeiQ AGS-20 are in Appendix A. If HeiQ does not meet the conditions set forth in this Decision Document, EPA will issue a notice of intent to cancel HeiQ's registration under section 6(e).

### **6.3.2 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. HeiQ provided information to the Agency regarding the economic benefits offered by its technology when compared to other options. EPA agrees that this information on conservation of the environment and consumer benefits contributes 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 antimicrobial products for use as materials preservatives. All antimicrobial 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 treated with 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 antimicrobial silver-based pesticide products currently contain a silver salt, [e.g., AgCl or AgNO<sub>3</sub>]. Compared to the amount of silver in HeiQ's product, most currently registered silver-based materials preservatives allow larger amounts of silver to be added to an article in order to provide a sufficient lifetime of activity for antimicrobial treatment. Therefore,

the overall potential environmental loading of silver resulting from the lower-volume use of the HeiQ product should be smaller than from a comparable use of currently registered silver-based pesticides.

For example, Geranio et al. (2009) reported that commercially available textiles treated with electrolytically deposited silver contained 21.6 mg/g silver as compared to textiles treated with AGS-20, which contained a maximum of 0.099 mg/g silver. EPA has reviewed the registered silver containing pesticides and found that for commercially available products, the amount of silver allowed in textiles ranges from 0.03 to 40 mg/g. By conditionally registering HeiQ, EPA is allowing a product in the market place where the amount of silver in AGS-20 is restricted to less than 0.1 mg/g or 0.01% as silver, which is on the low end of the spectrum for registered silver containing products. If AGS-20 displaces the silver-salt based materials preservatives currently on the market for comparable textile uses, which have significantly higher silver content, there exists the potential for a decrease in the net amount of silver entering the environment.

### Consumer Benefits

A nanosilver materials preservative should maintain its ability to reduce the number of odor causing bacteria longer than other silver active ingredients due to an expected gradual and controlled release of silver ions from the nanosilver as opposed to the rapid release of for example, silver ions from a zeolite structure or the immediate dissolution of the silver salt. While it may be that other silver active ingredients achieve greater silver concentrations, it is expected that their effects on odor causing bacteria are only short-lived due to rapid release of silver ions. In contrast, the Agency believes that AGS-20 will allow slow and controlled release of silver ions, potentially resulting in more prolonged antimicrobial activity (U.S. EPA, 2009). Data provided to the Agency by HeiQ supports this theory and will be confirmed by the required leaching study. Thus, consumers purchasing textiles treated with nanosilver may receive a more durable antimicrobial protection for AGS-20 treated textiles as compared to the alternatives even though there is less total silver in the AGS-20 treated textile, and such protection is believed to have equal ability to reduce the number of odor causing bacteria as 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 HeiQ application, EPA's conditional registration of HeiQ's AGS-20 product is in the public interest in that it will allow an innovative product to reach the market.

### **6.3.3 No Unreasonable Adverse Effects**

In assessing the potential risks to human health and the environment associated with the distribution and use of AGS-20 as a materials preservative, EPA relied on limited data submitted

by HeiQ 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 AGS-20 during the period of the conditional registration are small. Moreover, EPA expects the overall quantity of silver used in 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 following findings:

### Risks to Human Health

As discussed above, humans could be exposed to silver ions, to AGS-20, 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. These silver-salt preservatives are present in textiles at up to 3% silver by weight. Because the maximum amount of AGS-20 that can be used in textiles is limited to 0.01% silver by weight and because AGS-20 is meant to displace the silver-salt based materials preservatives, the Agency concludes that human exposure to silver ions from the use of AGS-20 and textiles treated with AGS-20 is acceptable.

With respect to AGS-20, the acute toxicity of AGS-20 is low by all routes of exposure. Because there are no subchronic or chronic oral or dermal toxicity studies available for AGS-20 or on the nanosilver that might break away from textiles treated with AGS-20, the subchronic or chronic toxicities were estimated using studies on analogous nanosilvers reported in the literature. The Agency relied on the results from a study in the scientific literature involving the washing of AGS-20 treated textiles to estimate exposure to AGS-20 and nanosilver that might break away from 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 textiles treated with AGS-20.

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 textiles treated with AGS-20. Thus, the Agency concludes that use of AGS-20 will not cause unreasonable adverse effects on the environment during the period when newly required data are being developed. The Agency does have a risk concern for occupational inhalation exposures because the calculated MOEs were less than the respective target MOEs. However, HeiQ amended its application to require that workers use personal protective equipment and engineering controls when handling AGS-20 powder which effectively mitigated EPA's risk concern.

### Risks to the Environment

The environment could be exposed to silver ions, to AGS-20, 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. These silver-salt preservatives are present in textiles at up to 3% silver by weight. Because the maximum amount of AGS-20 that can be used in textiles is limited to 0.01% silver by weight and because AGS-20 is meant to displace the silver-salt based materials preservatives, the Agency concludes that environmental exposure to silver ions from the use of AGS-20 and textiles treated with AGS-20 is acceptable.

With respect to the fate and ecotoxicity of AGS-20, because there are no fate or ecotoxicity studies available for AGS-20 or the nanosilver that might break away from textiles treated with AGS-20, the fate and ecotoxicity were estimated using studies on analogous nanosilvers reported in the scientific literature. Laundering AGS-20 treated textiles was anticipated to be the primary route by which AGS-20, silver ions, and nanosilver reach the environment. Because there was no adequate study available to determine the form of silver released from AGS-20 treated textiles during clothes washing, the Agency assessed impacts to surface waters assuming that nanosilver was the only compound released. Environmental exposure was assessed assuming that 300 million people (U.S. population) each purchased one t-shirt treated with AGS-20 at 0.1 mg/g silver and that all the silver in those t-shirts was released during a year as nanosilver. The Agency incorporated conservative assumptions when calculating Risk Quotients (RQs) for estimating risk to aquatic species from textiles treated with AGS-20. The RQs for *Daphnia magna* exposed to nanosilver ranged from 0.00005 to 0.016, which are not of concern for listed or non-listed organisms because they were less than the LOC of 0.5 and 0.05, respectively. EPA estimates, derived from down-the-drain modeling, of the concentrations of nanosilver resulting from the use of HeiQ AGS-20, 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 AGS-20 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 –  
Data Requirements, Enforceable Schedule, and  
Conditions of Registration for HeiQ AGS-20**

**December 1, 2011**

## **I. Introduction**

AGS-20 is a silver-silica composite that contains nanosilver sintered onto amorphous silicon dioxide (SiO<sub>2</sub>). The SiO<sub>2</sub> fine structure consists of aggregate matrix particles with an average diameter of approximately 1,000 nm or 1 micron. Each silica particle contains many small silver metal particles with a typical diameter between 1 and 10 nm (Egger et al., 2009) and some particles in the 50 nm range. Reference to the nanosilver in AGS-20 (whether when part of the composite or when broken away from the composite) is to the “new active ingredient” and reference to “AGS-20” or the AGS-20 “composite” is to the end use product.

## **II. Exposure Pathways**

The human and environmental exposures resulting from the use of AGS-20, and use and disposal of textiles treated with AGS-20 will largely be a function of what materials are available for inhalation or dermal exposure during treatment of textiles or what materials leach or might break away from the treated textile during use and disposal. EPA anticipates that humans and the environmental will potentially be exposed to the following materials:

1. AGS-20 particles;
2. Silver nanoparticles that might break away from AGS-20 particles; and
3. Silver ions released from AGS-20 particles.

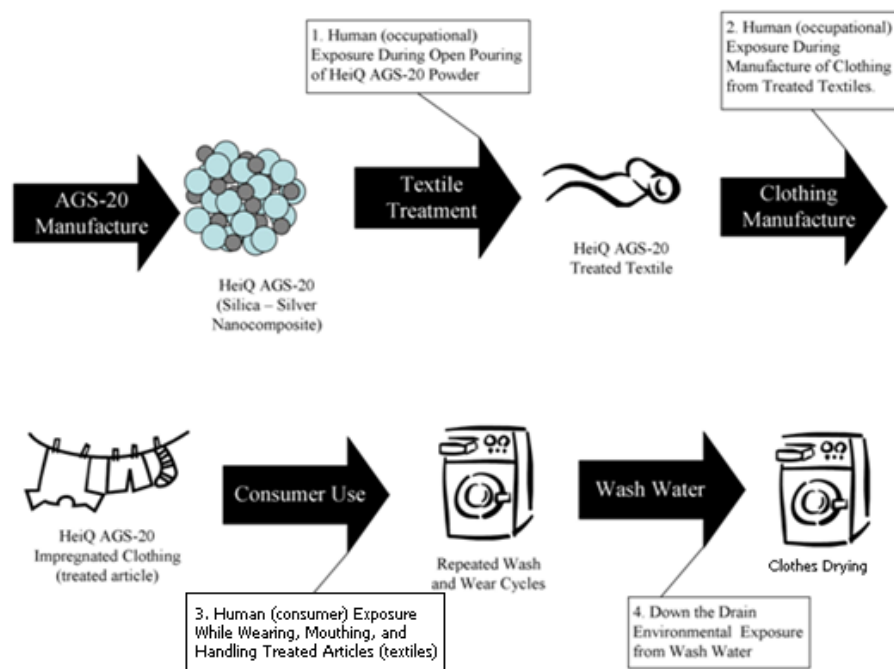
EPA expects occupational inhalation and dermal exposures to AGS-20 are likely to occur during the following use scenarios:

1. Mix, load, and apply AGS-20 powder during the treatment of textiles
2. Preparing textile articles from AGS-20 treated textiles
3. Laundering AGS-20 treated textiles

Based on the AGS-20 product use patterns as shown in Figure 1A, the following consumer exposure scenarios may be possible for AGS-20 treated textiles:

1. Inhalation exposure during laundry drying of AGS-20 treated fabrics;
2. Incidental oral exposure to AGS-20 treated fabrics; and
3. Dermal exposure while wearing AGS-20 treated fabrics.

Exposures to the environment can also occur if the particles are released into wash water (down-the-drain).



**Figure 1A – Product Use Analysis for HeiQ AGS-20**

### III. Data Needed to Confirm the Estimates for Risks of Exposure to AGS-20

As a condition of registration, EPA is requiring that HeiQ 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 conditional registration and discussed in the decision document. These tests include route-specific toxicity studies for occupational, residential, and environmental exposure scenarios. Data must be submitted within four years and according to the schedule provided in Table 3A 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 HeiQ 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 HeiQ’s registration under FIFRA section 6(e). Upon review of the data submitted, if EPA determines there is a risk of concern notwithstanding the terms and conditions on this conditional registration, HeiQ will need to propose risk mitigation measures to avoid potential cancellation of this registration under FIFRA section 6(b).

The following factors were considered in developing the data requirements for AGS-20:

- The nanosilver in AGS-20 is a new active ingredient and is therefore 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.

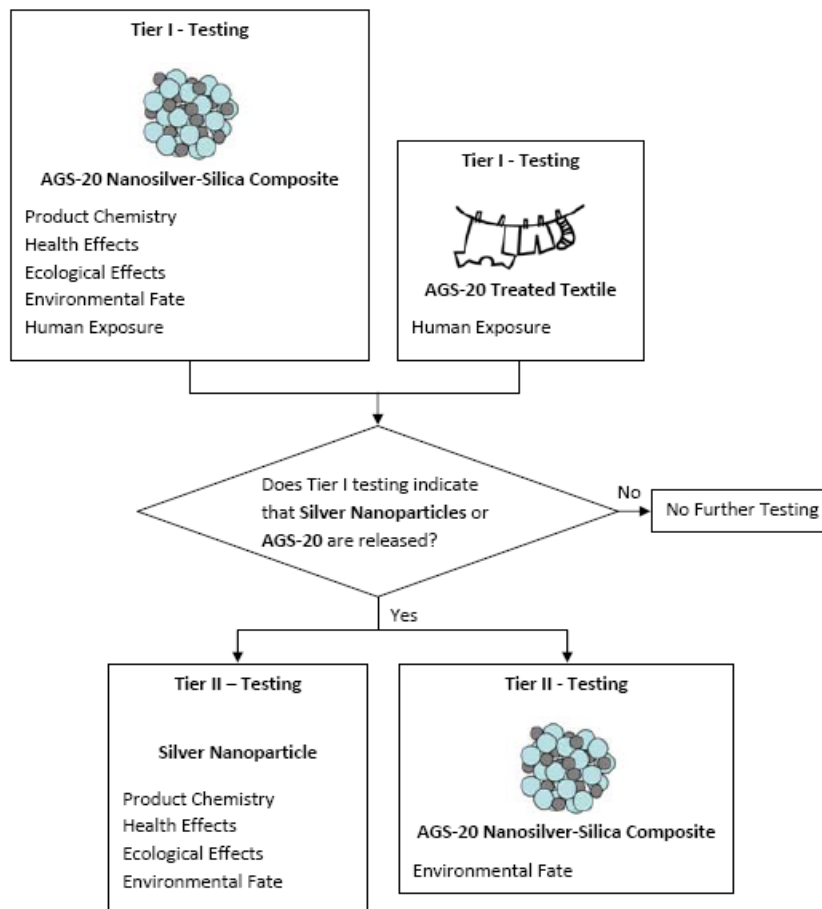
- Although some studies, such as those dealing with physical and chemical characteristics, are required for all use patterns, many of the data requirements are conditional based on the use pattern. Information provided by HeiQ and information from the literature was used to tailor the data requirements 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 AGS-20 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 conditional registration of AGS-20:

- Both nano-sized particles of silver (Ag) as well as ionic silver ( $\text{Ag}^+$ ) 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 AGS-20 is included in Tables 1A and 2A. The studies included in Table 1A are considered to be Tier I, meaning that their need is not based on the results of other studies. The studies listed in Table 2A are considered to be Tier II, because they may or may not be required depending upon the results of the Tier I studies. The AGS-20 composite and textiles treated with AGS-20 will be the test materials 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 2A contains a conceptual diagram outlining the Tier I and Tier II testing approach.



**Figure 2A - Test material and tiered approach for AGS-20. Only the testing category is shown; see Tables 1A and 2A for the specific tests required for each material.**

#### IV. NonData-Related Terms and Conditions of Registration

Consistent with HeiQ’s original and amended applications, HeiQ is required to state on the pesticide label that the application rates must be less than 100 ppm (mg/kg) or 0.01% silver on a weight basis for textile fibers which incorporate AGS-20 and less than 20 ppm (mg/kg) or 0.002% for textiles surface coated with AGS-20.

1. HeiQ is required to state on the pesticide label that workers who handle the AGS-20 powder shall wear the following personal protective equipment (PPE): Gloves that are chemically resistant to all of the components of the textile fiber master batch or coating formulations to which the AGS-20 powder is added.

2. A long-sleeve shirt, long pants, shoes plus socks, and overalls or a Tyvek® suit that cover the arms, legs and torso.

NIOSH certified full-face respirators with P100 or equivalent filter cartridges.

In addition to the PPE listed above, AGS-20 shall be applied in facilities equipped with engineering controls such as closed system loading or local exhaust ventilation systems that provide, at the least, a 10 fold reduction in the concentration of airborne AGS-20 as compared to the AGS-20 concentration generated without engineering controls. AGS-20 cannot be applied using open pouring methods.

## **V. Enforceable Schedule**

HeiQ agrees to take appropriate steps to secure the data listed in Tables 1A and 2A. As such, EPA has prepared an enforceable schedule that is presented in Table 3A. 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 AGS-20 (a difficult-to-test substance), which may cause a delay in testing. If such a case arises, HeiQ 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 HeiQ. In this case, the EPA shall submit a written statement to HeiQ 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 HeiQ 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 HeiQ's registration under FIFRA section 6(e). Specifically, HeiQ shall:

1. Submit protocols modified for AGS-20 to EPA's satisfaction for each of the data requirements listed in Table 1A.
2. Perform each test and submit the results from each test as described in Table 1A and 2A.

These items shall be submitted according to the schedule provided in Table 3A. If EPA determines that HeiQ has failed to initiate or to submit the required studies by the dates indicated in Table 3A, then EPA will issue notice of intent to cancel HeiQ's registration under FIFRA section 6(e).

EPA will use this data to confirm EPA's determination that the conditional registration of AGS-20 will not cause unreasonable adverse effects on the environment, taking into account the terms and conditions on the registration.

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<http://www.epa.gov/scipoly/sap/meetings/2009/november/110309ameetingminutes.pdf>

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

<b>Table 1A - Summary of Tier I Required Data for HeiQ AGS-20</b>			
<b>OSCPP Data Requirement (Note 1) Guideline Number: Study Title</b>	<b>Reason for Study</b>	<b>Test Material</b>	<b>Comments</b>
<b>Product Chemistry</b>			
830.1550: Product Identity and Composition	Required for antimicrobial pesticides per 40 CFR 161.	AGS-20	The topology of the nanocomposite needs to be fully described. Information is needed on how nanosilver is bound to and distributed on the silica matrix.
830.1750: Certified Limits		AGS-20	The submitted study only provides data on the average amount of nanosilver present in AGS-20. Data are also needed on the upper and lower limits of nanosilver present in AGS-20.
830.1800: Enforcement Method		AGS-20	Submitted method is based on analysis of total silver. Need method to include high resolution images of the nanosilver.
830.1900: Submittal of Samples		AGS-20	EPA is requiring samples for independent testing and confirmation of HeiQ test results.
830.6313: Stability to Normal and Elevated Temperatures, and Metals/Metal Ions		AGS-20	Also need to test stability to sunlight, detergents, and salinity. The results of these tests will dictate the test substance for the Tier II studies.
830.6317: Storage Stability		AGS-20	Originally submitted studies were not acceptable because they used an accelerated method. HeiQ is currently conducting one year studies.
830.6320: Corrosion Characteristics			
830.7050: UV-Visible Light Adsorption		AGS-20	UV-Vis data will be used to confirm that the material is in fact nano-sized. It may also provide data on the range of particle sizes of the nanosilver particles in AGS-20.
830.7840: Solubility		AGS-20	Submitted data for pH 7. Need data for pH 5 and 9.
Non-Guideline: Particle Size and Diameter (size) Distribution	Required to characterize product.	AGS-20	A literature study (Egger et al., 2009) provides some particle size and surface area data; however, it does not have the quality control information and documentation that is required for a product chemistry study.
Non-Guideline: Surface Area Determination	Required to characterize product.	AGS-20	

**Table 1A - Summary of Tier I Required Data for HeiQ AGS-20**

OSCPP Data Requirement (Note 1) Guideline Number: Study Title	Reason for Study	Test Material	Comments
<b>Human Exposure</b>			
875.1200 and 875.1400: Applicator, Indoor Exposure	Required to confirm assessment on occupational handler exposure during pouring of powder.	AGS-20	This study will provide EPA with information useful for evaluating the form, route, and level of exposure experienced by workers who handle AGS-20 powder in the process of treating textiles. The exposure data will be used in conjunction with the toxicology endpoints to confirm the Agency's evaluation of the risk of applicator exposure to AGS-20 powder. This risk assessment will be used to determine what, if any, further mitigation measures may be necessary to prevent adverse health effects in exposed workers.
Non-Guideline: Attrition Study	Required to confirm assessment on consumer and occupational exposure during laundry drying of AGS-20 treated textiles.	AGS-20 Treated Textiles	This study will provide EPA with information useful for evaluating the form, route, and level of exposure to silver experienced by consumers and workers based on the lint produced during drying of AGS-20 treated textiles.
Non-Guideline: Textile Leaching Study (Confirm Geranio et al., 2009 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.	AGS-20 Treated Textiles	A leaching study is needed to determine if materials other than silver ions are released from AGS-20 treated textiles under conditions of use. HeiQ has submitted both a leaching study they conducted and a leaching study from the literature (Geranio et al., 2009). HeiQ claims that these studies indicate that only silver ions were released from the AGS-20 or textiles treated with AGS-20. EPA has reviewed these studies and determined they do not support HeiQ claims of no silver nanoparticle release. An additional, follow-up leaching study is needed and this study could be done in the manner of Geranio et al. (2009) ISO wash-test with the addition of electron microscopy to characterize the particulate that is found to be released. The exposure data will be used in conjunction with the toxicology endpoints to confirm the Agency's evaluation of the risk of exposure to AGS-20.

**Table 1A - Summary of Tier I Required Data for HeiQ AGS-20**

OSCPP Data Requirement (Note 1) Guideline Number: Study Title	Reason for Study	Test Material	Comments
<b>Health Effects</b>			
870.3465: 90-Day Inhalation Toxicity (Rat) (Replace Sung et al., 2009 study results) Modified to include <i>in vivo</i> bone marrow assay and functional observational battery, motor activity and detailed neuropathology	Conditionally Required for antimicrobial pesticides per 40 CFR 161.340 (Required if use results in repeated inhalation exposure)  Occupational inhalation exposures to AGS-20 powder are anticipated during textile manufacturing and processing.	AGS-20	The inhalation study is a route-specific study and is more appropriate than a subchronic oral toxicity study. Inhalation studies of shorter duration (e.g. acute inhalation studies or repeated exposure inhalation studies less than 90 days) would not be sufficient to identify health effects such as pulmonary fibrosis, which has been observed with other nanoparticles, and which takes several months to develop. Guideline modified to include <i>in vivo</i> bone marrow assay and functional observational behavioral battery, motor activity and detailed neuropathology.
870.3250: 90-Day Dermal Toxicity (Rat) (Replace DAF of 0.1% and Park et al. 2010 study results)	Conditionally Required for antimicrobial pesticides per 40 CFR 161.340 (Required if human exposure via skin occurs)  Occupational dermal exposures to AGS-20 are anticipated during textile manufacturing and processing.	AGS-20	A 90 day dermal toxicity study is needed to assess the risks of these exposures. The dermal toxicity study is route-specific, and is more appropriate than a subchronic toxicity study via another route (e.g. oral). The use of studies via other routes requires dermal penetration studies to estimate dermal absorption.
Modified 870.3550/ OECD TG 421: Reproduction/Developmental Toxicity Screening Test	Conditionally Required for antimicrobial pesticides per 40 CFR 161.340 (Required for non-food use products resulting in human exposure over significant portion of human lifespan)  Occupational exposure to workers of reproductive age.	AGS-20	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.
Non-Guideline: <i>in vitro</i> micronucleus (MN) assay	Required for antimicrobial pesticides per 40 CFR 161.340.  Nanosilver known to generate radical oxygen species which can induce genetic mutations.	AGS-20	Genetic toxicity tests are used to screen chemicals for mutagenic or carcinogenic potential. The data from this test will be used to determine if AGS-20 is a potential mutagen or carcinogen.

<b>Table 1A - Summary of Tier I Required Data for HeiQ AGS-20</b>			
<b>OSCPP Data Requirement (Note 1) Guideline Number: Study Title</b>	<b>Reason for Study</b>	<b>Test Material</b>	<b>Comments</b>
<b>Ecological Effects</b>			
850.2100: Avian Acute Oral Toxicity	Required for labeling when shipping AGS-20.	AGS-20	These data are required for labeling packages which contain AGS-20 for shipment.
850.1010: Aquatic Invertebrate Acute Toxicity, Freshwater Daphnids			
850.1075: Fish Acute Toxicity Test, Freshwater and Marine			
<b>Environmental Fate</b>			
Non-Guideline: Dissolution Kinetics Study	Required to assess environmental exposure from wash water.	AGS-20	This is a primary study on the persistence of AGS-20 in the environment and the extent of silver ion or nanoparticle release. This test will dictate the test substance to be used for the Tier II studies.

<b>Table 2A - Summary of Tier II Required Data for HeiQ AGS-20</b>			
<b>OSCPP Data Requirement (Note 1) Guideline Number: Study Title</b>	<b>Reason for Study</b>	<b>Test Material</b>	<b>Comments</b>
<b>Product Chemistry</b>			
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			
<b>Human Exposure – No Tier II Studies are Required</b>			
<b>Health Effects</b>			
870.3100: 90-Day Oral Toxicity (Rat) (Replace Park et al. 2010 study results)	Residential incidental oral exposure to nanosilver.	Nanosilver	The oral study is a route-specific study required to evaluate the effects of ingesting nanosilver from incidental oral exposures.
870.3250: 90-Day Dermal Toxicity (Rat) (Replace DAF of 0.1% and Park et al. 2010 study results)	Occupational dermal exposures to nanosilver are anticipated during AGS-20 treated textile manufacturing and processing. Consumer dermal exposures to nanosilver are anticipated while wearing and mouthing AGS-20 treated textiles.	Nanosilver	A 90 day dermal toxicity study is needed to assess the risks of the exposures to nanosilver if released from AGS-20 treated textiles during Tier I studies. The dermal toxicity study is route-specific, and is more appropriate than a subchronic toxicity study via another route (e.g. oral). The use of studies via other routes requires dermal penetration studies to estimate dermal absorption.

**Table 2A - Summary of Tier II Required Data for HeiQ AGS-20**

OSCPP Data Requirement (Note 1) Guideline Number: Study Title	Reason for Study	Test Material	Comments
Modified 870.3550/ OECD TG 421: Reproduction/Developmental Toxicity Screening Test	Children’s exposure to nanosilver while wearing and mouthing AGS-20 treated textiles. Occupational exposure to nanosilver for workers of child bearing age.	Nanosilver	The combined repeated-dose toxicity study with the reproduction/developmental toxicity screening test will provide initial information on possible effects to reproduction and/or the developmental effects if nanosilver is released from treated textiles in Tier I studies. In addition, the study may also provide a toxicity endpoint applicable to a risk assessment for oral incidental exposure.
Non-Guideline: <i>in vitro</i> micronucleus (MN) assay	Nanosilver is known to generate radical oxygen species which can induce genetic mutations.	Nanosilver	Genetic toxicity tests are used to screen chemicals for mutagenic or carcinogenic potential. The data from these tests will be used to determine if the nanosilver released from the AGS-20 in Tier I studies is a potential mutagen or carcinogen.
<b>Ecological Effects</b>			
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 <i>Chironomus dilutes</i>	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 ( <i>Chironomus dilutes</i> , formerly <i>Chironomus tentans</i> ) with 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 <i>Hyalella azteca</i>	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 ( <i>Hyalella azteca</i> ) 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 <i>Leptocheirus plumulosus</i>	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 estuarine/marine benthic amphipod ( <i>Leptocheirus plumulosus</i> ) will be used to estimate potential risks to marine benthic organisms.

<b>Table 2A - Summary of Tier II Required Data for HeiQ AGS-20</b>			
<b>OSCPP Data Requirement (Note 1) Guideline Number: Study Title</b>	<b>Reason for Study</b>	<b>Test Material</b>	<b>Comments</b>
<b>Environmental Fate</b>			
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.
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 AGS-20 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 (Confirm HeiQ screening study)	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 AGS-20 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, dissolution/dispersability, and textile leaching studies. These materials include the AGS-20 composite and/or nanosilver released from the AGS-20 composite.

**Table 3A – Enforceable Schedule**

The AGS-20 composite and textiles treated with AGS-20 will be the test materials 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, Phase 4 – Health Effects, and Phase 5 – Ecologic Effects.

**Tier I: AGS-20 and AGS-20 Treated Textile Testing**

<b>Guideline</b>	<b>Phase 1 – Product Characterization</b>	<b>Prepare and Submit Protocols*</b>	<b>Perform Study and Submit Results*</b>
Non-Guideline	Particle Size Distribution	<b>Q1<sup>†</sup> 2012</b>	<b>Q1 2012</b>
Non-Guideline	Surface Area		
	<b>Phase 2 – Product Testing</b>	<b>Q1 2012</b>	<b>Q2 2012</b>
830.6313	Stability		
830.6320	Corrosion Characteristics		
830.7050	UV-Vis		
830.7840/7860	Solubility		
	<b>Phase 3 – Release Characteristics/Exposure</b>	<b>Q3 2012</b>	<b>Q4 2012 - Q1 2013</b>
Non-Guideline	Dissolution Kinetics		
Non-Guideline	Leaching Test of Textile		
875.1200	Dermal Exposure-Indoor		
875.1400	Inhalation Exposure-Indoor		
Non-Guideline	Attrition Test - Laundry Drying		
	<b>Phase 4 – Health Effects</b>	<b>Q4 2012 – Q3 2013</b>	<b>Q4 2013 – Q3 2014</b>
870.3465	90-Day Inhalation		
870.3250	90-Day Dermal Toxicity		
870.3550/ OECD 421	Reproduction/Developmental Toxicity Screening Test		
Non-Guideline	<i>in vitro</i> micronucleus (MN) assay		
	<b>Phase 5 - Ecologic Effects</b>	<b>Q2 – Q3 2013</b>	<b>Q4 2013 – Q1 2014</b>
850.2100	Avian Acute Oral Toxicity		
	Aquatic Invertebrate Acute Toxicity,		
850.1010	Feshwater Daphnids		
850.1075	Fish Acute Toxicity Test, Freshwater and Marine		

\*Submissions are due at end of quarter unless otherwise noted.

<sup>†</sup>Q1- Jan thru March; Q2 - April thru June; Q3 - July thru September; Q4 - October thru December

**Table 3A – 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 6 – Characterization will occur prior to developing protocols for Phase 7 – Health Effects, Phase 8 – Ecological Effects, and Phase 9 – Environmental Fate.

**Tier II: Testing for Nanosilver and/or AGS-20 Released during Tier I Tests**

Guideline		Prepare and Submit Protocols* Q2 2013 <sup>†</sup>	Perform Study and Submit Results* Q3 2013
	<b>Phase 6 – Characterization</b>		
830.7050	UV-Vis		
Non-Guideline	Particle Size Distribution		
Non-Guideline	Surface Area		
830.7840/7860	Solubility		
Non-Guideline	Zeta-potential		
	<b>Phase 7 - Health Effects</b>	<b>Developed during Phase 4</b>	<b>Q4 2013 – Q3 2014</b>
870.3100	90-Day Oral Toxicity		
870.3250	90-Day Dermal Toxicity		
870.3550	Modified Reproduction/Developmental Toxicity Screening Test		
Non-Guideline	in vitro micronucleus (MN) assay		
	<b>Phase 8 – Ecological Effects</b>	<b>Q3 - Q4 2013</b>	<b>Q1 – Q3 2014</b>
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 <i>Chironomus dilutes</i>		
Non-Guideline	Measuring the Chronic Effects of Freshwater Sediment-Associated Contaminants on <i>Hyalella azteca</i>		
Non-Guideline	Measuring the Chronic Effects of Marine and Estuarine Sediment-Associated Contaminants on <i>Leptocheirus plumulosus</i>		
	<b>Phase 9 – Environmental Fate</b>	<b>Q3 – Q4 2013</b>	<b>Q1 – Q3 2014</b>
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		

\*Submissions are due at end of quarter unless otherwise noted.

<sup>†</sup>Q1- Jan thru March; Q2 - April thru June; Q3 - July thru September; Q4 - October thru December