# Proposed Decision Document for the Registration of HeiQ AGS-20 as a Materials Preservative in Textiles

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**Environmental Protection Agency**
**Office of Pesticide Programs**
**Antimicrobials Division**

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## I. SUMMARY OF EPA'S PROPOSED ACTION

EPA is announcing and seeking public comment on its proposed decision to conditionally register a pesticide product containing nanosilver as an active ingredient. The product is the subject of an application submitted by HeiQ Materials Ag ("HeiQ") in September 2008. The product is named "HeiQ AGS-20," and the nanosilver active ingredient is intended for use as a preservative in textile products. Generally speaking, with respect to pesticides, EPA views a "nanoscale material" as an active or inert ingredient and any component parts thereof intentionally produced to have at least one dimension that measures between approximately 1 and 100 nanometers.

EPA proposes to grant a conditional registration to this product under section 3(c)(7)(C) of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). The Agency's basis for the conditional registration is as follows:

1) Insufficient time has elapsed for the generation of data since the requirement for that data was imposed;
2) Use of the pesticide is in the public interest; and
3) Use of the pesticide during the period that the newly required data is being developed and reviewed by the Agency will not cause unreasonable adverse effects.

Until very recently, EPA had not reached a position with regard to which types of data would be needed to evaluate the potential risks to human and the environment of AGS-20. 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. EPA has determined that more extensive product chemistry, toxicology, exposure, and environmental data are necessary. Because the list of data requirements is being finalized with today's action, insufficient time has elapsed for HeiQ to have generated the data. Therefore, the Agency is proposing to require these studies as a condition of registration, allowing sufficient time for the studies to be conducted and for the Agency to review them. Ultimately, the Agency will use these data to determine whether the ingredient can be registered under FIFRA section 3(c)(5).

The Agency has determined that the benefits expected from this proposed pesticide product, together with other considerations, justify the conclusion that granting a conditional registration is in the public interest. EPA believes HeiQ's product offers potential benefits in terms of both conservation of the environment, through less use of silver, and consumer benefits, through prolonged efficacy. Moreover, considerations relating to market equity and international trade and promoting innovation lend further support to a determination that a conditional registration is in the public interest. Specifically, some current registrants of silver-based antimicrobial products have recently reported that their products contain nanoscale silver material. While EPA approved these registrations without knowledge that these products contained nanoscale silver and without specifically assessing any potential risks that might be associated with the specific nanoparticles contained in those products, they are on the market. Arguably, this unfairly disadvantages HeiQ because HeiQ's competitors are in the market ahead of them. None of the nanosilver products have submitted the types of data that the Agency is proposing to require as a

condition for the registration of HeiQ's nanosilver product. The Agency believes that in connection with this newly required data, treating all of the registered nanosilver products equally is in the public interest. In light of this, and in concert with the Agency's request to HeiQ for additional data as part of the proposed conditional registration, the Agency intends to require that similar data be developed to support the continued registration of these other nanoscale products as well.

With respect to the third conclusion, EPA conducted a screening level risk assessment of risks to workers, consumers and the environment associated with the use of AGS-20 as a materials preservative. EPA also reviewed pertinent literature on silver and nanosilver toxicity and fate. Although the available data are limited, the EPA was able to complete a screening level risk assessment that indicated, with the exception of worker exposures, that the proposed use pattern for AGS-20 will likely lead to low levels of human and environmental exposure. Therefore despite considerable uncertainty about the risk assessment, EPA concludes that the proposed registration will pose relatively little risk to consumers and the environment. As a result, EPA believes that the use of AGS-20 during the period of the proposed conditional registration would not pose unreasonable adverse effects on the environment. To mitigate potential worker risks, EPA is requiring label language and engineering controls to reduce potential exposures.

As a condition of registration, EPA is requiring the company to conduct a number of studies, based on a tiered approach, which will allow the Agency to further identify and characterize any potential risks that may be associated with the continued use of HeiQ AGS-20. EPA is including a time limitation of four years on the registration to ensure the studies are completed in a timely fashion. The time 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 product will not cause unreasonable adverse effects to human health and the environment.

## II. BACKGROUND

### A. Regulatory History

In September 2008, HeiQ submitted an application for registration of a new antimicrobial pesticide product named HeiQ AGS-20, which is a silver-based product that is proposed for use as a materials preservative additive to coatings, polymers and textiles. HeiQ's application originally claimed that HeiQ AGS-20 is similar to other currently registered silver-based antimicrobial pesticide products. The company later amended its application to limit the proposed use of HeiQ AGS-20 for treatment only of textiles. EPA reviewed the product chemistry data for HeiQ AGS-20 and determined that the nanosilver active ingredient in the product differed from currently registered silver-based antimicrobial products. (EPA has not previously registered any pesticide products containing as an ingredient nanoscale silver-silica composite structures similar to AGS-20.) Therefore, EPA reclassified the application under the PRIA (Pesticide Registration Improvement Act) as one involving a "New Active Ingredient Registration." EPA published a Notice in the Federal Register announcing receipt of the HeiQ

application. See [Federal Register Vol. 75, No. 61, March 31, 2010, page 16109] EPA received comments on this notice, which are located in the docket for this action. EPA plans to address these comments in conjunction with comments on this proposed decision document.

### B. 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. The SAP released its report in January 2010 (http://www.epa.gov/scipoly/sap/meetings/2009/november/110309ameetingminutes.pdf).

In general, the SAP advised that the toxicity of nanosilver particles could differ from and might be higher than bulk silver or the silver ion and that nanosilvers with different physicochemical properties may behave differently in biological systems. In addition, the SAP commented that not enough literature is available to draw any firm conclusions regarding human (occupational or consumer) exposures. Potentially, three major routes exist for human exposure to nanoparticles: oral, inhalation, and dermal. Only a few studies are known that address the toxicity of nanosilver to humans 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 treated with AGS-20. The same situation exists for environmental fate and transport. For nanosilver, the concentration in the environment, exposure pathways, bioavailability, toxicity and potential impact on ecological systems are still not quantified. Furthermore, little or no information on the fate of nanosilver in soils and sediments is 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 would not 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.

## III. PRODUCT CHARACTERIZATION

HeiQ AGS-20 is a silver-silica nanocomposite material that contains silver nanoparticles imbedded in a matrix of amorphous silicon dioxide ($SiO_2$). The $SiO_2$ fine structure consists of aggregate matrix particles with an average diameter of approximately 1 micron. Each silica particle contains many small silver metal particles with a typical diameter between 1 and 10 nm (Egger et al., 2009).

HeiQ proposes to formulate its product as a powder which would be used as a materials preservative to treat woven, knitted or non-woven textiles. The textiles will be treated by application of HeiQ AGS-20 either as a surface coating or by incorporation into the starting

materials (i.e. the masterbatch) prior to textile manufacture. The types of textiles include those made from natural and synthetic fibers and which are used to manufacture indoor use articles such as sheets, blankets, towels, napkins, outerwear, sportswear, sleepwear, undergarmets, socks and hosiery and outdoor use articles such as sailcloth, tarps, tents and awnings.

EPA reviewed the following guideline studies submitted by HeiQ and found the data acceptable. (The numbers in parentheses preceding the study name refer to the EPA guidelines for conducting that kind of study.)

- (830.1000, 1620, 1650, 1670): Description of Production Process, Description of Formulating Process, Discussion on Formation of Impurities
- (830.1700) Preliminary Analysis, five batch analysis
- (830.6302): Color
- (830.6303): Physical State
- (830.6304: Odor
- (830.7000) pH
- (830.7200): Melting Point
- (830.7300): Density

NOTE: EPA's data requirements regulations often require other types of data on the physical or chemical properties of a product. EPA has determined however, that these types of data were not needed for an evaluation of AGS-20. Specifically, EPA proposes not to require the following types of data:

- (830.6314): Oxidizing/Reducing characteristics. Not required as the nanocomposite contains no oxidizing or reducing agent.
- (830.6315): Flammability. Not required as the product does not contain combustible liquid
- (830.6316): Explodability. Not required, the product is not potentially explosive
- (830.7100): Viscosity. Not required. Product is not a liquid.
- (830.7370): Dissociation constant. Not required. Material is not expected to dissociate.
- (830.7550/7560/7570):Partition Coefficient (Log $K_{ow}$). Not required. The product is a solid.
- (830.7220/7950): Boiling Point, Vapor Pressure. Not required. The product is a solid.

HeiQ submitted data to address the following guidelines, but the Agency has determined that additional information is needed as follows:

- (830.1550): Product Identity and Composition. The submitted data are acceptable, but EPA is requiring additional information about the nanocomposite (see Appendix A).

- (830.1800): Enforcement Method. The submitted method is based on analysis of total silver. Need the method to include high resolution images of the active.

- (830.7840): Water Solubility. The submitted data for pH 7 are acceptable, but additional data are required for pH 5 and 9.

- (830.1750): Certified limits. The certified limits of the nanocomposite are not accurately identified.
- (830.6317): Storage Stability. A Storage Stability study was submitted but it was not acceptable because it used an accelerated method. The Agency requires a 1-year study be submitted to support this guideline. The study is currently being conducted by HeiQ.
- (830.6320): Corrosion Characteristics. A Corrosion Characteristics study was submitted but it was not acceptable because it used an accelerated method. The Agency requires a 1-year study be submitted to support this guideline. The study is currently being conducted by HeiQ.
- (830.7520): Size and Size Distribution. Some particle size information is given in a published paper (Egger et al., 2009) however, this information is incomplete. Additional data must be submitted that adequately characterizes the size distribution of the AGS-20 nanosilver-silica composite and the nanosilver particles in the composite. (See Appendix A).
- (Non-guideline): Surface Area. Some surface area information is given in a published paper (Egger et al., 2009) however this information is incomplete. Additional data must be submitted that adequately characterizes the surface area of the AGS-20 nanosilver-silica composite and the nanosilver particles in the composite. Currently there is no Agency guideline available for this type of study, however, it is anticipated that this study could be accomplished using methods reported in the literature. (See Appendix A).

Additionally, the Agency is requiring the following product chemistry data

- (830.1900): Submittal of Samples
- (830.6313): Stability to normal and elevated temperatures, and metals and metal ions. Also need to test stability to sunlight, detergents, and salinity.
- (830.7050): UV-vis absorption spectra. This study is a reliable identification method for nanoproducts and one that the SAP recommended.

## IV. HUMAN HEALTH RISK ASSESSMENT

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 their product. In addition, EPA has also reviewed data and information from the public literature. This section discusses EPA's assessment of the potential risks to human health from the use of AGS-20. First the section addresses information concerning toxicity of the different substances to which people may be exposed. Then the section addresses potential levels of exposure and finally it concludes with a quantitative assessment of the risks for different exposed populations and a characterization of the uncertainties associated with the assessment. As part of these discussions, additional data that EPA proposes to require in order to reduce its uncertainty with respect to assessing the risks of HeiQ AGS-20 are identified.

### A. Toxicology

Based on the proposed use pattern, EPA anticipates humans could be exposed to the following substances: AGS-20 nanosilver-silica composite particles, silver nanoparticles that break away from the composite particle and silver ions released from the treated textiles.

#### 1. AGS-20 Nanosilver-Silica Composite

Acute Toxicology

HeiQ submitted data from the battery of six required acute toxicity studies, and these studies are listed in Table 1. The AGS-20 nanosilver silica composite exhibited low acute toxicity by all routes of exposure.

**Table 1 - Acute Toxicity Profile for HeiQ AGS-20**

| Study | Toxicity Category | Status |
|---|---|---|
| Acute Oral Toxicity | III | Acceptable |
| Acute Dermal Toxicity | III | Acceptable |
| Acute Inhalation Toxicity | III | Acceptable |
| Primary Eye Irritation | III | Acceptable |
| Primary Skin Irritation | IV | Acceptable |
| Dermal Sensitization | Non-Sensitizer | Acceptable |

It is important to note that these acute studies do not characterize the hazard of AGS-20 resulting from longer periods of exposure. These studies also do not evaluate a number of potential endpoints from acute exposure and they are conducted only with non-pregnant, adult animals.

Subchronic/Chronic Toxicology

Based on its assertion that its product was similar to currently registered, silver-based antimicrobial products and could rely on data supporting those registrations, HeiQ did not submit any subchronic or chronic toxicity data on AGS-20. For a non-food use pesticide, such as a

material preservative like AGS-20, EPA requires a subchronic toxicity study, a developmental toxicity study, and a battery of mutagenicity studies.

EPA proposes to require HeiQ to conduct studies to identify the subchronic hazard of the nanocomposite. EPA will use these data to confirm that the screening level occupational and residential risk assessments for AGS-20 described elsewhere in this document provide an accurate assessment of the risks.

Based on the proposed use pattern for AGS-20 and the possibility of occupational and residential exposures, the Agency has determined that route-specific subchronic inhalation and dermal toxicity studies, a combined repeated-dose oral toxicity study with reproduction and developmental toxicity screening test, and a battery of genetic toxicity studies are required as Tier 1 studies to assess the subchronic/chronic risks of this product to adults and children. Completion of these studies *with the AGS-20 nanosilver-silica composite as the test material* will provide the kinds of data typically required for a pesticide containing a new active ingredient intended for use as a materials preservative. Additional toxicity studies (Tier 2) may be required based upon the results of the Tier 1 test results and the residential exposure studies (see Appendix A).

## 2. Nanosilver Particles

In addition to potential exposure to the AGS-20 nanosilver-silica composite, humans may be exposed to silver nanoparticles that might break away from the nanosilver-silica composite. HeiQ did not conduct any research on the toxicological effects of nanoscale particles that may break away from AGS-20 by the relevant routes of exposure (inhalation, oral, and dermal). However, there are studies in the open scientific literature that investigate the toxicological effects of other nanosilver nanoparticles by these routes. It is unknown if AGS-20 silver nanoparticles would behave similarly (toxicologically) to these nanosilvers used in the literature studies because of potential differences in physicochemical properties (e.g. size, surface area, surface modifications). The FIFRA Scientific Advisory Panel (SAP) report specifically noted issues with regard to size: "for nanosilver specifically, the literature does suggest that silver nanoparticles in the range of about 1 to 20 nm do possess the greatest quantum properties, are most optically active and may have the potential to induce the greatest toxicities" Further, citing the literature, the SAP suggested "biological activity and penetration through biological barriers depends on particle size". However, in the absence of product-specific data and as further explained below, these studies do provide some insight into what kinds of hazards may be anticipated for any released AGS-20 silver nanoparticles.

HeiQ submitted a literature study (Sung et al., 2009) to support the assessment of subchronic/chronic toxicity of AGS-20 by the inhalation route of exposure. 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 inhalation of pure nano-sized (18-19 nm) silver particles for 13 weeks at the high-dose level. The Agency considers these effects adverse. Also, significant increases in the amount of silver nanoparticles in tissues, such as lungs, liver, olfactory bulb, brain, kidneys, and blood, was also reported, particularly in the lungs. 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 $\mu g/m^3$ ($1.4 \times 10^6$ particle/$cm^3$, the mid-level dose tested) was determined. This study indicates to the Agency that, if the AGS-20 nanocomposite releases silver nanoparticles which become airborne, and if such nanoparticles display toxicity similar to the nanoparticles used in this study, inhalation of sufficient quantities of silver nanoparticles that break away from AGS-20 may result in adverse health effects.

There is also a study in the open scientific literature that investigates toxicity of nanosilver via the oral route (Kim, et al, 2008). The reported findings after 28 days of repeated administration of 60 nm silver nanoparticles 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 nanosilver 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 increase in alkaline phosphatase (ALP) and cholesterol at 300 mg/kg/day (mid dose level), was determined. This study indicates to the Agency that, if the AGS-20 nanocomposite releases silver nanoparticles which are ingested, and if such nanoparticles display toxicity similar to the nanoparticles used in this study, ingestion of sufficient quantities of silver nanoparticles that break away from AGS-20 may result in adverse health effects.

There are no available subchronic dermal toxicity studies on nanosilver particles in animals. In the absence of dermal toxicity studies, the Agency normally uses extrapolation from another route of exposure (usually oral) combined with a dermal penetration study to determine the fraction of a topically applied dose that is available for systemic absorption (i.e. the dermal absorption factor, or DAF). The Agency typically uses *in vivo* studies to calculate a DAF. However, the Agency will consider *in vitro* studies when *in vivo* data are also provided that show the *in vitro* study is predictive. An *in vitro* dermal penetration study with nanosilver is available in the peer reviewed literature (Larese, et al, 2009) that utilized intact and damaged human skin. This study found limited penetration in intact skin (about 0.6%) and increased penetration in damaged skin (3.3%). No data were provided to confirm the predictivity of *in vivo* results. This study indicates to the Agency that, if they behave similarly to the nanoparticles used in this study, any silver nanoparticles that break away from AGS-20 may be absorbed to some degree through intact skin and that absorption may be greater through damaged skin.

The Agency considers the potential for adverse systemic effects of nanosilvers via dermal exposure to be low. There is a single published report of a burn patient with nanosilver-coated wound dressing, who 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). However, another study in 30 patients reported no adverse effects associated with the use of these dressings in the treatment of burns (Viachou et al., 2007). Systemic exposures to nanosilver would be expected to be much greater in burn patients than in normal subjects, since the dermal barrier is compromised.

### 3. Silver Ion

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 nanosilver particles. With respect to silver ions, the Agency notes that safe exposure levels for silver have been established by several regulatory agencies including FDA, OSHA, and EPA based on the common endpoint argyria and using the same human studies. Argyria is a blue-gray discoloration of the skin and is not considered as being of toxicological concern.

## B. Human Exposure Scenarios and Data Submitted

EPA expects both consumers and workers are likely to be exposed as a result of the use of AGS-20. Consumer exposures are likely to occur during the following scenarios:

1. Inhalation exposure during laundry drying of AGS-20 treated fabrics;
2. Dermal exposure while wearing AGS-20 treated fabrics; and
3. Incidental oral exposure from mouthing or sucking on AGS-20 treated fabrics.

Occupational dermal and inhalation exposures are likely to occur during the addition of the AGS-20 powder at textile manufacturing and treatment facilities and during subsequent work activities involving the treated textile products. The above exposures could be to AGS-20 composite particles, silver nanoparticles that break away from the composite particle, and silver ions released from the treated textiles.

### 1. Consumer Exposure

<u>Textile Leaching Studies Submitted</u>

A textile leaching study (MRID 477287-01) was submitted by HeiQ. The study measured the release of silver nanoparticles from textiles treated with their proposed product, AGS-20. The testing was conducted by agitating fabric samples in room temperature ultrapure water for 24 hours. The wash water was analyzed for silver ions using an ion specific electrode (ISE) and for particles using scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDX). According to HeiQ, no ionic silver was detected using ISE and no silver particles were detected using SEM/EDX. There were other particles detected, but they were confirmed to be non-silver using EDX.

EPA thinks this study is not sufficient to support HeiQ's conclusion that no ionic silver and no silver particles were released from AGS-20. The ISE method that was used to detect silver ions had a detection limit of 160 ppb which is much higher than other available methods such as Induction Coupled Plasma (ICP), which has a detection limit of 0.2 ppb. The magnification used for SEM, which was at the micron scale, was insufficient to detect the possible presence of silver nanoparticles that are 1-10 nm in diameter. It is also not known if the silver nanoparticles would have aggregated and become visible at lower magnifications. At best, this study supports the

limited conclusions that no micron sized AGS-20 composite particles were released and that the release of ionic silver did not exceed 160 ppb.

In addition to the study just mentioned above, HeiQ submitted a literature study (Geranio, 2009) that used their product, HeiQ AGS-20, in its testing. During this study, 9 textiles treated with silver, including two treated with HeiQ AGS-20, were machine washed using an International Standards Organization (ISO) wash-test method to determine the effect of pH, surfactants and bleaching agents. The HeiQ samples were polyester fabrics treated with 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 before and after filtration using 0.45 micron filters and 30 kDa membranes (~5 nm) to separate large and small particulate silver from silver ions.

As shown in Table 2, the amount of silver released was greater for the surface coating treated fabric which released thirty percent of its silver content during the 1st wash as compared to incorporated fabric which released 1.3 percent. The analysis of the filtered and unfiltered samples indicated that for the surface coated fabric, 80 percent of the released silver was composed of particulate >450 nm in size, while for the incorporated fabric, roughly 50 percent or more of the silver was composed of particulates >450 nm in size. The study suggests that the mechanical stresses related to the ISO wash-test method, which uses steel balls, were a major cause of particulate release.

**Table 2 - Silver Released During Washing of Polyester Fabric Treated with AGS-20**

| Designation in Geranio et al. 2009 | Designation in MRID 477287-01 | Treatment Type | Silver Content µg/g* | Silver Released (ug/g)# | | |
|---|---|---|---|---|---|---|
| | | | | 1st Wash | 2nd Wash | Bleach Cycle |
| NP-PES-SURF | Sample #2 | Surface Coating | 29 | 10.1 | N/A | N/A |
| NP-PES | Sample #3 | Incorporation | 99 | 1.3 | 0.35 | 2.7 |

* Units are in µg silver / g of textile
\# Units are in µg silver release / g silver in textile

EPA has concluded that the HeiQ treated textiles, particularly when treated with a surface coating, released silver in the form of coarse particulate, but it is not known if this particulate consists of nanosilver particles in fibers or coating fragments, aggregates of silver nanoparticles, or precipitates of ionic silver. EPA has determined that an additional leaching study, which includes electron microscopy to characterize the particulate, is needed. The characterization is needed to determine if nanoparticles could be released from the particulate.

## 2. Occupational Exposure

Occupational Exposure Studies Submitted

HeiQ submitted a research report that was published in peer reviewed literature (Demou et al., 2008). The purposes of the study that is the subject of the research report were to: (1) Measure occupational inhalation exposures during the pilot production of AGS-20 and determine what

control measures are needed to minimize these exposures and (2) Measure the penetration of AGS-20 nanosilver particles through commercially available respirator filters.

Occupational Inhalation Exposure Study

Inhalation exposures to particles were measured during the pilot scale production of AGS-20. 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™ aerosol monitor and a Scanning Mobility Particle Sizer (SMPS). Although these instruments cannot identify 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 was performed during AGS-20 production, maintenance and handling. Samples taken during this test occurred at the end of the process, therefore generating the highest exposure measurement. The results indicated that the highest particle concentrations occurred during production and that these concentrations were an order of magnitude above the background particle levels. (Note - heating system combustion byproducts, vehicle exhaust, and electric motors are background sources of nanoparticles.) It was reported by the authors that the production rate 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 concentrations did not increase when the equipment was operating and production was not occurring. Manual cleaning of the reactor with a vacuum cleaner was also a major source of particle emission. Mechanical handling of the powder before packaging and transferring of powder caused little or no increase in particle concentrations.

Respirator Filter Penetration

The penetration of particles that are generated during the production of AGS-20 through four types of respirator filters from two different manufacturers was measured in a filter chamber. Three of these respirator filters were certified to the EN 143:2000 P3 standard, 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 production process was drawn into the inlet end of the chamber across the respirator filter and out the outlet end. Particle concentration measurements were taken on each side of the filter using two Condensation Particle Counters. The initial chamber flowrate was set at one of two levels (1.0 $m^3$/hr and 2.15 $m^3$/hr) to represent the inhalation rates for 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 of the testing indicated that retention was 99.89 percent or greater for the P3 respirators and 96.66 percent for the P2 respirator. These results are consistent with other studies cited in the National Institute for Occupational Safety and Health Document Approaches to Safe Nanotechnology (NIOSH, 2009) that have reported very high retention rates for respirator filters challenged with nanoparticles.

Conclusions Regarding the Occupational Exposure Study

The submitted occupational exposure study discussed above provides some useful information regarding the exposure potential of AGS-20 but it does not provide quantitative exposure data. EPA proposes to require HeiQ to conduct an indoor applicator study to quantify the exposure potential of AGS-20. EPA will use these data to confirm that the screening level occupational risk assessments for AGS-20 described elsewhere in this document provide an accurate assessment of the risks.

## C. Assessment of Potential Risks to Consumers and Workers

EPA's Margin of Exposure (MOE) Approach to Assess Risk

EPA uses a Margin of Exposure (MOE) approach to assess risk in which a calculated MOE is compared 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 engineering controls and/or personal protective equipment (PPE) are employed until the calculated MOE exceeds the target MOE. These relationships are summarized below:

$MOE_{CALC} > MOE_{TARGET}$ = Risk is not of concern and mitigation is not required.
$MOE_{CALC} < MOE_{TARGET}$ = Risk is of concern and mitigation is required.

The Target MOE is determined by uncertainty factors. These commonly include a 10X intraspecies uncertainty factor (to account for variation within the human population) and a 10X interspecies uncertainty factor (to account for differences between animals and humans) as well as any database uncertainty factors (up to 10X). Thus, the Target MOE commonly ranges from 100-1000.

The calculated MOE is determined by dividing the toxicological point of departure (POD) by the estimated dose to which humans will be exposed. The POD is determined by the dose at the most sensitive endpoint in the most sensitive species for relevant duration and routes of exposure. Commonly, this is a NOAEL from a laboratory animal toxicity study. When a route-specific study (such as a dermal toxicity study) is not available, a NOAEL from a study using a different route of exposure may be used and the dose adjusted (such as application of a dermal absorption factor to a NOAEL from an oral toxicity study).

$$MOE_{CALC} = POD\ (mg/kg/day) \div Dose\ (mg/kg/day)$$

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.

Uncertainty Factors and Target MOE for AGS20

The Agency has determined that the Target MOE for AGS 20 is 1000 for all routes of exposure (oral, dermal, and inhalation) and all populations exposed (occupational and residential). The Target MOE includes the standard safety factors of a 10X for intraspecies variation and 10X for interspecies extrapolation as well as a 10X database uncertainty factor to account for the limited toxicological database for nanosilver.

**1. Consumer Risk Assessment**

There is the potential for dermal exposures to nanosilver by children and adults when textiles treated with AGS-20 are worn and for incidental oral exposures by children when these textiles are put in the mouth. Because there are no oral or dermal toxicology studies available for AGS-20 or the nanoparticles that might break away from textiles treated with AGS-20, these exposures were assessed using a 28 day oral toxicity study on 60 nm silver nanoparticles that is reported in the literature (Kim et al., 2007) as a surrogate. For an oral exposure by a toddler, the POD is the NOAEL from the Kim et al. oral toxicity study (30 mg/kg/day). For dermal exposures, the POD is the NOAEL from the oral toxicity study, divided by a dermal absorption factor (which was estimated from a literature study, as described below).

Dermal Exposure Calculation

The dermal exposure was calculated using the following formula:

**Exposure** = Application Rate * Cloth Density * Surface Area Exposed * Transfer Efficiency

Where:

- The application rate is 100 ppm silver when AGS-20 is incorporated during fiber production and 30 ppm silver when AGS-20 is applied as a coating. These application rates are based on the HeiQ and Geranio et al. leaching studies.
- The cloth density is 10 $mg/cm^2$ based on the density of mixed cotton and synthetics. This value is a standard assumption used in OPP risk assessments and was taken from the HERA Guidance Document Methodology (AISE/CEFIC, 2005)
- The surface area exposed is 5,700 $cm^2$/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 (EPA, 2008).
- The clothing-to-skin transfer efficiency factors of 3.1 and 10.1 percent from the HeiQ leaching study are based on a detection limit of 160 ug/liter, a washwater sample volume of 38 ml, a fabric sample weight of 2 grams and a silver content of either 100 ppm or 30 ppm, respectively.

Dermal Dose Calculation

The dermal dose was calculated from the dermal exposure using the following formula to account for dermal absorption:

**Dose** = [Exposure (mg/day)* Dermal Absorption Factor (1%)] / Body Weight (kg)

Where:

- The dermal absorption factor is estimated at 1.0 percent in intact skin, based on an *in vitro* dermal penetration study published in the peer-reviewed literature (Larese, *et al.* 2009) in which 0.46 µg/cm$^2$ nanosilver was found in the receptor fluid after application of 70 µg/cm$^2$ to human skin in diffusion cells (0.46/70 x 100% = 0.59%, rounded up to 1.0%).
- The body weight of a toddler (3 years old) is 15 kg which is a standard assumption from the Exposure Factors Handbook (EPA, 1997).

Dermal MOEs

The MOEs for dermal exposures were calculated from the dermal dose using the NOAEL of 30 mg/kg/day from Kim et al., 2007 as the POD. These MOEs are listed in Table 3 and range from 79,000 to 270,000 depending upon the application rate and transfer factor used. These MOEs all exceed the target MOE of 1000 which indicates that the risks are not of concern. Although these MOEs were calculated for children, they are protective of adults, since ratio of skin surface area to the body weight is greater for children than for adults.

**Table 3 - Dermal MOEs for Toddlers Exposed to AGS-20 Treated Textiles**

| Application Rate | Cloth Density (mg/cm$^2$) | Surface Area Exposed (cm$^2$/day) | Cloth to Skin Transfer Efficiency | Exposure (mg/day) | Dose (mg/kg/day) | MOE (mg/kg/day) |
|---|---|---|---|---|---|---|
| 100 ppm | 10 | 5,700 | <3.1% (HeiQ[A]) | <0.18 | 0.012 | 250,000 |
| | | | 1.1% (Geranio, No Bleach[B]) | 0.063 | 0.0042 | 710,000 |
| 30 ppm | 10 | 5,700 | <10.1% (HeiQ[A]) | <0.17 | 0.011 | 270,000 |
| | | | 33% (Geranio, No Bleach[C]) | 0.56 | 0.038 | 79,000 |

A. Based on an LOD of 160 ug/liter from the HeiQ leaching study.
B. Based on Geranio study data for Sample #3 (Incorporated, 1st Wash, No Bleach)
C. Based on Geranio study data for Sample #2 (Surface Coating, 1st Wash, No Bleach)

Incidental Oral Exposure Calculations

Incidental oral exposures were calculated using the following general formula:

**Exposure** = Application Rate * Cloth Density * Surface Area Mouthed * Saliva Extraction Efficiency

Where:

- The textile contains 100 ppm silver when AGS-20 is incorporated during fiber production and 30 ppm silver when AGS-20 is applied as a coating.
- The cloth density is 10 mg/cm$^2$ based on the density of mixed cotton and synthetics.
- The surface area of fabric that is mouthed per day is assumed to be 100 cm$^2$ (~16 in.$^2$) which is a standard assumption

The saliva extraction factors for mouthing fabric are based on the results of the HeiQ and Geranio leaching studies and are the same factors used for the dermal exposure calculations.

Incidental Oral Dose Calculation

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

**Dose** = Exposure (mg/day) / Body Weight (15 kg)

Incidental Oral MOEs

The MOEs for incidental oral exposures were calculated from the incidental oral dose using the NOAEL of 30 mg/kg/day from Kim et al., 2007 as the POD. The estimated MOEs for incidental oral exposures are listed in Table 4 and range from 45,000 to 410,000 depending upon the application rate and saliva extraction factor used. These MOEs all exceed the target MOE of 1000 which indicates that the risks are not of concern.

**Table 4 - Incidental Oral MOEs for Toddlers Exposed to AGS-20 Treated Textiles**

| Application Rate | Cloth Density (mg/cm$^2$) | Surface Area Mouthed (cm$^2$/day) | Saliva Extraction Efficiency | Exposure (mg/day) | Dose (mg/kg/day) | MOE (mg/kg/day) |
|---|---|---|---|---|---|---|
| 100 ppm | 10 | 100 | <3.1% (HeiQ leaching study[A]) | <0.0031 | <0.00021 | 140,000 |
| | | | 1.1% (Geranio, No Bleach[B]) | 0.0011 | 0.000073 | 410,000 |
| 30 ppm | 10 | 100 | <10.1% (HeiQ leaching study[A]) | <0.0030 | <0.00020 | 150,000 |
| | | | 33% (Geranio, No Bleach[C]) | 0.0099 | 0.00066 | 45,000 |

A. Based on an LOD of 160 ug/liter from the HeiQ leaching study
B. Based on Geranio study data for Sample #3 (Incorporated, 1st Wash, No Bleach)
C. Based on Geranio study data for Sample #2 (Surface Coating, 1st Wash, No Bleach)

Conclusions Regarding Risks to Consumers

The risk calculations indicate that the MOEs for dermal and incidental oral exposures range from 45,000 to 71,000 which exceed the target MOE of 1000. The exposure portion of the calculations is based on the existing leaching studies, the use of which is conservative because of uncertainties surrounding the detection limits, TEM magnification, and identity of the particulate fraction. If these uncertainties can be addressed by future studies and if leaching is confirmed as lower than these conservative estimates, exposures would be lower and the MOEs would be

higher, reflecting even greater confidence that consumer exposure is unlikely to pose a significant risk. The point of departure used was based on an oral toxicity study that was done using different silver nanoparticles, which have a larger particle size of 60 nm, and a dermal absorption factor of 1 percent from an *in vitro* study. The Agency recognizes the uncertainties regarding extrapolation from an oral route to a dermal route with nanoparticles, the use of an oral study with different sized nanoparticles than those that may be released from AGS-20, and the use of an *in vitro* dermal absorption study in which the predictivity for *in vivo* dermal absorption has not been confirmed.

## 2. Occupational Risk Assessment

Inhalation Exposures

The occupational handler inhalation exposures that occur during the mixing and loading of AGS-20 powder during textile treatment were assessed using the Pesticide Handlers Exposure Database (PHED) unit exposure values from the Wettable Powder, Open Mixing and Loading Scenario (EPA, 1998). These values are an approximation as the AGS-20 powder has a particle size of approximately one micron while wettable powder pesticide formulations have a high proportion of particles that are less than 5 microns (Matthews, 2000). To account for the use of full face respiratory protection as suggested in the Risk Assessment of AGS-20 (Submitted by HeiQ as MRID 479344-02) the unit exposure was divided by a protection factor of 50. This factor is listed in the Approached to Safe Nanotechnology (NIOSH, 2009).

The MOEs were calculated as shown in Table 5 by comparing the inhalation exposure to the point of departure of 133 ug/m$^3$, which is the NOAEL from a 90 day inhalation toxicity study (Sung, 2009). The MOE is 8 when no respirators are worn and it is 430 when full face respirators are worn. Both of these MOEs indicate a risk of concern because they are less than the Target MOE of 1000. It is important to note, however, that these MOEs are not precise estimates of risk because AGS-20 has a smaller particle size than the wettable powders that were used for the PHED studies and it is not known if the test material used in the inhalation toxicity study has the same properties as AGS-20.

**Table 5 – Inhalation MOEs for AGS-20 Occupational Handlers**

| Scenario | Amount of Textile Treated per day[A] | Application Rate[B] | Amount of ai handled per day | Unit Exposure[C] (ug/m$^3$/lb ai) | Exposure[F] (ug/m$^3$) | MOE[G,H] |
|---|---|---|---|---|---|---|
| Mix/Load AGS-20 Powder During Textile Treatment | 20,000 lb | 0.01% silver by weight | 2 lb | 7.8[D] | 16 | 8 |
| | | | | 0.16[E] | 0.31 | 430 |

A. Standard OPP assumption for textile treatment.
B. Rate for incorporation treatment included in studies submitted by HeiQ.
C. PHED unit exposure data converted to air concentration units based on the mean 8 hour TWA.
D. Assuming no respirator is worn.
E. Assuming a full face respirator is worn which provides a protection factor of 50.
F. Exposure = Amount ai Handled * Unit Exposure
G. MOE = NOAEL / Exposure where the NOAEL is 133 ug/m$^3$ from Sung, 2009.
H. The target MOE is 1000 which includes uncertainty factors of 10X for interspecies variation, 10X for intra-species extrapolation and 10X for database uncertainty. MOEs that are less than 1000 indicate risks of concern.

Dermal Exposures

Because there are no dermal toxicology studies available, route to route extrapolation from the oral toxicity study was used to calculate the risks from dermal exposures, as for toddlers (above). As shown in Table 6, the MOE for dermal exposures is 610,000 which exceeds the target MOE of 1000 and indicates that the risk is not of concern. It should be noted, however, that this MOE is calculated on the assumption that the workers would wear protective gloves while handling AGS-20.

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

| Scenario | Amount of ai added or handled[A] | Unit Exposure[B] (mg/lb ai) | Exposure[C] (mg/day) | Dose[D] (mg/kg/day) | MOE[E] |
|---|---|---|---|---|---|
| Mix/Load AGS-20 Powder During Textile Treatment | 2 lb | 0.17 | 0.34 | 0.0049 | 610,000 |

A. Based on the same assumptions that were used for the inhalation MOE (see previous table)
B. PHED unit exposure data for wettable powder assuming gloves are worn.
C. Dermal Exposure = Amount ai Handled * Dermal Unit Exposure
D. Dermal Dose = [Dermal Exposure * Dermal Absorption Factor (1%)]/ Body Weight (70 kg)
E. Dermal MOE = NOAEL/Dermal Dose where the NOAEL is 30 mg/kg/day

Conclusions Regarding Occupational Risks

The material to which the workers will be exposed depends upon the condition of the AGS-20 powder. If the powder remains intact during handling and silver nanoparticles do not break away from the aggregate, workers would be exposed only to micron sized particles which are similar to wettable powder. Although not conclusive, the submitted literature study suggests that this might be the case because the levels of nanoparticles did not greatly increase during the handling of the finished product, which would be expected if the silver nanoparticles broke away from the silica matrix. This finding will be confirmed when the required additional applicator indoor exposure study is completed.

The inhalation MOEs of 8 and 430 indicate risks of concern because they are less than the Target MOE of 1000. These MOEs, however, are not precise estimates of risk because it is not known if the test material used in the 90 day inhalation toxicity study has the same properties as AGS-20. The dermal MOE of 610,000 indicates that risks for dermal exposure to silver nanoparticles are not of concern even when the uncertainties are considered. Given that the calculated inhalation risks are of concern and given the uncertainties in the unit exposures and toxicological endpoints used in the inhalation exposure calculations, occupational handler inhalation exposures should be minimized by using 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, as a secondary method of exposure control, EPA proposes to require workers to wear full face respirators with high efficiency filter cartridges (i.e. P100); these respirators will provide a protection factor of 50 (assuming that they are properly fitted). The use of gloves and Tyvek suits to minimize dermal exposure will also be required given the uncertainties in the dermal risk estimates.

Given the engineering control and PPE requirements, the Agency anticipates that the inhalation MOEs will be greater than 1000 and the risks will not be of concern. The efficacy of the engineering controls such local exhaust ventilation is dependent upon their specific design characteristic but in general it can be assumed that local exhaust without enclosure will result in a 3 to 10 fold reduction in exposure (AIHA, 2006, Marquart, 2008, Burgess, 1995) while local exhaust with enclosure will result in a 30 to 100 fold reduction in exposure (Marquart, 2008, AIHA, 2006). With respect to closed system loading, the use of containment without local exhaust ventilation can result in a 3X reduction in exposure (Marquart, 2008) and the use of water soluble packaging can result in a 180X reduction in exposure (EPA, 1998).

## V. ENVIRONMENTAL RISK ASSESSMENT

Based on the proposed use pattern of HeiQ AGS-20 in treated textiles, the AGS-20 nanocomposite and nanosilver particles in singular or aggregate forms may enter the environment through washing or disposal processes. In addition, silver ions will be released from AGS-20 treated textiles or from particles that break away from the treated textiles. Wastewater from washing cycles may contaminate streams and surface waters causing adverse effect to aquatic species. Solid waste (biosolids) removed during wastewater treatment may be deposited on agricultural fields where it could adversely impact terrestrial animals and plants. When treated articles like fabric are disposed of by land filling or incineration, the nanocomposite, nanosilver, or an aggregate of nanosilver may contaminate the environment. Therefore, the characterization and identification of the nanocomposite, nanosilver, and its aggregates in each environmental media are critical factors in assessing the environmental fate and transport of HeiQ AGS-20.

This section discusses the environmental fate of silver nanoparticles then discusses the environmental hazards posed by silver and nanosilver particles and concludes with a quantitative assessment of the risks. As part of these discussions, additional data that EPA proposes to require in order to reduce its uncertainty with respect to assessing the risks of HeiQ AGS-20 are identified.

### A. Environmental Fate and Transport

HeiQ has not conducted any studies to characterize the environmental fate of HeiQ AGS-20. However, HeiQ has submitted a number of literature studies to address the issues outlined above. The Agency has reviewed these studies and has noted a number of limitations with respect to their applicability to support a complete assessment of the risk of AGS-20 to the environment.

a. ***"Nanoparticle Silver Released into Water From Commercially Available Sock Fabrics", Benn and Westerhoff, 2008.*** This study attempts to characterize the effects washing can have on various types of nanosilver-treated textiles like socks. The work was conducted on pre-leached and post- leached socks. Silver was observed to be released from the treated items. The study conditions, however, do not reflect typical washing scenarios and it is possible that sample preparation methods may have impacted the integrity of the study. Moreover, the analytical methods may not have of been adequate to identify all silver forms released from the treated items.

b. ***"Nitrification Inhibition by Silver Nanoparticles", Choi and Hu, 2009.*** This study monitors the effect that 15 nm nanosilver particles have on the nitrification process--oxidation of ammonia by certain bacteria in soils. Inhibitory effects of the evaluated nanosilver on nitrification process were greater than that observed for colloidal silver and silver ions. This study indicates that nanosilver in the size range of 15 nm is toxic to some microbes. However, it is unclear if the 15 nm nanosilver particles used in the study represent the size of the nanosilver particles present in HeiQ AGS-20.

c. ***"The speciation of Silver Nanoparticles in Antimicrobials Fabric Before and after Exposure to a Hypochlorite/detergent Solution", Impellitteri et al., 2009.*** The study monitors one type of sock treated with a nanosilver-based product in the presence of bleach and sodium chloride. The treated items are shown to be stable in the presence of sodium chloride; however, in the presence of bleach elemental silver was shown to oxidize and convert into insoluble silver chloride. This result supports the likelihood that nanosilver products will end up in waste water streams or surface water as insoluble silver chloride when exposed to oxidizing agents.

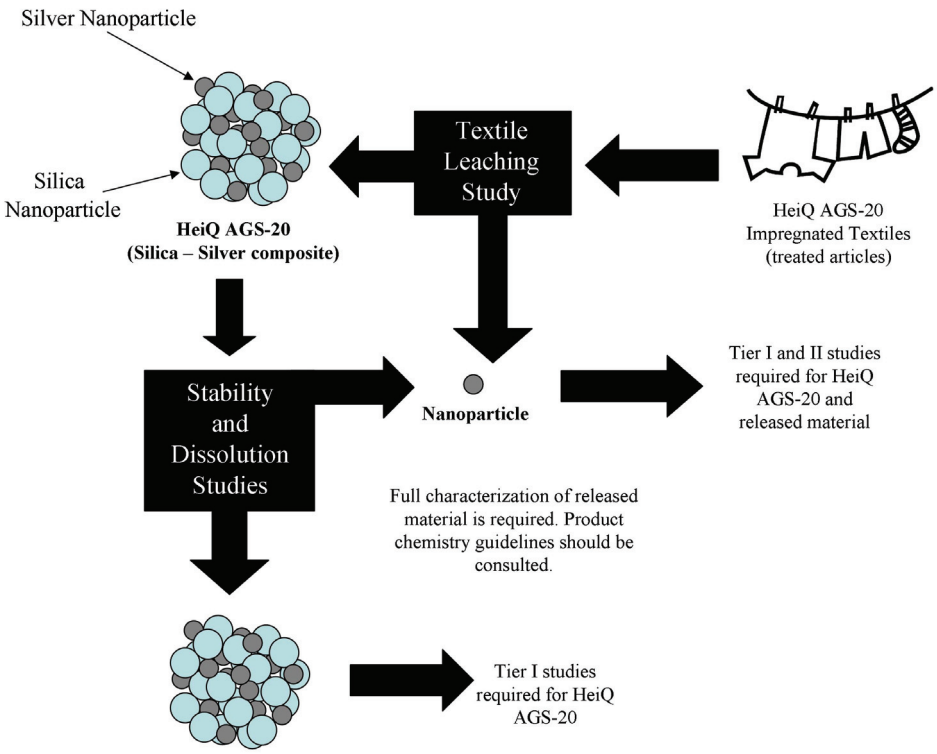
d. ***"Behavior of Silver Nano-Textiles during Washing", Geranio et al., 2009.*** In addition to the washing machine testing discussed in the residential exposure section, this study included dissolution studies on silver nanoparticles (NPs) and immersion testing on AGS-20 treated polyester textiles. The effect of pH, surfactants and oxidizing agents was evaluated. The results for the dissolution studies show that little dissolution of silver NPs occurs under conditions relevant to washing (pH 10) with dissolved concentrations 10 times lower than at pH 7. However, bleaching agents such as hydrogen peroxide or peracetic acid (PAA - formed by the perborate/TAED bleach system) can greatly accelerate the dissolution of silver NPs. The results of the textile immersion testing indicated that for the polyester with incorporated AGS-20, the silver ion concentration in the immersion water remained below the limit of detection of 10.7 ug/liter during the entire experiment, and for the polyester treated with AGS-20 in a surface coating the silver ion concentration rose to 107 ug/liter during the first 50 minutes and continued on a slight rise until the end of the experiment at 240 minutes. The rate of increase was not affected by the addition of 0.1 M PAA and was only slightly affected by the addition of 0.5M PAA. The analysis of the immersion water with and without filtration indicated that most of the silver released from the surface treated fabric was ionic while most of the silver from the incorporated fabric was particulate >450nm.

The Agency concludes that these studies are relevant and help with understanding the behavior of silver nanoparticles and silver nanoparticle-treated items under various environmental conditions. However, the studies do not necessarily represent HeiQ AGS-20 and are insufficient to fully characterize the environmental fate and transport for HeiQ AGS-20 and the silver moieties (nanoparticles, silver ions, nanocomposites, etc.) resulting from HeiQ AGS-20.

To this end, the Agency is requiring additional environmental fate and transport studies. This requirement is based on a tiered approach as shown in Figure 1. This tiered approach consists of two tiers (Tier 1 and Tier 2) and is based on a conceptual exposure model that takes into account

the proposed use of HeiQ AGS-20. Tier 1 studies will give preliminary data on potential environmental exposures. These studies must be conducted with HeiQ AGS-20. Tier 2 studies will provide quantitative data that can be used in the risk assessment conducted by the EPA for HeiQ AGS-20. These studies must be conducted on HeiQ AGS-20 as well as all materials released from it as determined from Tier 1 studies. Therefore, if needed, Tier 2 studies should be conducted following Tier 1 studies. The Agency is requiring a Tier 1 dissolution study for HeiQ AGS-20 and based on the results of this study, additional Tier 2 studies including a Fish Bioconcentration Factor (BCF) test and Adsorption/Desorption (batch Equilibrium) test may be required. Data justifications are provided in Appendix A.

**Figure 1 - Test Material and Tiered Approach for HeiQ AGS-20**



## B. Environmental Toxicity

EPA has considerable data on the environmental hazards posed by the release of silver ions from conventional silver-based products. For environmental concerns, the ecological fate database is defined; ecological toxicity data indicate that silver is toxic to fish, aquatic invertebrates and estuarine organisms. Because of use patterns for silver, however, EPA does not expect unreasonable adverse effects to the environment from the uses. Silver is currently being re-evaluated through the Agency's Registration Review program.

In contrast, the information available to assess the potential hazards of nanosilver to non-target wildlife is limited. The information below summarizes some of the available ecotoxicity data relating to nanosilver found in the literature.

a. ***"Toxicity of Silver Nanoparticles to Chlamydomanas reinhardtii", Navaro et al., 2008.*** This short-term toxicity study measured photosynthesis of *Chlamydomonas reinhardtii*, a freshwater algae, in a suspension of carbonate coated silver nanoparticles (Ag NPs; approximately 10-200 nm with a mean particle size around 25 nm) and silver nitrate ($AgNO_3$). The toxicity ($EC_{50}$) was higher for $AgNO_3$ compared to AgNPs, based on total silver concentration. When the $EC_{50}$ were calculated based on the concentration of the silver ion ($Ag^+$) present in each solution before incubation, the toxicity of the AgNPs was higher than $AgNO_3$. The authors also showed that the amount of bioavailable $Ag^+$ is directly related to its observed toxicity; supporting the free ion activity model for $Ag^+$ toxicity. However, the observed increase in toxicity of AgNP ($EC_{50}$ based on $Ag^+$ concentration) to *Chlamydomonas reinhardtii* cannot be explained solely by the concentration of $Ag^+$ present in each solution before incubation.

b. ***"In Vivo Imaging of Transport and Biocompatibility of Single Silver Nanoparticles in Early Development of Zebrafish Embryos" Lee et al., 2007.*** This study shows that citrate coated silver nanoparticles (AgNPs; 5– 46 nm) were transported into and out of Zebrafish embryos. Individual AgNPs were observed inside embryos at every developmental stage in normally developed, deformed, and dead zebrafish. AgNPs toxicity was shown to be dose-dependent with a critical concentration of 0.19 nM (0.021 ug/liter). At lower AgNP concentrations of 0.08 nM (0.0086 ug/liter) only a few dead and deformed zebrafish were observed; however, as AgNP concentrations increased, the frequency of deformed and dead zebrafish increased. This study indicates that the incidence of abnormalities in the zebrafish likely depends on the rates of passive diffusion of the silver nanoparticles, as well as accumulation of silver nanoparticles in the embryos.

c. ***"Toxicity of Silver Nanoparticles in Zebrafish Models" Asharani et al, 2000.*** Zebrafish embryos were incubated with Bovine Serum Albumin "BSA" or potato starch coated silver nanoparticles (AgNPs) to determine deleterious effects and distribution patterns. A dose-depended increase in mortality and hatching delay was observed for embryos incubated with AgNPs. Defects observed included bent and twisted notochord, in addition to accumulation of blood in the blood vessels near the tail, low heart rate, pericardial edema, and degeneration of body parts. Silver nanoparticles were found

distributed in the brain, heart, yolk and blood of embryos. No developmental defects were observed for embryos incubated with silver ion ($Ag^+$) or the capping agents but the concentrations used are not directly comparable to the AgNP concentrations used in the study.

d. ***"The Effects of Silver Nanoparticles on Fathead Minnow" Laban et al., 2010.*** This study shows that commercial silver nanoparticles (AgNPs; 20– 280 nm) are rapidly taken-up by fathead minnow during incubation. Exposure of fathead minnow embryos to AgNPs induced a variety of developmental abnormalities. Defects include absence of air sac, pericardial and yolk sac edema, hemorrhages, and lordosis. The study also reports that sonication increases the relative toxicity of AgNPs 10-fold. The authors attribute this increase to an increase in the bioavailability of the AgNPs and not the amount of silver released from the AgNPs. Based on the concentration of dissolved silver (silver concentration determined after filtration through a 0.02 µm membrane), the authors demonstrate that silver nitrate ($AgNO_3$) is more toxic than the AgNPs.

It is difficult to compare these studies because of the differences in the experimental conditions (e.g. water or growth media chemistry, nanoparticle size and capping agent variability, and taxa) and the differences and uncertainties in the methods that were used to quantify the dosages. In general, these studies indicate that exposure to silver nanoparticles can result in developmental defects and bioaccumulation of silver nanoparticles in aquatic organisms. It is also difficult to pinpoint the toxicity threshold for nanoparticles in comparison to ionic silver. Although limited ecotoxicity data are available for nanosilver, the existing data seem to indicate that its effects are different and/or more severe than for silver. Given this preliminary indication, the Agency requires additional data to assess the hazards resulting to fish, aquatic invertebrates, birds and algae resulting from exposures to AGS-20 nanosilver.

## C. Environmental Exposure Assessment

Screening Level Assessment Using the Down-the-Drain Model

The concentration of ionic silver in surface water resulting from the proposed use of HeiQ AGS-20 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:

- Production Volume: 4500 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: 356 days per year. This assumes that each t-shirt treated with HeiQ AGS-20 releases all its silver as silver ions over the course of one year.
- Waste Water Removal: 88%. This value is based on a study of Publicly Owned Treatment Works (POTWs) published by the US EPA Office of Water (US EPA, 2003).
- Stream Dilution Factor: 1.0X or 20.1X. These values are the 10th and 50th percentile values for the dilution that occurs during one day of lowest stream flow over a ten year period (1Q10).

- The toxicity value: An acute $LC_{50}$ of 0.19 ug/L for *Daphia magna* (MRID 000545-96). This value is protective of other organisms which are less sensitive to silver.
- Level of Concern for the RQ: The 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 7 and these results were divided by the LC50 of 0.19 ug/L for *Daphia magna* to obtain risk quotients (RQs). The RQ of 0.068 at the worst case stream dilution factor of 1.0X indicates a risk concern for listed species; however, it does not indicate a risk of concern for non-listed species. It is important to note that the stream dilution factor of 1.0X means that the stream is fed entirely by the waste water treatment plant and has no other source of water. The RQ of 0.0034 at the 50$^{th}$ percentile stream dilution factor of 20.1X is not of concern for either listed or non-listed species.

**Table 7 - Down-the-Drain Estimates of Silver from AGS-20 in Surface Water.**

| Waste Water Treatment Removal[A] | Stream Dilution Factor | Surface Water Silver Concentration (ug/liter) | Risk Quotient[D] | RQ Exceeds LOC?* |
|---|---|---|---|---|
| 88% | 1.0X [B] | 0.013 | 0.068 | Yes, for listed species |
| | 20.1X[C] | 0.00065 | 0.0034 | No |

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

Notes for Table 7

A. Silver removed from wastewater during treatment before discharge to a water body (e.g. lake, river etc.).
B. 10% Percentile dilution factor for 1Q10 stream flow.
C. 50% Percentile dilution factor for 1Q10 stream flow.
D. RQ = Surface Water Concentration / $LC_{50}$ for *Daphnia magna* (0.19 ug/liter)

Refined Assessment Using the Biotic Ligand Model

The Biotic Ligand Model (BLM) Version 2.2.3 (Hydoqual Inc., 2007) was used to refine the risk quotients obtained from the down the drain screening level assessment. This model uses water chemistry data from 811 surface water sites in the United States to calculate RQs that incorporate the biotic ligand into a chemical equilibrium framework that includes aqueous metal complexation, the relation between free metal ion concentrations and toxicity. The following values were used as inputs:

- Silver surface water concentration: 0.013 ug/liter. This is the highest concentration that was obtained from the down the drain model.
- Nitrate Concentration: 10 mg/liter. This is the National Recommended Water Quality Criteria for Non-Priority Pollutants (EPA, 2009a). A value of 45 mg/liter was also tested in the model and did change the resultant RQs.
- *Dapnia magna* was selected as the target organism because it is the most sensitive species.

The RQs obtained from the BLM are shown in Table 8. The RQs range from 0.0024 to 0.021 depending upon which of the 811 sites is used by the BLM in the calculations. All of these RQs are less than 0.05 which means that the risks are not of concern for listed species.

**Table 8 – Summary of BLM RQs for Silver from AGS-20 in Surface Water.**

| Silver Surface Water Concentration | Nitrate Concentration | Minimum RQ* | Average RQ* | Maximum RQ* | RQ Exceeds LOC?* |
|---|---|---|---|---|---|
| 0.013 ug/liter | 10 mg/liter | 0.0024 | 0.012 | 0.021 | No |

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

Neither the Down the Drain Model or the BLM can distinguish between silver nanoparticles or silver ions and for the purposes of this assessment, it is assumed that all of the silver is released from AGS-20 as ionic silver. In addition, this assessment only considers silver that could be released by AGS-20 and does not include the other known sources of silver which will all contribute to the environmental loading of silver. Lastly, it is important to note that the toxicity values are based on ionic silver and it is not known if they are applicable to silver nanoparticles.

## VI. PROPOSED REGULATORY ACTION

### A. 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 the statute requires EPA to make three findings in order to grant a conditional registration for a pesticide product containing a new active ingredient:

1. Insufficient time has elapsed for the generation of data since the requirement for that data was imposed;
2. Use of the pesticide is in the public interest; and
3. Use of the pesticide during the period that the newly required data is being developed and reviewed by the Agency will not cause unreasonable adverse effects.

## B. Findings and Proposed Decision

The Agency is proposing to issue a conditional, four year time limited registration for HeiQ AGS-20 in accordance with FIFRA section 3(c)(7)(C) and to require as a condition of registration that the company provide data from a number of studies that will allow the Agency to more confidently characterize the potential risks associated with this material. 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.

In order to mitigate occupational risks, label language is required to reduce potential exposures. Specific label revisions to the labeling as currently proposed will include the following:

1. The application rates must be limited to 100 ppm silver by weight.

2. Engineering controls such as closed system loading or local exhaust ventilation must be used when handling AGS-20 powder.

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.
   - Coveralls that cover the arms, legs and torso.
   - NIOSH Certified Full face respirators with P100 or equivalent filter cartridges.

## C. Basis for Conditional Registration

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

### 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 made a final determination that HeiQ's product contained nanosilver, and that EPA would treat nanosilver as a new active ingredient. Throughout the application review process, EPA and HeiQ have discussed what data might address the Agency's concerns. Although HeiQ has attempted to provide EPA with data in an effort to address Agency questions and concerns, until very recently, EPA had not reached a position with regard to which types of data would be 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 a screening level risk 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 screening level risk assessment were not conducted with AGS-20; rather the studies were conducted using other silver nanomaterials. As indicated in the 2010 SAP report, nanomaterials such as HeiQ AGS-20 are physically and chemically different from single molecules or bulk materials of the same substance, and these differences may impart unique 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 that the screening level risk assessments for the AGS-20 nanosilver-silica composite provide an accurate assessment of the risks. 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 proposing to require these studies as a condition of registration, allowing sufficient time for the studies to be conducted and for the Agency to review them. Ultimately, the Agency will use these data to determine whether the ingredient can be registered under FIFRA section 3(c)(5).

As discussed above, a listing of the studies that are needed for the registration of HeiQ AGS-20 under FIFRA section 3(c)(5), are in Appendix A.

## 2. Public Interest

As required under FIFRA section 3(c)(7)(C), the Agency has determined that the benefits expected from this proposed pesticide product, together with other considerations, justify the conclusion that granting a conditional registration 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. Moreover, considerations relating to market equity and international trade and promoting innovation lend further support to a determination that a conditional registration is in the public interest. These latter two points are discussed in more detail below:

<u>Conservation of the Environment</u>

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 additive, and subsequently 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 (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 $AgNO_3$]. Compared to the amount of silver in HeiQ's product, most currently

registered silver-based materials preservatives require 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 exposure to 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.

Consumer Benefits

A nanosilver materials preservative should maintain its efficacy longer than other silver active ingredients due to an expected gradual and controlled release of silver from the nano material as opposed to the rapid release of for example, silver from the zeolite structure and the immediate dissolution of the silver salt. While other silver active ingredients are more effective in applications where high silver concentrations are required immediately, their effects are only short-lived. In contrast, the Agency believes that AGS-20 will allow slow and controlled release of silver ions, resulting in more prolonged antimicrobial activity as described in the Efficacy Review posted in the docket for today's action (EPA, 2009). Data provided to the Agency by HeiQ supports this theory and will be confirmed by the required leaching study. Thus, consumers purchasing textile treated with nanosilver should receive a durable antimicrobial protection with less silver than other conventional silvers on the market.

Market Equity and International Trade

The regulatory framework established in FIFRA generally reflects a policy of providing equitable or fair consideration of pesticides that are similarly-situated, so that competing products are given the same opportunity to enter the marketplace. Evidence that FIFRA intends to protect similar interests equally includes the Section 3(c)(5) bar on denying a pesticide registration because it is not essential. Further, the authority to conditionally register pesticides was added to FIFRA in 1978 to address perceived unequal treatment in registering similar pesticides. See S. Rep. No. 95-334 (1977). Previous to the addition of this authority there was a "needlessly anti-competitive" "double standard" where "some producers enjoy[ed] an advantage over those who wish[ed] to enter the market by virtue of the date of their registrations." Id. Moreover, due to improvements in scientific knowledge which over time lead to new data requirements, an anomaly exists such that the Agency "often knows more about the safety of a new pesticide which is excluded from registration and use until all data are in than [the Agency] does for a previously registered pesticide that stays on the market." Id. By adding the authority to register a new pesticide while imposing conditions to deal with missing data, Congress sought to eliminate these barriers and intended to level the playing field between already registered and new pesticides. See id.

Specifically, Section 3(c)(7)(A) of FIFRA provides that the Agency may conditionally register pesticides that are identical or substantially similar to currently registered pesticides or pesticides that differ only in ways that would not significantly increase the risk of unreasonable adverse effects on the environment.

Recently, some current registrants of silver-based antimicrobial products have reported that four of their products contain nanoscale silver material. On the surface, these products appear to be

somewhat similar in composition and use to the product HeiQ is proposing, although not similar enough to warrant considering the HeiQ application under the FIFRA section 3(c)((7)(A) standard. At least one of the currently registered products identified as containing nanosilver has been on the market for many years. While EPA approved these registrations without knowledge that these products contained nanoscale silver and without specifically assessing any potential risks that might be associated with the specific nanoparticles contained in those products, they are on the market. Arguably, this unfairly disadvantages HeiQ because HeiQ's competitors are in the market ahead of them.

While the HeiQ nanosilver is a new active ingredient and thus does not meet the criteria of Section 3(c)(7)(A), it is similarly situated because it is a nanosilver active ingredient, and EPA now has information indicating that there are some nanosilver products already registered. None of the nanosilver products have submitted the types of data that the Agency is proposing to require as a condition for the registration of HeiQ's nanosilver product. The Agency believes that in connection with this newly required data, treating all of the registered nanosilver products equally is in the public interest. In light of this, and in concert with the Agency's request to HeiQ for additional data as part of the proposed conditional registration, the Agency intends to require that similar data be developed to support the continued registration of these other nanoscale products as well.

Finally, the AGS-20 nanosilver composite product has been approved for sale in Switzerland and is produced by HeiQ, a Swiss based company. EPA does not want to create an unintentional disadvantage for any registrant. EPA attempts to provide fairness in its regulatory activities so that companies making comparable products receive equal treatment and have the same opportunities to market their products.

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.

Developing new technologies can, however, be expensive and unpredictable, and meeting regulatory requirements can add costs and delay the marketing of new products. Overly burdensome regulatory requirements and / or unnecessarily lengthy regulatory reviews could discourage technology providers from pursuing the development of beneficial new applications of nanotechnology in the field of pesticides. Consequently, EPA thinks it is appropriate to consider the potential impacts of its regulatory process and decisions on the incentive to pesticide developers to pursue the application of nanotechnology.

In the case of the HeiQ application, EPA's conditional registration of HeiQ's product and the imposition of additional data requirements is reasonable in that it will ensure EPA receives the information it needs to confirm its screening level assessment of the safety of AGS-20, while

allowing an innovative product to reach the market already being served by competitors in a reasonable period of time.

### 3. No Unreasonable Adverse Effects

As noted in section III, EPA lacks information to conduct a complete assessment of the potential risks to human health and the environment associated with the use of AGS-20 as a materials preservative. Based on available information, however, EPA conducted a screening level assessment of risks to human health and the environment associated with the use of AGS-20 as a materials preservative. As a result, EPA believes that the likely risks from the use of AGS-20 during the period of the proposed conditional registration appear to be small and the overall exposure from such use is lower than exposures resulting from existing uses of nanosilver. At the same time, the use of AGS-20 would provide benefits both from the decreased release of silver to the environment and from likely extended antimicrobial efficacy compared to other silver-based antimicrobial products, as described in the Public Interest section above. After weighing the potential risks and benefits, EPA concludes that the proposed registration would not cause unreasonable adverse effects on the environment during the period of the proposed conditional registration. This conclusion is based on the following findings:

<u>Risks to Human Health</u>

The risk to humans depends on both the toxicity of a substance and the level and duration of exposure to a substance. As discussed above, humans could be exposed to silver ions, to AGS-20 (the nanosilver-silica composite), and to nanosilver particles. With respect to silver ions, the Agency notes that safe exposure levels for silver have been established by several regulatory agencies including FDA, OSHA, and EPA based on the common endpoint argyria and using the same human studies. Argyria is a blue-gray discoloration of the skin and is not considered as being of toxicological concern. The Agency has completed a risk assessment (EPA, 1993) of conventional silver and silver salts, and the risks are acceptable. In light of the estimates of low dermal and oral risks, findings from the textile leaching studies, as well as the absence of any incidents of argyria associated with the use of currently marketed nanosilver consumer products, the Agency concludes that human exposure to silver ions from the use of AGS-20 and textiles treated with AGS-20 is not likely to approach a level that causes argyria.

With respect to AGS-20, the acute toxicity of AGS-20 nanosilver silica composite is low by all routes of exposure and the textile leaching studies indicate that exposure to AGS-20 will be low. Because there are no subchronic or chronic oral or dermal toxicity studies available for AGS-20 or on the nanoparticles that might break away from textiles treated with AGS-20, these exposures were estimated using a study on silver nanoparticles that is reported in the literature (Kim et al., 2007) as a surrogate. The risk calculations indicate that the MOEs for dermal and incidental oral exposures exceed the target MOE of 1000 by at least an order of magnitude. The Agency recognizes the multiple uncertainties regarding these estimates. Even so, the Agency considers it unlikely that either AGS-20 or nanosilver particles that break away from AGS-20 would exhibit significantly greater toxicity compared to the nanosilver materials for which data are available, such that they would pose a risk of concern during the period of conditional registration. Whatever occupational risks may exist will be significantly reduced through the

required use of engineering controls and PPE. Thus, EPA concludes that neither acute occupational nor acute residential exposure would pose a risk of concern during the period of conditional registration.

With respect to nanosilver and nanoscale particles of the silver-silica composite, EPA lacks data to assess completely either the toxicity or the human exposure to such materials. The available leaching data indicate, however, that release of these substances is generally limited. To the extent that these materials might be released during the acute toxicity studies with AGS-20, they did not cause significant acute toxic effects.

Apart from the studies specifically considered in the screening level assessment of the HeiQ product, EPA has considered information on other nanosilver products. The Agency expects that the overall exposure from use of AGS-20 would be lower than the corresponding exposures resulting from existing uses of nanosilver. The Project on Emerging Nanotechnologies (PEN) has compiled an inventory of all consumer products reported by their manufacturers to contain nanomaterials. Based on the information PEN has compiled, nanosilver is the most common nanoscale material currently used in consumer products. Approximately a quarter of the identified consumer nanomaterial products are claimed to contain nanosilver. Nanosilver products are sold in the U.S. and in foreign markets such as Asia, Europe, Australia, and South America. As discussed in the Market Equity and International Trade section, while EPA cannot confirm the assertion, four registered antimicrobial products have recently been identified by the registrants as containing nanosilver. In addition, FDA has approved the marketing of several medical devices containing nanosilver. Specifically, FDA has authorized the sale of bandages treated with nanosilver to control post-operative infections in surgical wounds and the sale of catheters and other devices, which may come in contact with human tissues, treated with nanosilver to control the growth of microorganisms.

While EPA does not have data to quantify relative exposure to nanosilver from these products vs. HeiQ's product, EPA thinks it is reasonable to expect that nanosilver exposure resulting from the multiple consumer products and FDA-approved uses is likely to be considerably higher than the residential exposure that would result from wearing treated textile products.

In sum, the current presence of nanosilver products on the market, potentially including registered antimicrobial products, allows the Agency to anticipate that conditionally registering the HeiQ product will not add sufficient incremental human exposure to nanosilver that would cause significant adverse effects while the required additional data are being generated.

Risks to the Environment

With regard to environmental exposure, the Agency does not have enough information to determine whether exposure from the HeiQ product is to silver ions, silver nanoparticles, the silica-silver composite or any combination of the three, and to what levels the environment is exposed. The ecological fate database for silver is defined; silver is toxic to fish, aquatic invertebrates and estuarine organisms. Because of use patterns for registered silver products, however, EPA does not expect unreasonable adverse effects to the environment from silver based antimicrobial products.

EPA estimates, derived from down-the-drain modeling, of the concentrations of silver resulting from the proposed use of HeiQ AGS-20, do not exceed the Agency's estimate of the highest concentration of ionic silver in surface water to which an aquatic community can be exposed briefly without resulting in an unacceptable effect. These estimates do not take into account any potential higher toxicity of silver nanoparticles than ionic silver. Nonetheless, the available leaching data indicate that environmental release will be generally limited, and would involve relatively smaller masses of silver than other silver-based antimicrobial products. Moreover, the Agency expects that the overall environmental exposure from use of AGS-20 would be lower than the corresponding exposures resulting from existing uses of nanosilver. For the same reasons as discussed above, EPA expects conditionally registering the HeiQ product will not add sufficient incremental environmental exposure to nanosilver that would cause significant adverse environmental effects while the required additional data are being generated.

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# Appendix A –
# Data Requirements for the Registration of HeiQ AGS-20 as a Materials Preservative in Textiles

## 1.0 Introduction

HeiQ AGS-20 is a novel silver-silica nanocomposite material (Egger et al., 2009) that contains silver nanoparticles embedded in a matrix of amorphous silica which prevents agglomeration of the silver particles. This material will be formulated as a powder which will be used as a materials preservative to treat textiles by application as a surface coating or by incorporation into the starting materials (i.e. the masterbatch) prior to fiber manufacture. The human and environmental exposures that result from the use of AGS-20 and the use and disposal of textiles treated with AGS-20 will largely be a function of what materials leach or break away from the treated textile during use and disposal. The materials could include:

- Micron sized particles of the nanosilver - silica composite,
- Silver nanoparticles that break away from the composite and
- Silver ions released from the silver nanoparticles.

## 2.0 AGS-20 Product Use and Exposure Pathways

**Manufacture** - AGS-20 will be manufactured using a proprietary process where the starting materials are dissolved in a solvent and subjected to flame spray pyrolysis. The resulting material consists of silver nanoparticles embedded within a matrix of amorphous silica as shown in Figure 1. The silver nanoparticles have a typical diameter of 1 to 10 nm and the silver-silica composite material has an average diameter of approximately one micron (Egger et al., 2009). As is the case with all pesticides, the worker exposures that might occur during the manufacture of AGS-20 are outside the purview of FIFRA and fall under the jurisdiction of the Occupational Safety and Health Administration OSHA.

**Application** - It is proposed that AGS-20 will be formulated as a powder. This powder will be used to treat textiles either by application as a polymeric coating or by incorporation into the fiber during manufacture. Occupational handler exposures will occur during the addition of the AGS-20 powder at end user textile manufacturing and treatment facilities and during subsequent work activities involving the treated textile products. The highest exposures will occur as workers pour the powder into coating mix tanks for textile surface treatment or into blending hoppers for melt spinning textile fibers. Both dermal and inhalation exposures are anticipated. Therefore, personal protective equipment and/or engineering controls will be needed.

**Consumer Use** - The AGS-20 treated textiles will be used to manufacture consumer items such as clothing and it is intended that the AGS-20 treatment will provide a slow release of silver ions during the life of the treated item. These items will be purchased and used by consumers in a manner that is similar to untreated clothing with the exception that they may be washed less often due to the reduced growth of odor-causing bacteria. Exposures to humans and the environment can occur if the particles leach or break away from the treated textile. The particles could consist of either silver-silica composite particles, silver nanoparticles that break away from the composite or silver ions.

The following consumer exposure scenarios may be possible for AGS-20 based on the proposed use pattern:

- Dermal and incidental oral exposures from wearing and mouthing treated clothing.
- Inhalation exposures during machine drying.

Exposures to environment can also occur if the particles are released into the washwater.

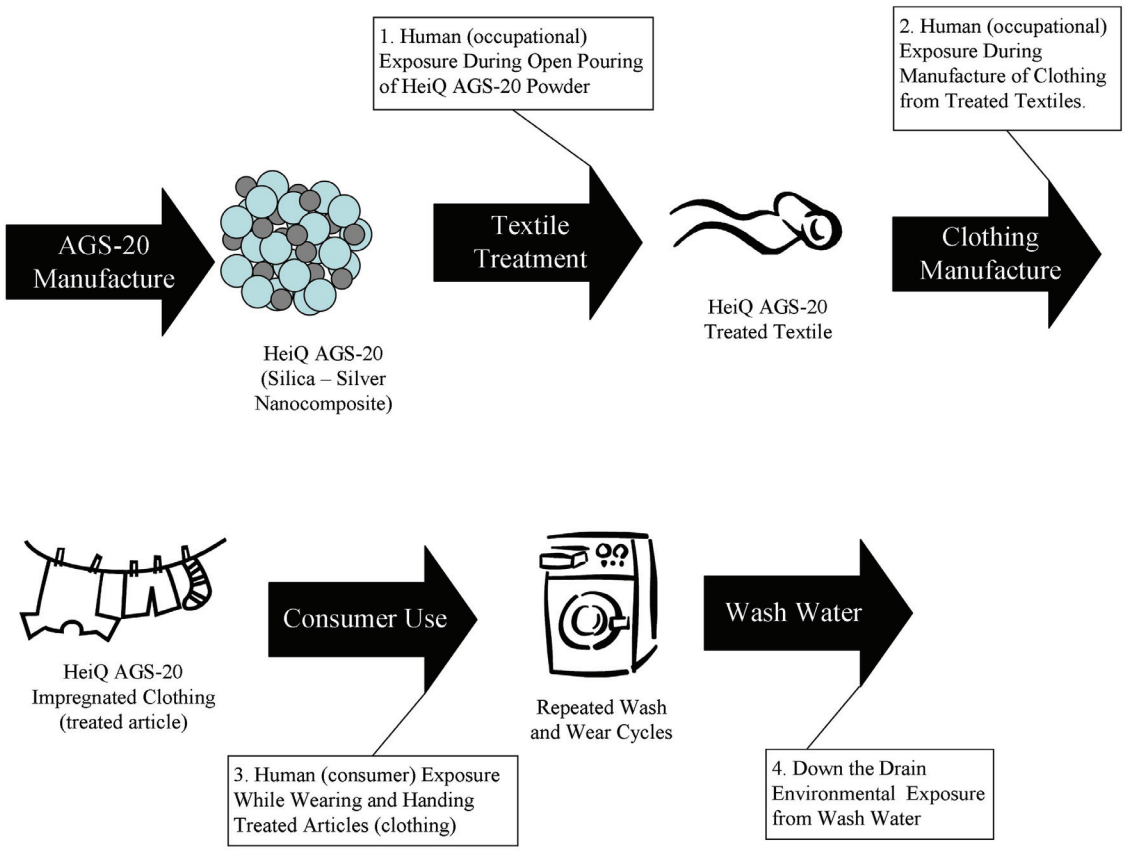


**Figure 1 – Product Use Analysis for HeiQ AGS-20**

## 3.0 Data Needed to Assess the Risks of Exposure to HeiQ AGS-20

The nanosilver in HeiQ AGS-20 is physically and chemically different than single molecules of elemental silver or bulk silver, and this difference may impart unique properties to the nanomaterials, affecting structure, function, and ultimately their environmental fate. For this reason, the data necessary for the Agency to make the required statutory safety finding for this product are provided in this document. The following factors were considered in the development of these data requirements:

- 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, non target 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 potential for exposure. Information provided by HeiQ and information from the literature was used to tailor the requirements to the proposed use pattern based upon the anticipated exposures.
- Additional studies in the area of physical and chemical characterization that are not included in 40 CFR Part 161 are needed because AGS-20 contains nanosilver. These studies are needed because nanosized materials 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 (SAP Minutes No. 2010-01) and by the MINChar Initiative (ISO TC229 WG3/PG5 Project).

In addition, the following recommendations from the FIFRA SAP report were considered in the development of the data requirements for AGS-20:

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

- Particle size substantially impact particles 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 HeiQ AGS-20 is included in Tables 1 and 2. The studies included in Table 1 are considered to be Tier 1 meaning that their need is not based on the results of other studies. The studies listed in Table 2 are considered to be Tier 2 because they may or may not be required depending upon the results of the Tier 1 studies. In the Tier 1 studies the test material will be the AGS-20 composite, however, for some of the Tier 2 studies, the test material will be dependent upon the results of the Tier 1 leaching and dissolution studies. These studies may indicate that the silver nanoparticles, in addition to the AGS-20 composite, should be tested in the Tier 2 studies. A diagram that outlines this approach is included in Figure 2.

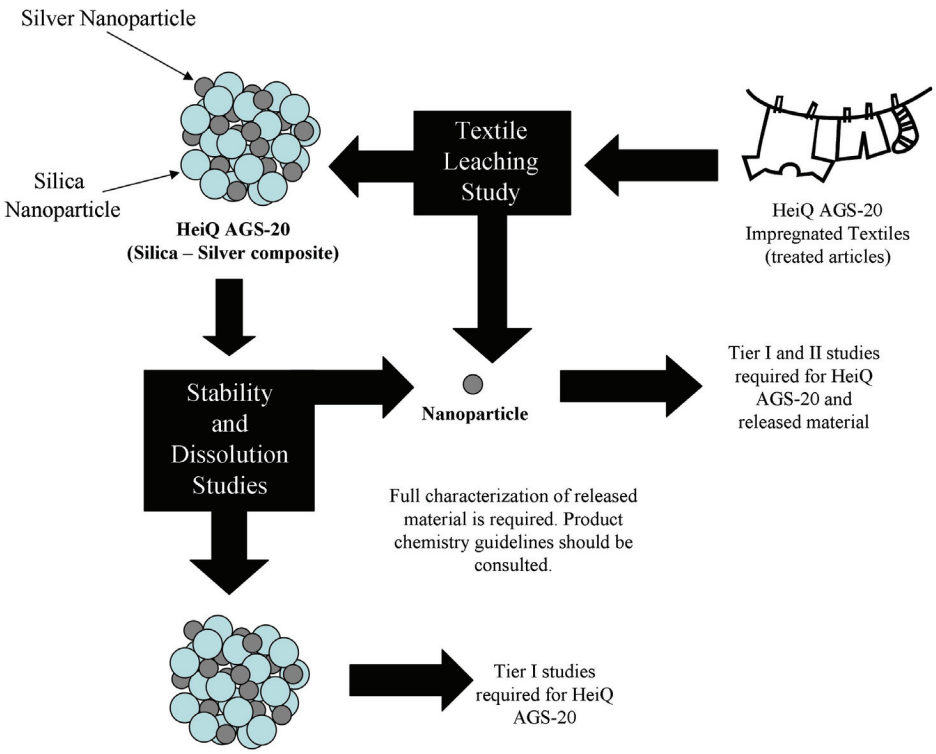


**Figure 2 - Test Material and Tiered Approach for HeiQ AGS-20**

## 4.0 Submission of Protocols

Because the guidelines are based on conventional chemicals used in industry and agriculture, they provide only general guidance on study design issues that might arise when testing nanomaterials. Due to the unique properties of nanomaterials, the Agency is requiring HeiQ to submit protocols for the majority of the studies as noted in this Appendix. Once approved, the protocols will be made available on the EPA website. 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.

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| Table 1 - Summary of Tier 1 Required Data for HeiQ AGS-20 | | | | |
|---|---|---|---|---|
| **OSCPP Data Requirement (Note 1) Guideline Number: Study Title** | **Study Status** | **Data Trigger** | **Test Material** | **Comments** |
| **Product Chemistry** | | | | |
| 830.1550: Product Identity and Composition | Incomplete | These studies are 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 the silica and nanosilver are bound and on the distribution of nanosilver in the silica matrix (uniform or heterogeneous). |
| 830.1750: Certified Limits | Incomplete | | 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 | Incomplete | | AGS-20 | Submitted method is based on analysis of total silver. Need method to include high resolution images of the active. |
| 830.1900: Submittal of Samples | No Data | | AGS-20 | |
| 830.6313: Stability to Normal and Elevated Temperatures, and Metals/Metal Ions | No data | | 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 2 studies. |
| 830.6317: Storage Stability | Not Acceptable | | 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 | No data | | 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. |
| 830.7520: Particle Size, Fiber Length, and Diameter (size) Distribution | Incomplete | | 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. |
| No Guideline: Surface Area Determination | No data | | AGS-20 | |
| 830.7840: Solubility | Incomplete | 40 CFR 161 | AGS-20 | Submitted data for pH 7. Need data for pH 5 and 9. |

| Table 1 - Summary of Tier 1 Required Data for HeiQ AGS-20 | | | | |
|---|---|---|---|---|
| **OSCPP Data Requirement (Note 1) Guideline Number: Study Title** | **Study Status** | **Data Trigger** | **Test Material** | **Comments** |
| **Environmental Fate** | | | | |
| No Guideline: Dissolution Kinetics Study | No data | Needed to assess environmental exposure from wash water. | AGS-20 | This is a fundamental 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 2 other studies. |
| **Environmental Effects** | | | | |
| 850.1010: Acute Toxicity Freshwater Invertebrates.<br>850.1075: Freshwater Fish Toxicity<br>850.2100: Avian Oral Toxicity<br>850.5400: Algal Toxicity, Tier 2 | No data | Needed to assess environmental exposure from wash water. These studies are also conditionally required per 40 CFR 161.490. | AGS-20 | The proposed use of AGS-20 may lead to surface water contamination that could impact fish, invertebrates, avian, and algal communities. These studies will address concerns regarding the potential risks of AGS-20 and will provide toxicity endpoints applicable to ecological risk assessment. |
| **Human Exposure** | | | | |
| 875.1200 and 875.1400<br>Applicator, Indoor Exposure | Supple-mental | Needed to assess 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 in the process of treating textiles. The exposure data will be used in conjunction with the toxicology endpoints to assess the risk of applicator exposure to AGS-20. This risk assessment will be used to determine what mitigation measures will be needed to prevent adverse health affects in exposed workers. |
| No Guideline: Textile Leaching Study | Supple-mental | Needed to assess human and environmental exposure. This test will determine what test substance should be used for other studies. | AGS-20 Treated Textiles | A leaching study is needed to determine what, if any, materials 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, 2009). HeiQ claims that these studies indicate that no particles were released. EPA has reviewed these studies and determined they do not support HeiQ claims of no particle release. An additional, follow-up leaching study is needed and this study could be done in the manner of Geranio et al. with the addition of electron microscopy to characterize the particulate. |

| Table 1 - Summary of Tier 1 Required Data for HeiQ AGS-20 | | | | |
|---|---|---|---|---|
| **OSCPP Data Requirement (Note 1) Guideline Number: Study Title** | **Study Status** | **Data Trigger** | **Test Material** | **Comments** |
| **Toxicology** | | | | |
| 870.3465 90-Day Inhalation Toxicity (Rat) | No Data | Inhalation toxicity studies are conditionally required per 40 CFR 161.340. In this case, the trigger for this requirement is the potential for inhalation exposure to AGS-20 based upon its formulation as a powder and its use in textiles. | AGS-20 | It is proposed that HeiQ AGS-20 will be formulated as a powder. Occupational inhalation exposures will occur during the handling of this powder during textile treatment and during manufacturing of clothing from the treated textiles. Inhalation toxicity data are needed to assess the risks of these exposures.<br><br>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. |
| 870.3200 21-Day Dermal Toxicity (Rat) | No Data | Dermal toxicity studies are conditionally required per 40 CFR 161.340. In this case, the trigger for this requirement is the potential for dermal exposure to AGS-20 based upon its formulation as a powder and its use in textiles. | AGS-20 | Occupational dermal exposures could occur during the handling of this powder during textile treatment and during manufacturing of clothing from the treated textiles. Consumer dermal exposures could occur while wearing treated clothing. A 21 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. |
| 870.3650 Combined Repeat Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test | No Data | Oral developmental and reproductive studies are conditionally required per 40 CFR 161.340. In this case, the trigger for this requirement is the potential for incidental oral exposure to AGS-20 based upon its use in textiles. | AGS-20 | There is potential for children's incidental oral exposure to AGS-20 during the wearing and mouthing of treated clothing. The combined repeated-dose toxicity study with the reproduction/developmental toxicity screening test will provide initial information of potential developmental, reproductive, and other hazard concerns of AGS-20. In addition, the study may also provide a toxicity endpoint applicable to a risk assessment for oral incidental exposure. |
| Genetic Toxicity Tests<br>870.5100, 5300, 5375, 5385, 5395 | No Data | These studies are required per 40 CFR 161.340. | AGS-20 | Genetic toxicity tests are used to screen chemicals for mutagenic or carcinogenic potential. The data from these tests will be used to determine if AGS-20 is a potential mutagen or carcinogen. |

| Table 2 - Summary of Tier 2 Required Data for HeiQ AGS-20 | | | | |
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| **OSCPP Data Requirement (Note 1) Guideline Number: Study Title** | **Study Status** | **Data Trigger** | **Test Material** | **Comments** |
| **Product Chemistry** | | | | |
| 830.7050: UV-Visible Light Adsorption | No data | These studies are needed if silver nanoparticles are released during the Tier 1 stability, dissolution or leaching studies. | Silver Nano-particles | |
| 830.7520: Particle Size, Fiber Length, and Diameter (size) Distribution | | | | |
| No Guideline: Surface Area Determination | | | | |
| 830.7840: Solubility | | | | |
| No Guideline: Zeta Potential and Surface Charge Determination | | | | |
| **Environmental Fate** | | | | |
| 850.1730: Fish BCF | No data | These studies are needed if AGS-20 or silver nanoparticles are released during the Tier 1 stability, dissolution, or leaching studies. | See Note 2 | The proposed AGS-20 use pattern may lead to bioconcentration/bioaccumation of AGS-20, or constituents of it, which may leach or break away from AGS-20 treated items. Open literature studies indicate that nanometals are not excreted by biota and can accumulate. Bioconcentration studies will assess the potential that the test substance will accumulate in fish and therefore its potential to impact the food chain. |
| 835.1230: Adsorption/Desorption (Batch Equilibrium) | No data | | AGS-20 | If the material is removed during waste water treatment, it may be deposited on land through the deposition of sewer sludge on land (i.e. land farming). If the material is not removed during waste water treatment it may be released into aquatic environments and may bind to sediment. For this reason, EPA is requesting adsorption/desorption equilibrium studies to be conducted with AGS-20. This information will be used to assess the sorption of a test material on soil/sediment and to estimate exposure. |
| No Guideline: MITI Glucose | No Data | | See Note 2 | Silver from industrial processes (e.g., film processing) has been shown to reduce microbial activity in waste water treatment systems. The purpose of the study is to assess the impact of AGS-20 or its constituents on microbial activity in waste water treatment. EPA will use this information to determine adverse effects on microbial communities in waste water treatment. |

| Table 2 - Summary of Tier 2 Required Data for HeiQ AGS-20 | | | | |
|---|---|---|---|---|
| **OSCPP Data Requirement (Note 1) Guideline Number: Study Title** | **Study Status** | **Data Trigger** | **Test Material** | **Comments** |
| **Environmental Effects** | | | | |
| 850.1010: Acute Toxicity Freshwater Invertebrates<br>850.1075: Freshwater Fish Toxicity<br>850.2100: Avian Oral Toxicity<br>850.5400 Algal Toxicity, Tier 2 | No data | These studies are needed if silver nanoparticles are released during stability, dissolution, or leaching studies. | Silver Nano-particles | The proposed use of AGS-20 may lead to the release of silver nanoparticles into surface water. This could impact fish, invertebrate, avian, and algal communities. These studies will address concerns regarding the potential risks of silver nanoparticles from AGS-20 and will provide a toxicity endpoint applicable to ecological risk assessment. |
| **Human Exposure –** No Tier 2 Studies are Required | | | | |
| **Toxicology** | | | | |
| 870.3465 90-Day Inhalation Toxicity (Rat) | No Data | This study is needed if silver nanoparticles are released from AGS-20 powder during the occupational exposure study. | Silver Nano-particles | 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. |
| 870.3200 21-Day Dermal Toxicity (Rat) | No Data | This study is needed if silver nanoparticles are released from AGS-20 powder during the occupational exposure study, or if silver nanoparticles are released during the textile leaching study. | Silver Nano-particles | Occupational dermal exposures to silver nanoparticles could occur while handling AGS-20 powder and consumer dermal exposures could occur while wearing treated clothing. A 21 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.<br><br>Although the weight-of-evidence presently indicates that nanoparticles do not penetrate intact human skin, the possibility cannot be completely ruled out. There is scientific evidence indicating that, under certain conditions (e.g., formulation, size, and/or composition), nanoparticles are able to penetrate skin and be absorbed into the body (Mortensen et al, 2008; Wu et al., 2009; and Larese et al, 2009). Due to concerns about human exposures from intact, or more likely, abraded skin (which is common), a dermal toxicity study on silver nanoparticles is required to assess the hazards of dermal exposure. |
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| Table 2 - Summary of Tier 2 Required Data for HeiQ AGS-20 | | | | |
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| **OSCPP Data Requirement (Note 1) Guideline Number: Study Title** | **Study Status** | **Data Trigger** | **Test Material** | **Comments** |
| 870.3650 Combined Repeat Dose Tox Study with Repro/Dev. Tox Screening Test | No Data | These studies are needed if silver nanoparticles are released during the leaching studies. | Silver Nano-particles | There is potential for children's incidental oral exposure to silver nanoparticles during the wearing and mouthing of treated clothing. The combined repeated-dose toxicity study with the reproduction/developmental toxicity screening test will provide initial information of potential developmental, reproductive, and other hazard concerns of the silver nanoparticles. In addition, the study may also provide a toxicity endpoint applicable to risk assessment for oral incidental exposure. |
| Genetic Toxicity Tests 870.5100, 5300, 5375, 5385, 5395 | No Data | | Silver Nano-particles | The data from these studies will be used to determine if silver nanoparticles are a potentially mutagenetic or carcinogenetic. |

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

**Note 2:** The test material should include materials that are released during the stability, dissolution/dispersability, or textile leaching studies. These materials include the AGS-20 Composite or Silver Nanoparticles released from the AGS-20 Composite.