UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

January 26, 2010

MEMORANDUM

SUBJECT: Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting held November 3 - 5, 2009 on the Evaluation of Hazard and Exposure Associated with Nanosilver and Other Nanometal Pesticide Products

TO: Steven Bradbury, Ph.D.
Acting Director
Office of Pesticide Programs

FROM: Joseph E. Bailey, Designated Federal Official
FIFRA Scientific Advisory Panel
Office of Science Coordination and Policy

THRU: Laura Bailey, Executive Secretary
FIFRA Scientific Advisory Panel
Office of Science Coordination and Policy

Frank Sanders, Director
Office of Science Coordination and Policy

Attached, please find the meeting minutes of the FIFRA Scientific Advisory Panel open meeting held in Arlington, Virginia on November 3 - 5, 2009. This report addresses the consultation meeting held on the Evaluation of Hazard and Exposure Associated with Nanosilver and Other Nanometal Pesticide Products.

Attachment

cc:

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James J. Jones
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Vicki Dellarco
William Jordan
Margie Fehrenbach
Keith Matthews
Donald Brady
William Diamond
Jack Housenger
Tina Levine
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OPP Docket

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Carey N. Pope, Ph.D. (Session Chair)
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Il Je Yu, Ph.D.

SAP Minutes No. 2010-01

A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding:

Evaluation of the Hazard and Exposure Associated with Nanosilver and Other Nanometal Pesticide Products

November 3 - 5, 2009
FIFRA Scientific Advisory Panel Meeting
Held at the Environmental Protection Agency
Conference Center
Arlington, VA

Notice

These meeting minutes have been written as part of the activities of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP). The meeting minutes represent the views and recommendations of the FIFRA SAP, not the United States Environmental Protection Agency (Agency). The content of the meeting minutes does not represent information approved or disseminated by the Agency. The meeting minutes have not been reviewed for approval by the Agency and, hence, the contents of these meeting minutes do not necessarily represent the views and policies of the Agency, nor of other agencies in the Executive Branch of the Federal Government, nor does mention of trade names or commercial products constitute a recommendation for use.

The FIFRA SAP is a Federal advisory committee operating in accordance with the Federal Advisory Committee Act and established under the provisions of FIFRA as amended by the Food Quality Protection Act (FQPA) of 1996. The FIFRA SAP provides advice, information, and recommendations to the Agency Administrator on pesticides and pesticide-related issues regarding the impact of regulatory actions on health and the environment. The Panel serves as the primary scientific peer review mechanism of the Environmental Protection Agency, Office of Pesticide Programs (OPP), and is structured to provide balanced expert assessment of pesticide and pesticide-related matters facing the Agency. FQPA Science Review Board members serve the FIFRA SAP on an ad hoc basis to assist in reviews conducted by the FIFRA SAP. Further information about FIFRA SAP reports and activities can be obtained from its website at http://www.epa.gov/scipoly/sap/ or the OPP Docket at (703) 305-5805. Interested persons are invited to contact Joseph E. Bailey, SAP Designated Federal Official, via e-mail at bailey.joseph@epa.gov.

In preparing these meeting minutes, the Panel carefully considered all information provided and presented by EPA, as well as information presented by public commenters. This document addresses the information provided and presented by EPA within the structure of the charge.

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Evaluation of the Hazard and Exposure Associated with Nanosilver and Other Nanometal Pesticide Products

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Held at the Environmental Protection Agency
Conference Center
Arlington, VA

Carey N. Pope, Ph.D.
FIFRA Session Chair
FIFRA Scientific Advisory Panel
Date: January 26, 2010

Joseph E. Bailey
Designated Federal Official
FIFRA Scientific Advisory Panel
Date January 26, 2010

Federal Insecticide, Fungicide and Rodenticide Act
Scientific Advisory Panel Meeting
November 3 - 5, 2009

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INTRODUCTION

The Federal Insecticide, Fungicide and Rodenticide Act Scientific Advisory Panel (FIFRA SAP) has completed its review of the Evaluation of the Hazard and Exposure Associated with Nanosilver and Other Nanometal Pesticide Products. Advance notice of the meeting was published in the *Federal Register* on September 16, 2009. The review was conducted in an open Panel meeting held in Arlington, Virginia, on November 3 - 5, 2009. Dr. Carey Pope chaired the meeting. Joseph E. Bailey served as the Designated Federal Official.

Companies with an interest in marketing products that contain nanosilver and/or other nanometals or nanometal oxides as pesticides regulated under FIFRA have approached the Office of Pesticide Programs (OPP) seeking product registration. Based on OPP's understanding of the scientific literature, it appears that there may be potential for pesticides containing nanoscale materials to pose different risks to humans and the environment than pesticides that do not contain nanomaterials. The Agency held a consultation meeting with the FIFRA Scientific Advisory Panel (SAP) to obtain advice and recommendations on the following issues associated with the identification and assessment of these potential risks:

1. Scientific evidence that nanosilver and other nanometals/nanometal oxides with at least one dimension in the range of 1 - ~100 nm have unique behavior under conditions relevant to human and environmental risk assessment and other properties (in addition to size) that may influence this behavior.
2. Recommendations regarding the types of data that OPP should require to evaluate the risks to humans and the environment for products containing free nanosilver and/or nanometals or nanometal oxides, or products with incorporated nanosilver and/or nanometals or nanometal oxides with variable potential to leach and the relative priorities for obtaining recommended types of data.
3. Recommendations regarding how OPP should conduct risk assessments of pesticide products containing nanosilver and/or nanometals or nanometal oxides.

Steven Bradbury, Ph.D., Deputy Office Director for Programs, Office of Pesticide Programs provided opening remarks at the meeting. The Agency's presentations were given by William Jordan, Dennis Edwards, Najm Shamim, Ph.D., Melba S. Morrow, Ph.D., Jenny Tao, M.D., Jessica P. Ryman-Rasmussen, Ph.D., Tim Dole, C.I.H., and Edward Odenkirchen, Ph.D., all of the Office of Pesticide Programs.

PUBLIC COMMENTS

Oral statements were presented as follows:

Jaydee Hanson, Ph.D., on behalf of the International Center for Technology Assessment
James E. Hutchison, Ph.D., University of Oregon, on behalf of Dune Sciences, Inc.
Murray Height, Ph.D., on behalf of the Silver Nanotechnology Working Group
Samantha Dozier, Ph.D., on behalf of People for the Ethical Treatment of Animals
Andrea Dreisbach, Ph.D., Noble Biomaterials, on behalf of the U.S. Silver Task Force
Gordon Pedersen, Ph.D., on behalf of American Biotech Laboratories, LLC

Written Statements were provided by:

Approximately 236 written comments were submitted to the public docket (EPA-HQ-OPP-2009-0683) at www.regulations.gov by individuals representing themselves or various organizations.

SUMMARY OF PANEL DISCUSSIONS AND RECOMMENDATIONS

The Panel was not aware of any information that suggested that silver ions released from silver nanomaterials would behave differently than silver ions generated by any other source. However, the Panel believed that the rate of silver ion production, as well as the distribution of silver in tissue, may differ substantially between silver nanomaterials and other forms of silver. Nanomaterials can deliver ions directly to specific tissues, cell membranes or inside cells. The biological effects of silver nanomaterials (including temporal pattern for ion delivery), as well as their environmental fate, can be affected by other materials present in the preparation (e.g. surfactants). Nanosilver can also potentially act as a carrier for other toxic chemicals. These issues led the Panel to suggest that the hazard profile of silver nanomaterials may differ from other forms of silver.

The Panel agreed that particle size has a substantial impact on particle properties, including rate and concentration of silver ion release, reactivity and catalytic efficiency, plasmon resonance, and quantum effects. The effects of size are generally most observable in particles below 20 nm and largely below 10 nm, leading to some concern about biological effects of very small silver nanoparticles. However, the effects of silver nanoparticle size on biological responses are less defined. In general, studies with other particles suggest that smaller particle size is associated with increased organismal uptake and distribution. Several studies suggest that this is also true for silver nanoparticles. However, at this time, direct determination of particle size on biological response is often confounded by agglomeration of particles under test conditions. Other physicochemical properties, such as shape, charge and surface coating, are also likely to impact biological response and environmental fate. Studies clearly demonstrate an impact of charge on cellular uptake of nanomaterials. The lack of a clear understanding of how particle size and other physical properties affect hazard profiles led most Panel members to be unsupportive of bridging among silver-based materials with different properties. Several Panel members felt that some bridging would be appropriate for materials of similar size with other physical properties being essentially identical. The Panel suggested that an appropriate set of metrics which incorporated size in conjunction with physicochemical or biological parameters, such as surface area or polydispersity, may be appropriate in bridging exercises, but appropriate physical or multi-criteria decision models should be developed. The Panel also suggested that available data on existing colloidal silver materials should be investigated.

The Panel indicated that they were not aware of studies which definitively answered the question whether or not agglomerated silver nanoparticles in the range of 100 nm to 1,000 nm pose different hazards than larger sized particles. Many existing studies actually exposed test systems to polydispersed nanomaterial suspensions. Improved characterization of materials under test conditions are needed. The Panel identified the following three factors which may influence the response of agglomerates relative to larger single particles: 1) differences in density and aerodynamic (hydrodynamic) parameters that are likely to influence airway deposition and environmental distribution; 2) agglomerates may have increased activity due to higher accessible surface area; and 3) agglomerates may deagglomerate leading to exposure to dispersed smaller sized nanomaterials.

Regarding exposure from "realistic use" scenarios, the Panel indicated that there are limited data available. It was agreed that virtually all uses of nanosilver will result in some release of silver, as ionic, nanoparticulate, or composites. The extent of release and nature of the silver released will depend on a variety of factors including the nature of the silver product and its use. Human exposure is likely to occur by inhalation, oral ingestion, and dermal exposure routes and will vary with the product used. Primarily, exposure is likely to be from aggregated forms of nanosilver or nanosilver associated with structural material (e.g., fibers and plastics), although inhalation exposure to dispersed nanomaterials is also possible, especially in manufacturing settings and with products that are coated to reduce agglomeration.

Environmental fate of released silver nanomaterials is somewhat unclear, though "down-the-drain" disposal will result in introduction to sewage treatment plants, where much of the material is likely to be retained in sludge. Land application of these solids or release of effluents containing particles will then lead to introduction into the environment. There was a need to reevaluate OPPT protocols for assessing interaction of nanomaterial forms of pesticides (nanosilver) with wastewater biosolids. Environmental fate of silver nanomaterials remains unclear, though the Panel again suggested evaluation of existing data on fate of products containing colloidal silver, including those used in photography.

The Panel stated that most existing models are not appropriate for use with silver nanomaterials and will not accurately predict nanosilver exposure scenarios. Most aquatic and sediment models are not adequate for nanosilver, but are adequate for ionic silver. It may be possible to modify some existing models to include additional parameters that are pertinent to nanomaterials, but uncertainty and variability of model parameters for nanomaterials makes adjustment of existing models very difficult. New models implementing novel approaches to predict environmental exposures to nanoparticles should be created. Two approaches were discussed by the Panel. The first approach includes developing very simplified exposure models using tools of multi-criteria decision analysis to allow classification of different nanomaterials (including nanosilver products with different properties) in different risk groups. The second approach would require developing complex mechanistic models utilizing the best knowledge on mechanisms governing nanomaterial behavior incorporating the following: 1) additional metrics for dose and exposure; particle size (mean and distribution); surface area; number and mass concentration; and 2) additional parameters such as particle size/distribution; particle shape; agglomeration state and rate of agglomeration/de-agglomeration or stabilization in application environments; surface chemistry (coating, charge, reactivity) of nanoparticles; and rate of dissolution. For example, information is lacking on 1) dissolution rates for nanoforms of pure Ag^0 and Ag^0 with impurities, surface imperfections or non-crystalline or amorphous coatings; and 2) effects of organic (or inorganic) surface coatings.

The Panel reiterated some of the existing data that suggest differences in toxicokinetics and toxicodynamics for nanoscale materials. The Panel strongly believed that in addition to current data requirements under FIFRA, additional assays which compared nanoscale and bulk materials would be most beneficial in addressing this question. These studies should include appropriate material characterization, such as that outlined in the draft ISO TC229 WG3/PG5 project "Guidance on physicochemical characterization of engineered nanoscale materials for toxicologic assessment" (ISO/AWI TR 13014, available at:

http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=52334) and the Minimum Information for Nanomaterial Characterization Initiative (MINChar Initiative: www.characterizationmatters.org). It is also necessary to know chemical composition and hazard properties of the end-use products, including inert ingredients (e.g., propellants, carriers, odorizers and plasticizers) in order to account for possible synergistic effects. Data to characterize exposure properties should include information on dislodging of nanoparticles from support materials, ion release under differing conditions, metabolism and mobility in the environment. Studies should be conducted with relevant forms of materials under relevant exposure conditions as defined by exposure characterization.

The Panel believed that the lack of existing data suggests that it would be necessary to conduct research on a case-by-case basis to evaluate whether silver nanomaterials remain associated with a substrate. The Panel indicated that current data requirements for antimicrobial pesticide products are a starting point, but the general use patterns and test guidelines require adjusting to accommodate the novel properties and novel uses that will likely evolve through the application of nanotechnology. In general, the Panel discussed the need for studies that provide information about the degradation of substrates containing nanomaterials; metabolism and the transformation of dislodged nanomaterials; and fate information related to leaching, dissipation and bioaccumulation. The Panel recommended that a system of metrics for environmental exposures be developed including mass (total silver), particle number and surface area concentrations. A life cycle analysis is needed to determine stability of nanosilver products over time.

The Panel disagreed that nanosilver applied to a substrate will permanently bind with the substrate and concluded that, given the state of science and measurement standards currently available, it will be especially challenging to determine that there is no release of nanomaterials from a substrate. Even if nanosilver would not be released from a substrate, the potential environmental impact of nanosilver products should be carefully evaluated based on the product life cycle. The Panel suggested use of tests to simulate realistic use of products and potential nanosilver release with subsequent quantitative life cycle assessment and risk assessment.

The Panel agreed that additional data are needed before the scientific community can conclude whether or not particles at the smaller end of the size scale (e.g., 1 nm) are likely to behave like particles at the larger end of the scale (e.g., 100 nm). 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. It was noted though that how particles are used in a particular application will affect the associated risks. The Panel noted at least two observations that could lend credence to the existence of a relationship between particle size and potential hazard: 1) biological activity and penetration through biological barriers depends on particle size and 2) particle dynamics, which affect transport and exposure, depend on size. The Panel suggested considering concentrations of particular particle sizes falling within specific ranges of sizes within a product when assessing hazard.

The Panel cautioned about extrapolating from one nanosilver formulation to another when assessing hazards. Moreover, extrapolations between nanomaterials based on different

metals could be of particular concern. Differences in particle formulation (e.g., coating and inert ingredients) are likely to affect particle properties and associated biological activity. One Panel member presented alternative risk assessment approaches that could be used for bridging. Three analytic approaches including risk assessment, life cycle assessment and multi-criteria decision analysis (MCDA) could be integrated into a framework for synthesizing both objective scientific information and subjective values-driven information relevant in decision contexts.

The Panel believed that environmental conditions can affect the properties of nanoparticles, including silver. For instance, literature indicates that ionic strength of water, natural organic matter content and pH affect particle size distribution, dissolution, aggregation rates and fate. In physiological media, particles will be rapidly coated with adsorbed proteins and other materials and the physicochemical properties of particles are likely to change as the particle moves from one physiological compartment to another. The Panel reported that published literature does indicate that the physicochemical properties of some nanoparticles do change based on the suspension matrix, temperature and pH while other nanoparticles do not change. The Panel suggested that each nanomaterial should be characterized in the primary phase (pristine, dry state), secondary phase (aqueous solution) and tertiary phase (in vitro or in vivo fluid, cells or tissues) on a case-by-case basis.

Finally, the Panel acknowledged that data gaps about potential exposures and hazards related to nanosilver are broad and there is very little information about nanosilver in the environment related to fate, transport and transformation, including what may be released from products or facilities producing nanosilver-enabled products. Data reported in current exposure and toxicity studies vary greatly with respect to control samples, particle characterization, composition, size, aggregation information and solubility of particles. Further, there is a lack of comparative toxicity studies addressing effects related to particle size, aggregation state, particle dissolution, etc. Moreover, frameworks that could be used to integrate available fragmented data to assess product risk are not available. The Panel provided a detailed framework to help guide the assessment of nanosilver products in two areas: 1) environmental fate, transport and transformation needs and 2) toxicity assessment. In addition, the Panel provided recommendations on scientific studies in support of an integrated risk-based decision framework for regulatory decision making.

PANEL DELIBERATIONS AND RESPONSE TO CHARGE

Charge Question 1A:

Potential Risks from Nanosilver Materials Issue: Whether pesticide products containing nanosilver as the active ingredient pose potential hazards and exposures to humans and the environment that are different from those associated with products containing conventional silver.

Available scientific literature indicates that nanosilver products may exert an antimicrobial effect by releasing silver ions, and that these ions may pose potential hazards to humans and the environment. The Agency is unaware of any information that would suggest exposures to silver ions released from nanosilver products differ from the hazards of silver ions released by non-nanosilver (hereafter referred to as silver) based products and, therefore, might present a different hazard profile. Is the Panel aware of any information inconsistent with this determination?

Panel Response: The Panel agreed that silver ions released into the environment by nanosilver are unlikely to present different hazards than those created through dissolution of silver salts or silver compounds in macroscopic forms. However, recent studies suggest that both "nano-sized particles of Ag", as well as "ionic Ag^+", contribute to the toxic effects of silver nanoparticles (Griffitt et al., 2009). For instance, when compared as a function of the Ag^+ concentration, toxicity of silver nanoparticles appeared to be much higher than that of silver nitrate.

Moreover, there are several major differences that could potentially result in a distinct hazard profile for nanosilver. The rate at which silver ions are released from nanosilver, nanoparticle aggregation, ion delivery at specific tissues, functionalization and surface modification of nanoparticles will likely affect the acute or chronic toxicity of nanosilver. However, it is important to note that there are no experimental studies that provide definitive conclusions in this matter and more research is required.

1. ***Nanosilver particles can deliver ions directly to specific tissues, cell membranes or inside cells.*** Nanoscale particles including nanosilver have been shown to be capable of penetrating biological barriers such as cell membranes (Verma et al., 2008) and can enter into the cells themselves (AshaRani, et al., 2009; Miura and Shinohara, 2009; Kim et al., 2009; Hussain, et al., 2005). Nanoparticles are able to attach to cell membranes, producing changes in membrane permeability, redox cycling in the cytosol, intracellular radical accumulation, and dissipation of the proton motive force for ATP synthesis. Each of these has been reported as a possible mechanism for nanoparticle toxicity (Sondi and Salopek-Sondi, 2004; Morones et al., 2005; Lok et al., 2006; Nel et al., 2006). Evidence from scanning transmission electron microscopy also shows that smaller particles (< 10 nm) may enter the cell directly to inhibit microbial growth (Morones et al., 2005, Lee, 2007) For comparison, the inhibitory effect of Ag^+ is believed to be due to its sorption to the negatively charged bacterial cell wall, generating reactive oxygen species (ROS) and deactivating cellular enzymes, disrupting membrane

permeability, and ultimately leading to cell lysis and death (Ratte, 1999; Matsumura et al., 2003; Sambhy et al., 2006)

2. Temporal pattern for ion delivery by nanosilver particles can be different from bulk silver. Silver nanoparticles have ability for protracted ion release that could result in different toxic impacts. Uncertainty remains regarding the impact of agglomeration on the rate of ion release, though the scientific consensus is that ion release from aggregates of small particles will be greater than from individual particles of similar size. The issue of acute vs. chronic impact of nanosilver and specific toxicity end points (e.g., alteration of morphology, viability and oxidative stress) related to aggregation has not been studied. There is, however, a rich history of exposure to colloidal silver through waste from products used in photography as well as other products that contain colloidal silver. Relatively little is known about these exposures (particle size, charge, dose metric utilized), but they could potentially form a useful knowledge base for understanding biological effects as well as environmental fate and transport.

3. Nanosilver can also potentially act as carriers for other toxic chemicals. Pesticide end-use products contain other chemicals (inert ingredients and impurities) and contaminants that could act synergistically. Such unintended effects have been demonstrated for polymer fumes (Johnston et al., 2000) and diesel particulates (Wallace et al., 2007). In addition, nanoparticles are intentionally utilized as carrier "platforms" in the medical field for targeted delivery of therapeutics (McNeil, 2009).

4. Nanoparticle and resulting differences in exposure and environmental fate could modify hazard profile. The concentration of silver ions may be higher in the media of nanosilver suspensions compared to the concentration of silver ions in micron-sized silver suspensions because of the increased release of silver ions from nanoscale material. If there are more ions released, then the potential exposure to ionic silver is increased and, therefore, a potential for increased response. Many studies have shown that nanoparticle risk is mostly associated with the formulation. Research at the US Army Engineer and Research Development Center has shown strong evidence of the ability of surfactants to modify nanosilver behavior (e.g., stabilization of dispersion). Such interactions suggest (though not experimentally proven) enhanced mobility through soils and in waters. An association between small particle size and enhanced nanosilver dissolution has been reported (Chappell et al. Submitted for publication).

Charge Question 1B:

Available scientific literature also indicates that, in addition to any hazards resulting from the release of silver ions, nanosilver particles themselves may present hazards that differ from those of silver particles. What, if anything, does the existing scientific literature indicate about the potential for nanosilver materials with specific particle sizes in the range of 1 to 20 nm, 21 to 50 nm, and 51 to 100 nm to pose different hazards than those of larger-sized particles of the same material, particularly nanosilver vs. silver? What does the existing scientific literature indicate about the potential for particular physicochemical properties of nanosilver materials (e.g., shape, surface characteristics, composition, etc.) to pose hazards that are different from larger-sized particles of the same material? Does existing literature support "bridging" data? In other words, can hazard or exposure data

developed on 1 to 20 nm silver particles or silver composites be used to assess the risks for 51 to 100 nm silver particles or silver composites?

Panel Response: Existing data clearly indicate that many properties of particles change with particle size, including rate of release of ionic forms of metal, reactivity or catalytic efficiency, plasmon resonance, and quantum effects. Changes in these properties are most apparent at sizes below 20 nm and markedly so below 10 nm (Elder et al., 2008 and Auffan et al., 2009). Along with size, it is also apparent that actual formulation of silver nanomaterials can have significant influence on the rate of ion release and particle reactivity.

The effect of particle size on biological responses to particle exposure is less well defined. Several studies have compared nano particulate silver particles to larger particles (hundreds of nm up to micron sized) and generally concluded that nanosized particles result in greater response than larger particles (Hwang et al., 2008). It is unclear whether the differences are due to lack of availability of larger particles, rate of release of ionic silver or other mechanisms. Existing data also suggest that silver nanoparticles can elicit effects that are not solely due to dissolution and release of silver ions (Kawata et al., 2009; Griffitt et al., 2009). The mechanism responsible for effects due to silver nanoparticles is unclear, but is likely to involve bound forms of oxidized silver on the cell surface, uptake and release of high concentrations of silver ions within cellular compartments, and disruption of membranes through catalytic or physical processes. The effect of particle size on these actions is still not well understood.

While there are limited *in vivo* data to help determine the effect size may have on the effects of silver nanoparticles, data clearly indicate that inhalation exposure to smaller particles is associated with greater pulmonary effects in mammalian models, though this is largely ascribed to increased particle surface area. Similar effects have been observed in aquatic systems with silica (Van Hoecke et al., 2009). Particle size has been shown to affect deposition in lungs and translocation from lung to blood for other particles although overall translocation is low (less than 1%) (Kim et al., 2008; Sung et al., 2008; Kreyling et al., 2002; Chen et al., 2006). Similar effects of size would be expected with silver nanomaterials.

In vitro and *in vivo* studies have demonstrated intestinal uptake of nanoparticles. Generally, uptake increases with decreasing size and is also affected by surface modification (Florence et al., 1995). Data suggest that mucus is a significant barrier to particle uptake in vivo (Behrens et al., 2002). No studies have specifically addressed *in vivo* translocation of silver nanoparticles following oral administration. A recent study reported the presence of silver nanoparticles on the surface of luminal epithelia as well as within cells and on the lamina propria of intestine following 28-day administration by gavage of 60 nm silver nanoparticles at doses up to 1,000 mg/kg (Jeong et al., 2010). Particles were associated with increased levels of mucus, altered mucus composition and intestinal cell shedding.

The role of particle size in dermal absorption has been demonstrated in intact skin using gold nanoparticles (Sonavane et al., 2008). There is a potential role for size in response to dermal exposures, where interactions with varying wavelengths of light (i.e., UVA, UVB, and visible light) could produce differential amounts of leached metal ions, ROS and heat.

Recent repeated dose inhalation and oral administration studies with silver nanoparticles suggest possible translocation of nanomaterials due to elevated tissue silver content, but it is unclear whether silver measured in these tissues is particulate. Even though routes of administration are different, hazard data produced by using 15-20 nm and 60 nm nanosilver showed similar toxic endpoints; however, the degree of toxicity may be different. A 90-day inhalation study using 18 nm nanosilver (Sung et al., 2008) and a 28-day oral ingestion study using 60 nm nanosilver (Kim et al., 2008) showed similar endpoints although lung inflammation and change in lung function was also found in the inhalation study. Both studies showed similar liver toxicity (bile duct hyperplasia). Both studies showed similar tissue distribution of silver exhibiting blood circulation with a dose-related increase of silver concentration in every tissue examined. Consistent gender differences in the accumulation of silver in the kidney were observed in both studies. A recent 90-day GLP oral study using 60 nm silver nanoparticles at doses of 30, 125, 500 mg/kg has been performed and submitted to the Korean FDA (KEMTI, 2008, unpublished report). This study also reported consistent increases in blood alkaline phosphatase and cholesterol. Tissue distribution of nanoparticles showed blood circulation with a dose-related increase of silver concentrations in various tissues. Consistent gender differences in the accumulation of silver in the kidney were also observed. The NOAEL for the 90-day study was between 30 and 125 mg/kg/day.

It is also possible that particle size will affect distribution and elimination in mammals. One study demonstrated that smaller nanodevices (5 nm) had much longer half-lives than larger devices (11 and 22 nm) with the same coating (Balogh et al., 2007). Distribution to other tissues and route of elimination were also size dependent.

Nanoparticles appear to enter cells by several routes, with size and charge playing a role in mechanism of uptake (Zhang and Montiero-Riviere, 2009; Lu et al.,2009). In vitro studies have demonstrated apparent size dependence of silver particles on cytotoxicity and generation of ROS with small particles (15 nm) being substantially more effective than larger particles (30 or 55 nm) when adjusted to the same mass (Carlson et al., 2008). It is uncertain from this study if response is due to increased cellular uptake, surface area, or increased ROS production by particles. ROS generation may be due directly to particle effects or indirectly by subsequent cellular injury. It is important to appreciate that almost immediately upon exposure to a biological fluid, nanomaterials will be coated with proteins, lipids, and polysaccharides. The nature of this corona is quite likely to influence cellular recognition and response to a nanomaterial. Recent studies clearly demonstrate an effect of size on protein corona (Lundqvist et al., 2009). It is unclear how coating with environmental (e.g., natural organic matter) or biological molecules will influence ion release and dispersion of materials.

Several studies have shown that nanosilver toxicity is size dependent (Choi and Hu, 2008; Bar-Ilan et al., 2009). Nanosilver particles of 1-10 nm were preferentially bound to cell membranes and were incorporated into bacteria (Morones et al., 2005). By examining the correlation among nanoparticle size distribution, intracellular ROS accumulation, and nitrification inhibition, it was observed that inhibition of nitrifying organisms correlated with the fraction of silver nanoparticles less than 5 nm in the suspension. It appears that sizes of nanoparticles (< 10 nm) could be more toxic to bacteria than any other fractions of nanoparticles or their counterpart bulk species (Choi and Hu, 2008).

In aquatic systems, silver nanoparticles have been demonstrated to cause toxicity to Daphnia, fish, algae and bacteria. Toxicity is clearly influenced by water chemistry and underlying susceptibility to silver toxicity. Work with zebrafish embryos suggests minimal effect of size on response (Bar-Ilan et al., 2009). However, it is important to note that in many studies, effects of size are difficult to ascertain because in virtually all *in vitro* exposures, as well as oral suspensions and aquatic exposures, particles are present as a mixture of dispersed and agglomerated particles. Studies with stable dispersions of particles having relatively narrow particle size distributions and careful monitoring of agglomeration are needed to define the potential for particular physicochemical properties of nanosilver materials (e.g., shape, surface characteristics, composition, etc.) to pose hazards that differ from larger-sized particles of the same material.

- **Physicochemical properties** - There is significant evidence that particles with substantial surface charge, especially negative charge, are taken up more rapidly and to a greater extent than uncharged particles. It is likely that this may also be true for silver nanoparticles; however, it is important to note that particle properties change substantially after contact with natural waters or biological fluids. A number of reports indicate that particles exhibit similar charges after addition to cell culture media, regardless of initial values (Alkilany et al., 2009). The effects of shape on particle uptake or toxicity are less clear. Recent work suggests that dendritic nickle aggregates are more toxic than other forms of nickel in zebrafish embryos, with effects primarily on intestines (Ispas et al., 2009). Other coatings are also likely to affect toxicity (Ahamed et al., 2008). It is also likely that the physical form of a particle preparation may affect responses. The catalytic nature of silver nanoparticles with dyes has been demonstrated and activity is enhanced by tethering to silica particles, thereby reducing aggregation of silver particles which would otherwise slow or stop catalysis (Jiang et al., 2005).
- **Bridging data** - There was some diversity of opinions among the Panel regarding the extent of bridging which would be appropriate. Hazards of silver nanomaterials are likely due to a combination of release of silver ions and effects due to the particles themselves. For the portion of the hazard due to silver ion release, bridging is feasible based on the amount of silver ion released. However, because the mechanism of toxicity of silver nanoparticles and nanocomposites is not currently known, it is difficult to determine if bridging data for effects of nanoparticles is appropriate. As indicated above, the literature suggests that particle physicochemical properties such as size, surface functionalization and chemical composition, may affect uptake, distribution and magnitude of toxicity for silver nanoparticles. Existing data on different materials suggest that there may be differential routes of exposure at the cellular level when comparing nanosilver with micron-sized silver or comparing nanosilver with silver salts. More specifically, when silver is delivered in its water-soluble salt form, the leached silver ions interact with a cellular membrane via passive or active transport (i.e., ionic channels) across the cytoplasmic membrane. In the case of silver nanoparticles, the nanoparticles are taken up into cells via lipid rafts (i.e., clathrin-mediated endocytosis) and accumulate in endosomes in the cytosol. Once these nanoparticles and nanoaggregates are in endosomes, the pH drops, accelerating the leaching of silver ions. This exposure is different than exposure to silver salt in that leached silver ions inside the

cell could react with organelles in a different manner than silver ions leached outside of the cytoplasmic membrane. These factors argue against use of bridging data for materials with differing physicochemical properties.

As indicated above, existing studies using different sizes of silver nanoparticles in mammals reveals relatively similar toxicity profiles (Sung et al., 2008; Kim et al., 2008). Thus, it may be possible to use 51 -100 nm nanosilver data to estimate toxicity of 1-20 nm particles. The particles used in these studies averaged 20 nm or 60 nm, but in both cases were not monodispersed, so overlap of particle size distributions for the 20 nm and 60 nm studies is possible. It is important to note that, depending on particle size, the toxicokinetics of nanosilver could vary with differences in tissue distribution or elimination.

There has been considerable use of colloidal silver products with sizes ranging from approximately 2 nm up to perhaps 50 nm in a variety of products including pesticides, dietary supplements and those used in photography. As indicated in the Panel's response to Charge Question 1A, available information on biological effects as well as environmental fate and transformation resulting from use of these materials should be investigated. Current evidence suggests that there are similar toxicological profiles for colloidal and spherical silver nanomaterials. With additional data, there is a potential for information obtained from colloidal products to be used to bridge to nanomaterials provided the physicochemical properties are essentially identical. Again, it is important to note that formulation is likely to play a significant role in the kinetics and dynamics of nanosilver toxicity. Many variables in addition to size need to be considered in any bridging exercise, including shape, coating, and use of surfactants or other dispersing agents that may be present with the nanomaterial.

Charge Question 1C. What, if anything, does the existing scientific literature indicate about the potential for nanosilver particles in the range of 100 nm – 1000 nm (or "agglomerated" nanosilver or nanometals/nanometal oxides) to pose hazards that are different from larger-sized particles of the same material?

Panel Response: The Panel was unaware of any dispositive literature that establishes differences in the toxicology or elicited biological response between nanosilver particles less than 100 nm diameter, aggregates of nanosilver with a dimension greater than 100 nm or individual particles of nanosilver larger than 100 nm in any dimension. There are studies suggesting differences in behavior between dispersed nanoparticles and aggregated forms. Differences in clearance and macrophage uptake following inhalation exposure to 15 nm silver aerosol and instilled agglomerates of the same material, with faster clearance of inhaled material, was reported (Takenaka et al., 2001). Nevertheless, special note was made of the general variability in the quality of material characterization in the studies purporting to use nanosilver. Many studies with poorly characterized material may, in practice, expose the experimental system to polydispersed test articles composed of nanosilver, aggregates of nanosilver and/or larger silver particles. A growing, but still relatively small, number of studies include characterization of test articles both prior to, during, and at the end of the experimental period (Sayes and Warheit, 2009). Such careful measurement, quantification and documentation of chemical composition, size, shape, crystallinity, surface properties, and surface reactivity

characteristics are required for extrapolation (bridging) exercises from which inferences on hazards of similar, untested materials may be drawn.

There are at least three factors differentiating larger continuous and nanostructured (agglomerated and aggregated) particles in the range of 100-1000 nm, which have relevance to their risk profiles. They include the following:

1. Differences in densities between larger continuous and nanostructured particles would result in differences in aerodynamics and, therefore, deposition properties affecting exposure processes.

2. Nanoscale particles agglomerated into clusters with overall size in the range 100 nm - 1,000 nm can have higher biological activity due to higher accessible surface area compared to continuous similar-sized particles (Oberdörster et al., 1992; Driscoll 1996; Lison et al., 1997; Donaldson et al., 1998; Brown et al., 2001; Duffin et al., 2002, Lok et al., 2007). Higher specific surface area on a per mass basis leads to a higher concentration of sites for interaction with biological moieties and higher solubility of the materials, thereby, releasing more ions into the environment.

3. Nanoscale particles agglomerated into clusters with overall size in the range 100 nm to 1000 nm have the potential to de-agglomerate under ambient conditions such as natural aqueous solutions (Mackay et al., 2006; Zhang et al., 2009; Gao et al., 2009). In addition, another report indicated that agglomerates held together relatively weakly (for example, by van der Waal's forces) can de-agglomerate in the presence of surfactant-like substances with properties similar to those found in the human respiratory system (Maynard, 2002).

Charge Question 1D:

If nanosilver particles present different hazards than either silver or agglomerated nanosilver, the potential risks to human health and the environment will depend on the extent of exposure. Several types of nanosilver pesticide products are described in the attached Background Paper, and other types seem possible in the near future (e.g., sanitizers and disinfectants; and chemicals used in or on industrial, commercial or residential systems, such as slimicides, preservatives, antifoulants, metal working fluids, etc.). What do available data on the release, fate, transport and transformation of nanosilver particles suggest regarding potential human or ecological systems exposure to nanosilver particles (individual or agglomerated) under realistic use scenarios?

Panel Response: There have been very few studies that directly assess human exposures to nanosilver particles under realistic use scenarios or the contribution of silver to ecological systems through nanotechnology applications. Currently, there is not enough information available to make conclusions about human (occupational or consumer) and environmental exposure related to "realistic use" scenarios. Discussed below are studies that currently attempt to address the issue and factors that may influence human and environmental exposure.

Studies on Potential Occupational Exposure: The release of silver nanoparticles into the workplace during manufacturing processes has not been characterized clearly. One publication measured airborne levels of silver nanoparticles in a manufacturing facility. Characterization of potential human exposure was outlined during various portions of the liquid-phase synthesis process (Park et al., 2009). Depending on the monitoring location, stages of production and particle size/agglomerate size, the concentrations ranged from a low end of 4.0 - 6.0 x 10^4 particles/cm^3 up to a high end of 1.0 - 1.2 x 10^5 particles/cm^3. Ongoing monitoring studies in silver nanoparticle manufacturing facilities have indicated that concentrations to which workers may have been exposed were lower than the current silver dust threshold limit value (TLV) of 100 ug/m^3 and sizes ranged from 15 to 710 nm representing individual nanoparticles and agglomerated and aggregated particle exposure (Lee and Yu, 2009b). The release of silver from consumer products to ambient air has not been studied and there are no standard methods to evaluate such release. Therefore, actual particle sizes and concentrations of airborne nanosilver released from consumer products are not known. However, even in extreme cases, it seems likely that airborne concentrations will not exceed 1,000,000 particle/cm^3, which is the NOAEL suggested by current inhalation studies (Sung et al., 2009).

Studies on Consumer Product Exposure – human and environment: Overall, there is clear evidence that forms of silver will be released during use of products containing nanosilver, resulting in potential human and environmental exposure. With regard to leaching of silver nanoparticles from consumer products and possible human and environmental exposure, the background data provided to the Panel described and summarized the results of studies on the leaching of nanosilver from socks and textiles (Benn and Westerhoff, 2008; Geranio et al., 2009). These studies indicated that the degree of leaching was variable and depended on the item, the number of times the item was washed or exposed and the water chemistry to which the item was exposed.

Other data suggested that the use of titanium dioxide nanoparticles in exterior coatings resulted in release of nanoparticulate material (Kaegi et al., 2009). Similar results would be expected from comparable nanosilver preparations. The use of silver nanoparticles in sanitizers and disinfectants in the form of foams or sprays will almost certainly result in release of nanosilver. No information on the release of nanosilver from plastics, polymers or coatings was found. Surface coatings on food containers and paints can wear or shear off and lead to non-ionic silver exposures. Some products, such as humidifiers, may contain nanosilver, colloidal or ionic silver and discharge the materials into the air as mists.

Only one study was found which provided information on silver ion release from commercially available silver wound dressing in patients (Lansdown, et al., 2005). This study concluded that silver bound to the wound scale and debris was proportional to the amount of silver ions released. In addition, a review of two other studies noted silver exposure in patients after treatment with Acticoat® wound dressing (Wijnhoven et al., 2009; Trop et al., 2006; Vlachou et al., 2007). A case of treatment-related silver poisoning (elevated urine and blood levels, grayish discoloration of skin, fatigue, lack of appetite) due to silver ion release from Acticoat® release was described (Trop et al., 2006). Also, the absorption of silver into the blood without any indicators of toxicity has been reported (Vlachou et al., 2007). Cytotoxicity has been

reported following *in vitro* testing of silver wound dressing containing silver nanoparticles or silver ion (Paddle-Ledinek et al., 2006).

Route(s) of human exposure: In each case, the specific type of nanoparticulate employed and its method of use will have a significant impact on human and ecological exposure. Exposure to these materials may occur *via* oral, dermal and inhalation routes in humans as delineated by EPA in the background material and their presentation. As indicated above, without stabilization, released nanosilver is very likely to be present in an agglomerated state (either as homo- or heteroaggregates) in most exposure situations. However, the use of stabilized dispersions of silver nanoparticles in spray applications may result in human exposure to individual nanoparticles by inhalation. Following inhalation, ingestion of particles cleared by mucociliary action will occur.

Additional considerations for exposures: The size of the particle will determine deposition of inhaled nanoparticles. It was reported that 70 - 80% of particles 20 nm in size will deposit in the respiratory tract (Kim, 2000). Some proportion of nanoparticles will be deposited in the nasal tract and may lead to transport via the olfactory nerve and accumulation of silver in the olfactory bulb (Ji et al., 2007; Sung et al., 2009). Metal transport to the olfactory bulb via the olfactory nerve has also been demonstrated with other metals, such as cadmium and manganese (Tjalve et al., 1996; Yu et al., 2003). Inhaled nanoparticles can be transported to essentially all tissues by blood circulation. Several nanoparticle inhalation studies clearly indicated that nanoparticles can be translocated to all tissues, although dissolution of the particles and translocation of silver ions to the tissues cannot be ruled out (Ji et al., 2007; Sung et al., 2009). Studies with gold nanoparticles, which are unlikely to dissolve, indicated similar, but slightly different, distribution as seen in silver, suggesting that nanoparticles themselves can be translocated (Korea FDA, 2008). Size difference can make the magnitude of deposition in the respiratory tract greater; 20 nm particles can be deposited 4 times higher than 500 nm particles. A 90-day inhalation study showed that surface area in relation to size contributed to nearly a 20% decrease in lung function at 515 $\mu g/m^3$ (silver nanoparticle) compared with a similar decrease in lung function at 100 mg/m3 (welding fume particle). This study considered welding fume exposure in which the average particle size was 100 nm (Sung et al., 2004).

Ingestion of nanosilver is possible from its use in dietary supplements or for medicinal purposes, as well as release from nanosilver-impregnated water purification filters. Nanosilver also can be ingested if there is release from nanosilver-coated toys or baby bottles. The genital and anal areas may be exposed if nanosilver is released from nanosilver impregnated diapers.

Studies with oral administration of 60 nm silver particles demonstrated initial deposition in the stomach, followed by the liver, kidneys and, to some extent, the lungs. Presumably, the majority was excreted in feces. Nanosilver administered both by inhalation and oral ingestion showed gender differences in accumulation of silver in the kidneys, with 2 - 3 times more silver accumulated in female kidneys than those of males (Kim et al., 2009). Female rats showed a higher accumulation of silver nanoparticles in all kidney regions, including cortex, outer medulla, and inner medulla. In particular, the glomerulus in the cortex contained a higher accumulation in females than males. Silver was also preferentially accumulated in the basement membranes of the renal tubules in the cortex, middle and terminal parts of the inner medulla, and

outer medulla. In addition, silver was detected in the cytoplasm and nuclei of interstitial cells in the inner medulla of the kidney. Orally administered silver was also accumulated in the lamina propria, the connective tissue under the epithelia, in both the small and large intestine, and also in the tip of the upper villi in the ileum and protruding surface of the fold in the colon (Jeong et al., 2009). Rats treated with silver nanoparticles exhibited higher numbers of goblet cells that had released mucus granules than the controls, resulting in more mucus materials in the crypt lumen and ileal lumen. Lower amounts of neutral and acidic mucins were found in the goblet cells, plus the amount of sialomucins increased while the amount of sulfomucins decreased. In particular, in the colon of the treated rats, sialylated mucins were detected in the lamina propria. These results suggest that silver nanoparticles induce the discharge of mucus granules and cause the production of an abnormal mucus composition in the goblet cells in the intestines. Some effects on microbial flora in the gastrointestinal tract were also expected. Although the distribution following exposure to nanosilver does not clearly reveal whether silver reaches tissues and organs as silver nanoparticles or as silver ions, it is suggested that silver nanoparticles can be translocated to tissues and organs by blood circulation, as evidenced by distribution of gold nanoparticles (which are not ionized) after 90-day inhalation (Korea FDA, 2009).

In examining dermal exposures, the *in vitro* application of silver nanoparticles of 25 nm size caused a significant increase of silver in the skin and in receptor fluid in damaged skin (Larese, 2009). Transmission electron microscopy showed that silver nanoparticles were located in the deepest part of the stratum corneum and the silver permeation appeared mainly as ions. In intact skin *in vitro*, the silver permeation after application of silver nanoparticles was very low. Based on these data, it appears that silver nanoparticle permeation through the skin is low; however, under normal conditions the skin is frequently damaged (dry, atopic, or mechanical- or chemical-impaired skin). After repeated contact with a textile containing silver nanoparticles, one can assume silver permeation could potentially be increased by sweat released during exercise, damage to the skin, and by release of silver ions/silver nanomaterials from textiles.

Following absorption, some data suggest that nanosilver can be eliminated. Silver accumulated in tissue following 28 days of nanosilver treatment was eliminated gradually (Lee and Yu, 2009a, unpublished data).

Modeling of Environmental Fate: The literature on environmental fate and transport of silver nanomaterials (contributing sources, concentrations, pathways, bioavailablity, toxicity, and potential impact on ecological structure and function) has been reviewed (Luoma, 2008). Using the Rhine River as a case study, the risk to fresh water ecosystems from silver that was released from nanotechnology manufacturing installations that incorporate nanosilver into plastics and textiles (biocidals) was analyzed (Blaser et al., 2008). It was estimated that 15% of the silver released into the fresh waters of the European Union is due to release from biocidal textiles and plastics. Some of the silver released will enter sewage treatment systems. Silver cannot be transformed into innocuous products (e.g., mineralized, etc); therefore, the silver forms (ionic, nano or colloidal) will end up in biosolids or wastewater effluent – both of which can enter the environment. Silver in other products may end up in landfills.

There is little information on the fate of silver nanoparticles in soil or sediment relative to realistic use scenarios. In ecological systems, nanosilver is likely to form aggregates rapidly and

deposit in sediments. Investigation of the fate, transport and effects data that are extant for photographic and other colloidal silver uses should be utilized as a starting point for nanosilver. Currently, the location of colloidal silver that has been used in products is not known. It may be present in many ecological compartments, but probably in sediments as ionic, precipitated, sorbed, or colloidal forms. EPA may want to inventory and evaluate uses of past and currently registered products in order to attempt to determine the locations where residuals can be detected. Such sites would be instrumental in assessing the fate of silver from "down-the-drain" or other "end use" products and the accumulation of silver from such products in the environment. The possibility of persistent environmental contaminants being sorbed to nanoparticle surfaces should also be considered when evaluating environmental fate. Finally, it should be noted that silver is an effective pesticide with relatively low risk to humans. If it is not used, other synthetic organic chemicals may be used instead.

Charge Question 1E.

The Agency would like the Panel's advice as to whether the models currently used by the Agency would be appropriate to predict potential environmental exposures to nanosilver and if not, what, if any, modifications would be necessary.

Panel Response: There was consensus among the Panel that most models are not appropriate for predicting environmental exposure to nanosilver, though some may be useful with the addition of appropriate parameters. The charge question was viewed, as instructed by the EPA, to focus on exposure models and not biological models (e.g., types of organisms to test, etc).

Existing EPA models are poorly suited to predict nanosilver exposure scenarios. Pollutant exposures to specific organisms are generally assessed through fate and transport models (product release, water, sediment, air), many of which are described in the EPA Center for Exposure Assessment Modeling (CEAM), and specifically for the Office of Pesticide Programs at http://www.epa.gov/opp00001/science/models_db.htm. These models require the definition of input parameters specific to test material (e.g., physicochemical properties), environmental properties (e.g., soil properties), and exposed population (e.g., intake rates), as well as describing the interaction of test materials with the environment and the potential exposure to populations at risk through mathematical equations. Uncertainty associated with parameters that are necessary to describe nanosilver is high and the many parameters required to run the models are unknown.

Most aquatic and sediment models are not adequate for nanosilver, but are adequate for ionic silver. A few examples of aquatic models and their potential deficiencies follow:

- Many of the general fate models (e.g., EPIWIN: see http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm) assess fate of many organic pollutants rather than metals or nanomaterials. EPIWIN actually gives erroneous output for silver regarding its octanol water partition coefficient and biodegradation when silver's CAS number (7440-22-4) is inserted. Other models (e.g., AQUATOX: see http://www.epa.gov/waterscience/models/aquatox/) focus on organic pollutants and nutrients and their exposure, toxicity or other effects on foodwebs, but are not

suitable for silver. The primary reason is that these models are based largely upon the solubility of organic chemicals in water (e.g., partition coefficients such as LogKow).

- WASP (see http://www.epa.gov/athens/wwqtsc/html/wasp.html) or MINEQL$^+$ (see http://www.mineql.com/) has been used for heavy metals and may be valid for predicting silver forms, including nanosilver, in surface waters, although no case studies or validation studies have been reported. Most existing EPA models that account for metals permit computations related to metal speciation such as aqueous species, precipitation or complexation, but cannot predict particle size dependent or kinetic processes like dissolution or aggregation.
- EPD-RIV1 (see http://epa.gov/athens/wwqtsc/html/epd-riv1.html) can be used for iron and manganese, but has not been applied for silver.
- Biotic Ligand Model (Bielmyer et al., 2007; Choi et al., 2009) has been successfully applied to predict the toxicity of ionic Ag^+ to aquatic organisms.
- Theoretical models developed for aggregation (e.g., electric double layer models for computing energies of interaction) are valid for very clean systems (Zhang et al., 2008b; Chen and Elimelech, 2007; Chen and Elimelech, 2006), but models incorporating polydispersed solution (e.g., Smoluchowski model) are not widely applied. They are available in applications such as coagulation methods for drinking water treatment (Lawler, D.F., 1986).
- The COMET model (CML model) (Mills et al., 1992) may provide a useful platform as it includes metal and colloid transport. Other models of colloid transport of metals such as that by Massoudieh and Ginn (2008) and the MNM1D model (Tosco and Sethi, 2009) may be useful for surface and subsurface fate and transport.
- Most models do not account for aggregation, stabilization, dissolution, etc. that are influenced by ionic strength, ionic composition, dissolved organic matter, pH, dissolved oxygen, redox conditions or a wide range of other water quality parameters or processes (biological reactions, photolysis, etc). Models to predict the interactions between nanosilver and particulates (suspended or sediment) in natural environments are lacking.
- Many ecosystem models (water, sediment, organism) are semi-equilibrium models and do not reflect parameters that vary over time (e.g., changing nanosilver size which affects dissolution rates) which would be required to predict dynamic exposures.

The Panel agreed that models for colloidal silver in the environment do not currently exist. New models to predict environmental exposures to nanoparticles should be created. Such models would be valuable not only for engineered nanomaterials, but other environmentally relevant colloids that include, but are not limited to, the following: viruses, radionuclides, prions, carbon black, colloidal silver, etc.

- EPA has modeled the fate of metallic pollutants associated with particles (e.g., transport in the subsurface – EPA/600/R-02/082).
- The Multiple Pathway Deposition Model (MDEP) by CIIT (Anjilvel and Asgharian, 1995) is probably valid for assessing exposure to nanosilver in air. This model predicts lung deposition from breathing through the mouth and nose.

- The Exposure Assessment Tools and Models from EPA/OPPTS (http://www.epa.gov/oppt/exposure/ index.htm) are probably applicable for nanosilver. For example, with addition of appropriate parameters, ChemSTEER and EFAST may be useful models for estimating releases of nanosilver during manufacture and use.

Only a few studies have considered how colloids (of nanoscale size) are transported through watersheds. These studies have focused on facilitative transport of pollutants by colloids, but there is a chance such models could track the colloid itself as a nanoparticle. However, such models have not been validated. For engineered nanosilver, these models should incorporate 1) additional metrics for dose and exposure such as particle size (mean and distribution); surface area; number and mass concentration; and 2) additional parameters such as particle size/distribution; particle shape; agglomeration state and rate of agglomeration/de-agglomeration or stabilization in application environments; surface chemistry (coating, charge, reactivity) of nanoparticles; and rate of dissolution. For example, information is lacking on dissolution rates for nano-forms of 1) pure Ag^0; 2) Ag^0 with impurities, surface imperfections or non-crystalline or amorphous coatings; and 3) the effects of organic or inorganic surface coatings.

Many EPA protocols that are used to develop important fate and transport parameters for pollutants have not been validated for nanosilver, but would be applicable for ionic silver. For example, EPA protocols (EPA/600/R-05/074) for partition coefficients for metals in surface water, soil and wastes exist for dissolved forms of metals, but have not been shown to be applicable for nanosilver. Other protocols that assess "down-the-pipe" fate of pollutants in publicly owned treatment works (Fate, Transport and Transformation Test Guidelines (OPPTS 835.1110 Activated Sludge Sorption Isotherm) have not been validated for nanosilver. Critical issues that need to be addressed in these protocols are related to nanosilver pseudo-equilibrium assumptions (i.e., dissolution, stabilization, aggregation kinetics), critical water conditions (pH, ionic strength, nanoparticle concentrations), and operating conditions (time, filtering, adequate controls, etc).

One Panel member outlined two approaches that EPA might take in revising its modeling for nanosilver. The first approach could include developing simplified exposure models using multi-criteria decision analysis tools (Linkov et al., 2009; Tervonen et al., 2009; NIOSH, 2009). This approach allows classification of different nanomaterials (including nanosilver products with different properties) into different risk groups. The second approach requires developing complex mechanistic models utilizing the best knowledge on mechanisms governing nanomaterial behavior (e.g., colloidal transport in soil).

Data Requirements Issue: If the Panel believes that nanosilver is different in terms of hazard/exposure what type of data (studies) would EPA need to adequately assess the potential risks associated with the use of an antimicrobial pesticide containing nanosilver particles, when the product is intended for use as an additive to various substrates (e.g., textiles, plastics, ceramics) to impart antimicrobial properties to the treated substrate? In liquid, spray form?

Charge Question 2A: What types of data on the nanoscale material would be sufficient to adequately evaluate whether the hazard and exposure properties of the nanoscale material were comparable to that of a macroscale/bulk form of the same material? Could EPA rely on toxicity data for the bulk material to assess the risk of the nanoscale material?

Panel Response: At the present state of knowledge it is difficult to extrapolate existing data related to the use of molecular and/or macroscopic materials as a pesticide to a new nanoscale pesticide with the same chemical identity. Toxicity data for the bulk material might be used to assess the hazard of the nanoscale material provided that the mechanism of biological activity does not change as particle size decreases to nanoscale and no additional synergistic effects resulting from use of nanoparticles are evident. However, these properties would not necessarily be known *a priori* for a new nanoscale pesticide. For related details, please see answer to Charge Question 1A through B.

Several studies have implicated particle size as an important factor in silver toxicity (Carlson et al., 2008; Choi and Hu, 2008), and a toxicity study on a soil microbe has shown that nanosilver may be more toxic than the bulk silver counterpart (Gajjar et al., 2009). A number of other ecotoxicology studies have demonstrated greater toxicity of some nanometals compared to their bulk material counter parts, although not all nanometals tested were found to exhibit this characteristic (Heinlaan et al., 2008; Jiang et al., 2009; Kasemets et al., 2009; and Wang et al., 2008). In light of these studies and those discussed in Charge Question 1, as well as the variations that exist between studies (i.e., dose, particle shape, degree of agglomeration, surface chemistry, solubility), it is difficult to make the assumption that toxicity data from bulk material studies could be used to assess the risk of the same nanoscale material.

Comparative studies are necessary to evaluate whether hazard and exposure properties of nanoscale silver are similar to macroscale/bulk material, preferably following a specific regimen to identify particle characteristics. Comparison of physicochemical properties of the nano vs bulk materials are needed, as well as comparisons of the particles' properties in different media where exposures may occur due to lifecycle fate and transport properties. Particle transformation over the lifecycle may also differ between nano and bulk materials and should be assessed. If toxicity is due to ion release, the Panel questioned how particle delivery and translocation related to release of ion may vary between the two size ranges. A series of comparative toxicity studies are needed in target and non-target organisms. Importantly, these studies should be performed at concentrations relevant to the exposure scenario, such as those that may occur in occupational settings versus consumer settings versus ecological settings (a current knowledge gap for nanosilver). It is also important that testing evaluate additional components of product formulations (e.g., dispersants, stabilizers) which may influence the results.

A number of Panel members indicated that future EPA data requirements for nanomaterial products should balance costs and benefits and allow for sustainable nanotechnology development. In addition to the present data requirements under FIFRA, the following data would be necessary to assess risk of new nanomaterial pesticides:

1. To characterize hazard properties, it is necessary to characterize physicochemical properties as outlined in the MINChar Initiative (see draft Minimum Information for

Nanomaterial Characterization Initiative, ISO TC229 WG3/PG5 project "Guidance on Physico-chemical Characterization of Engineered Nanoscale Materials for Toxicologic Assessment" (ISO/AWI TR 13014, available at http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=52334). The MINChar Initiative suggests characterizing the following: particle size and size distribution; agglomeration state and aggregation; shape; overall composition (including chemical composition and crystal structure); surface composition; purity (including levels of impurities); surface area; surface chemistry (including reactivity and hydrophobicity); surface charge; stability and context/media (http://characterizationmatters.org/parameters/). Also necessary is knowledge of chemical composition and hazard properties of the end-use products, including inert ingredients such as propellant, carriers, odorizers or plasticizers to account for possible synergistic effects since nanosilver could act as a carrier vehicle for other chemicals.

2. To characterize exposure properties, it is necessary to have information on the following:

a. dislodging of nanoparticles from the support during normal use and also during environmental degradation of the support and generation of particles and their characteristics in non-supported application scenarios;
b. silver ion release from products under different situations (physiological solution, synthetic sweat, temperature, pH);
c. data on metabolism and mobility of degradation products in the environment (air, soil and aquatic);
d. a comparison study of absorption, distribution, metabolism and excretion (ADME) because of the variety of products that exist between nano and bulk materials;
e. ecotoxicological data to consider ion generation and particle nature of silver nanoparticles; and
f. for spray products it is necessary to obtain information on particle size and distribution in air.

3. Additional needs include modifications to the product Material Safety Data Sheets (MSDS) so that they are specific for products containing nanomaterials to protect workers, manufacturers, and downstream users. One Panel member indicated that current MSDS for nanomaterials are lacking in hazard communication information

Charge Question 2B: What types of data would be sufficient to:

1) Evaluate whether the nanoscale material, once it has been applied to or incorporated within a substrate, remains associated with the substrate through the whole-life cycle of that substrate to such an extent that there would be essentially no human or environmental exposure to the nanoscale material or nanosized composite, and

2) Measure and characterize exposures to nanomaterials that may leach from treated materials.

Panel Response: Existing data suggest that it is necessary to take a product-by-product approach to evaluate whether silver nanomaterials remain associated with the substrate. Required data can be obtained through research aimed at establishing models or through data submission as part of the registration process. In the latter case, current antimicrobial pesticide data requirements (49 FR 42881, Subpart D – Data Requirement Tables, §161.202) include relevant data on environmental fate, degradation, metabolism, mobility, dissipation and accumulation for assessing exposure. These data requirements should apply to nanomaterials. However, general use patterns and test guidelines might need to be adjusted to accommodate novel properties and novel uses such as antimicrobial pesticides containing nanomaterials that may be incorporated into clothes and household items. For example, the general use pattern "indoor" might not be specific enough to adequately characterize uses of nanomaterials. In order to assess the potential human and environmental exposure associated with the use of a substrate coated with or containing a nanoscale material, the following studies should be performed:

1. degradation studies describing under what scenarios and in what form and rate can bound nanomaterials dislodge from the support. Such data could be derived from hydrolysis, photolysis and tribolysis (which in this context can be defined as product degradation upon mechanical treatment) studies;
2. studies describing transformations of nanomaterials once dislodged from the support;
3. mobility studies describing leaching and adsorption/desorption;
4. dissipation studies describing exposures resulting from reentry into treated areas, residues in crops and other food sources, the loss of land and water resources;
5. accumulation studies describing pesticide residue levels in food supplies.

Study guidelines would need to be adjusted in order to account for the particulate nature of nanomaterial pesticides and/or their degradation products. New standard methods would need to be developed that include consideration of the following parameters:

1. Exposure metrics: Metrics most appropriate for characterizing risk might be different for nanoparticles. The Panel discussed characterizing exposure using mass (total silver), particle number and surface area concentrations (NIOSH 2009; OECD 2009). In addition, the Panel believed that morphology of nanoparticles and some form of "reactivity" (magnetic, optical, ROS, etc.) should be characterized. One Panel member suggested that in aqueous environments, size distribution could be characterized approximately by utilizing a standard membrane filter (e.g., 450 nm).
2. Exposure duration: Since nanomaterials can potentially elicit chronic effects, chronic exposures should be considered in addition to acute exposure.
3. Particle/ion release conditions: Silver nanoparticle release (leaching) and silver ion release over time using different conditions, depending on the proposed/reasonable use and disposal of the product. Various conditions that should be considered include the following:

    a. In physiological solutions or synthetic sweat at body temperature – to verify silver ion release and silver nanoparticle leaching for materials that may come in contact with the human body;

b. At different pH and temperature conditions – for plastics that may come in contact with food;
c. In physiological solutions and in tissues and animals (*in vivo*) – for prostheses containing silver nanoparticles that are implanted into the body;
d. Under conditions of stress – i.e., stretching, flexing of materials;
e. In aquatic environments – to support fate assessments;
f. In ambient air – to evaluate nanosilver and silver-ion release from consumer products and inhalation exposure;
g. During burning – to assess fate of products containing nanomaterials during disposal;
h. Under conditions that examine the type and strength of the bond between a nanoparticle and a composite material (like textile or plastic) – to assess the likelihood of particle dissociation from composite materials. One Panel member expressed the opinion that silver forms potentially released from products might be reasonably measured using a toxicity characteristic leaching procedure (TCLP) test.

4. Life Cycle Assessment (LCA): Data on stability of applied or incorporated nanosilver products and related exposures should be considered over the life of the product using standard methods developed to consider the many use (especially for coating formulations) and possible misuse patterns. Standard LCA methods should be adopted to include quantitative characterization of environmental impacts through the product life cycle (Seager and Linkov, 2008).
5. Data to support estimation of NOEL or NOEC for nanosilver products should be obtained to support regulatory exposure limits.

Standards for characterizing nanomaterials in the air, along with some recommendations, are being developed. For example, ISO TC146/SC2 "Air Quality/Workplace Atmospheres" is developing a series of standards for use in characterizing nanoparticles in the air. ISO TC229/WG3 "Nanotechnologies/Health, Safety and Environment" is preparing a technical report summarizing standard techniques available for characterizing nanoparticles in different media.

It is also necessary to develop new test guidelines to adequately characterize novel applications allowing for novel degradation mechanisms. For example, in the case of antimicrobial pesticide nanomaterials applied to clothes, it is necessary to develop new test guidelines to characterize degradation upon wear, washing and drying. Some standards in this area are already under development. For example, ISO TC 229/WG2/PG10 is developing International Standard "General Framework for Determining Nanoparticle Content in Nanomaterials by Generation of Aerosols."

There is a significant need to standardize terminology that differentiates between ionic, nanoscale, colloidal and particulate forms of silver or composites containing silver. It should be determined whether size or unique function defines something as nanoscale.

Charge Question 2C: Assuming appropriate studies could adequately show that nanosilver, which is applied to a substrate, would bind with that substrate to such an extent

that there is essentially no exposure to the nanosilver, does the Panel think that other types of data (such as toxicity studies on the nanosilver particles or composite) would be needed? Similarly, if only silver ions are released from substrate containing nanosilver, would consideration of the potential risks associated with the silver ions be sufficient or would additional data be needed to assess hazards and exposure to human health and the environment from nanosilver?

Panel Response: The Panel generally disagreed with the statement that nanosilver applied to a substrate would permanently bind with the substrate *via* any existing physical/chemical modifications. Concluding that there is no release of nanomaterials from a substrate is especially challenging given the current state of the science and measurement standards. There is a certain release of the nanosilver when the nanotechnology-enhanced products become commercially available. For instance, nanosilver is released into water from commercially available sock fabrics after washing (Benn and Westerhoff, 2008). Current analytical detection methods for silver (~ 0.2 ppb) equate to billions or trillions of nanoparticles per liter. Therefore, the phrase "essentially no exposure to the nanosilver" is of concern because products containing nanosilver for disinfection purposes will almost always result in the deposition of silver on the surface of treated areas. A test protocol is needed to simulate realistic use, including the effects of transformations due to sunlight, different temperatures, etc. that could alter the product during normal and expected use. The permanent covalent binding mechanisms of nanosilver with fabrics may significantly reduce the amount of nanosilver released to water. Regardless of the release patterns of nanosilver products, studies have predicted that more than 60% of nanosilver products will eventually be disposed of in landfills with the rest mainly released to water bodies (Mueller and Nowack, 2008). Even if nanosilver is not released from bound substrates and there is no exposure to nanosilver, the potential environmental impact of the significant use of nanosilver products should be carefully determined based on a life cycle assessment (Seager and Linkov, 2008).

Similarly, silver ions are likely not the only form of silver species released from substrates containing nanosilver. If only silver ions are released, then adequate exposure and fate models can be applied (as currently used for ionic silver). While the potential risks associated with the silver ions are heavily studied and may be relatively easily predicted (Bianchini et al., 2002; Bielmyer et al., 2007), additional data for nanosilver will be needed to assess its hazards and exposure to humans and the environment. This is because: (1) the mode of antimicrobial action of nanosilver is likely different from that of ionic Ag^+; and (2) continuous release of Ag^+ due to oxidative dissolution of nanosilver will result in prolonged/chronic toxicity to aquatic organisms.

The Agency presented three alternatives for establishing data requirements for pesticide applications based on nanosilver. Those alternatives could be considered as three elements of the same decision tree. The first element determines if there is no exposure. If there is exposure or it is difficult to make such a determination, the second element is to determine if existing data support hazard characterization. The third element would apply if it is not possible to characterize hazard using existing data. At this point in time, the lack of standardized and validated techniques to measure or model exposures to nanosilver and to extrapolate hazard data for existing formulations to new formulations would cause the Agency to require a full data set

as part of the registration process. However, this approach should be reassessed as new data are generated and new techniques and models are established.

The Panel agreed that there are many beneficial applications of nanosilver products. However, in order to minimize their negative impact to public health and the environment, the Agency needs to consider that nanoproducts should be characterized, particularly in terms of particle size, formulation (surface modification) and/or surface area when they are registered. The Panel recommended that the Agency should also consider the shelf life of the nanoproducts. Aging of nanosilver products, particularly prepared in aqueous suspension, could result in increased particle size due to hydrophilic/hydrophobic self-aggregation or increased ionic Ag^+ concentration due to continuous dissolution.

3. Other Risk Assessment Issues.

Charge Question 3A: Products developed using nanotechnology may contain a distribution of particle sizes. Please comment on how information concerning the percentage of the particles in a product falling within the nanoscale range (e.g., 1 - ~100 nm) could affect the risks of a product. Are particles at the lower end of the range (e.g., 1 nm) likely to behave like particles at the upper end of the range (e.g., 100 nm)?

Panel Response: In general, the Panel agreed that more data and interpretations of data are needed before the scientific community can say if particles at the lower end of the range (e.g., 1 nm) are likely to behave like particles at the upper end of the range (e.g., 100 nm). However, in the case of nanosilver specifically, the literature suggests that nanosilver particles in the size range 1 to ~20 nm possess the greatest quantum properties, are the most optically active, and may potentially induce the greatest toxicities (Auffan et al., 2009). NOTE: The lower limit of this particular size range should be defined by the science, much like the upper range of nanosilver particles. However, the Agency should consider at what size are particles no longer nanoparticles. For sizes less than 1 nm, nanoparticles become more of an atomic phenomenon. For pure silver, selecting a lower bound size limit based upon science may preclude future terminology issues. The Panel suggested the Agency take advantage of defining size ranges to clarify the lower bound.

The way in which the percentage of particles below 100 nm in a product may affect risk is largely dependent on how the product is used. If the particles are encased in a solid support with essentially no particle release, then the major effect of small particles will increase release of silver ions relative to mass of silver. If intact particles are released, then higher proportions of smaller particles may be associated with greater environmental distribution and increased uptake as indicated above.

The Panel also noted that there are at least two observations that could lend credence to the existence of a relationship between particle size and potential risk. They are as follows:

1) Biological activity and penetration through biological barriers, such as cell membranes and skin, depend on particle size. It has been demonstrated that biological activity of silver materials depends on the size of particles (Lok et al., 2006; Carlson et al., 2008;

Cha et al., 2008; Choi & Hu, 2008). In addition, it has been shown that nanoparticles in the lower nanoscale range can penetrate cell membranes (Verma et al., 2008) and can potentially translocate systemically upon inhalation exposure (Semmler et al., 2004). Such nanoparticles can also potentially penetrate through the skin (Larese et al., 2009, Baroli et al., 2007).

2) Particle dynamics, which affect transport and exposure, also depend on size. A lower mass of particles at the lower nanoscale range will affect nanoparticle motion in the environment and be more relevant to molecular dynamics. Specifically, small nanoparticles will move diffusively. Particles in the lower nanoscale size range might also have enough thermal energy to overcome attractive van der Waal's forces and, therefore, might exist un-agglomerated or in individual forms for longer time periods. For similar reasons, a singularity in dissolution and condensation dynamics, which is often expressed in terms of "critical nuclei," exists at around 1 nm and will affect changes in the size of nanoparticles and nanoagglomerates.

The Panel recommended that when assessing hazards of a particular product it might be appropriate to consider concentrations of particles in the product within several particle size ranges (or size bins) as appropriate for a particular mechanism of biological activity. For example, if one considers the so-called Poorly-Soluble-Low-Toxicity materials such as titanium dioxide, where biological activity appears to be a function of the total surface area of particles, two particle size ranges can be sufficient to characterize hazards using mass-based metrics of exposure: ultrafine (nanoscale) and fine (microscale) (NIOSH 2005). For a distribution of particles, the weights of each particle size range (or size bin) should be determined. *NOTE: The Panel noted that it will be difficult to develop data and characterization tools based on size. A possible approach is to test multiple products with good characterization to develop a multi-parameter assessment such as Principal Component Analyses (PCA). Statistical analysis tools, such as PCA, would aid in assigning these weights. A conversation between experimentalists and mathematicians to design experiments at their onset is suggested.*

Panel discussion on the question of how information concerning the percentage of the particles falling within the lower nanoscale range could affect the risks of a product centered on three conceptual issues. The first issue involves the idea that the effect of dosing an organism (biological or activity) may depend on the particle size through a "response function". The Panel discussion centered on issues of the functional shape of this relationship. Several Panel members indicated that effects could take a linear, non-linear or even discontinuous form (Figure 1). Since the true relationship is never known, predictions of effects from the model would be accompanied by estimation (prediction) of uncertainty.

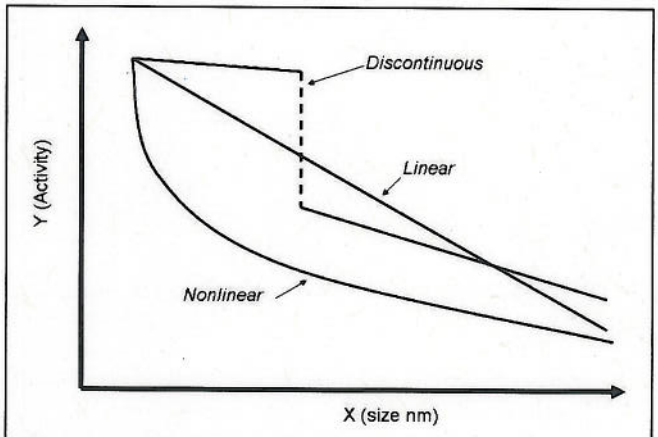

Figure 1. Theoretical relationship forms to describe how size (X) relates to activity (Y).

Collecting data to determine the correct form for this relationship is complicated by the second issue discussed, that most products do not contain one-size or homogeneously sized particles, but actually consist of a distribution of sizes. Thus, the

particle size distribution of the product translates to a distribution of effects. The proportion of particles falling in a specific range of sizes could affect the "amount" or the "likelihood" of an effect, depending on whether the effect function describes a continuous condition or a binary response.

The third issue discussed was that size is not the only characteristic of products containing nanoscale materials that is likely to drive its activity or effects. Hence, the functional relationship is multi-dimensional in character, further increasing the difficulty of modeling the activity relationship.

In summary, the Panel agreed that the following general recommendations should be considered:

- Test the products on a case-by-case basis.
- Use a meta-analysis on the products to understand trends in life cycle analyses.
- Close attention should be given to products that claim a non-ionic silver mode of action as an antimicrobial agent.

Charge Question 3B: Please comment on the extent to which the scientific literature indicates that data on one form of a nanosilver particle or other nanometal/nanometal oxide particle can be used to assess the potential hazards and exposures of another form of nanosilver that has different physicochemical properties (e.g., is a different size or shape or has different surface properties). For example, if nanosilver is reacted with a non-metal material to form a nanosilver complex or composite, to what extent could data developed for the nanosilver be used to predict the toxicity of the complex or composite?

Panel Response: Research data indicate that nanoparticle size and shape (Pal et al., 2007), size (Brown et al., 2001; Carlson et al., 2008; Ji et al., 2007) and surface coating (Malik et al., 2000, Ryman-Rasmussen et al., 2007) can all dramatically affect biological activity. Therefore, at present, caution should be exercised in extrapolating from one nanosilver formulation to another and especially between nanomaterials based on different metals. As more research data become available, it might become possible to develop predictive Quantitative Structure Activity Relationship-like models for nanomaterials. However, such models do not exist at this time.

Two forms of nanosilver are mentioned in the charge question: complex and composite. The Panel expressed concern for complexes containing nanoscale particles (e.g., silica core coated with silver; silver functionalized with PEG) because this would affect the mobility within humans or environmental exposure. Nanosilver contained in a complex is likely to have a different nature, environmental fate and interactions with biota than nanosilver itself (Jiang et al., 2005). The fate and effects of the complex will be controlled to a significant extent by the properties of the complex (i.e. size, chemistry, etc). For inert composites that may be used to manufacture items such as cutting boards or plastic wrap, the Panel recommended testing the active ingredient, i.e., nanosilver.

As suggested above, extrapolation from one form of nanosilver particle to another may be possible with appropriate information, but care should be exercised as differences in particle

formulation (e.g., coating and inert ingredients) are very likely to affect results. Extrapolation from other metal or metal oxides to silver is unlikely to yield beneficial information with regard to biological effects. Numerous studies have demonstrated that the toxicity of particles with similar sizes, but differing compositions, is quite divergent (Bar-Ilan et al., 2009; Griffitt et al., 2008). It should be possible to apply findings regarding effects of physicochemical properties of particles on fate and cellular uptake obtained with other particles to silver.

One Panel member presented an oral presentation entitled "Alternative Risk Assessment Approach – Integration with Multi Criteria Decision Analyses and Life Cycle Assessment" which discussed the serious challenge nano-structured materials present to traditional models of risk analysis. The high degree of variability and uncertainty with regard to different materials engineered at the nanoscale greatly expands data needs with regard to dose-response and environmental fate and transport. Even a massive expansion of risk assessment efforts is unlikely to keep pace with the rapid introduction of new nano-based materials and products (Linkov et al., 2009). Therefore, it is essential to have an integrated framework for prioritizing research efforts and interpreting new findings. In keeping with the National Research Council recommendations that toxicological risk assessment be decision-driven, a decision-analytic framework for prioritizing data needs related to the risks of nanostructured materials in the environment is necessary. Three analytic approaches: risk assessment, life cycle assessment, and multi-criteria decision analysis (MCDA) should be integrated into a framework for synthesizing both objective scientific information and subjective, values-driven information relevant in decision or policy contexts.

There are several advantages to framing risk analytic research in a decision-analytic context – especially in applications of high variability and uncertainty (Linkov et al., 2009). Whereas the objective of technical analysis is often an absolute characterization of intrinsic material properties, a decision-directed approach emphasizes comparative bases for relative assessment (Robichaud et al., 2005) and thus, data needs may be less intensive. For example, research efforts can be focused on reducing uncertainties that relate directly to the risk management decision, conserving investigative resources that are unlikely to result in a change in eventual decision-maker preferences. However, a decision-directed approach requires coupling risk analytic investigations with a structured decision aid, such as cost-benefit analysis or MCDA. This necessarily requires an expansion of knowledge and expertise to include social and decision sciences (McDaniels and Gregory, 2004) in addition to the physical and toxicological sciences.

A MCDA framework has already been put forth in the context of environmental risk management for nanomaterials (Linkov et al., 2007) and a stochastic approach to prioritizing materials for either precautionary measures or further research has been related to several nanomaterial characteristics (Tervonen, et al., 2009). However, the emphasis to date has been on characterization of the nanomaterials themselves, whereas there is widespread agreement that the proper perspective for management of nanomaterial risks should be that of the entire life-cycle, including extraction of basic materials, beneficiation or purification, manufacturing, distribution, use, and lastly, end-of-life (Sweet and Strohm, 2006; Köhler et al., 2008). From this broader perspective, it becomes clear that efforts to reduce risk at one life-cycle stage (e.g., use) may simply result in shifting or exacerbating risks in other stages (e.g., distribution or manufacturing

(Seager and Linkov, 2008). Therefore, a more complete decision scope would not only understand the important attributes of nanomaterials that relate to risk, but also the comparative importance of traditional environmental risks concomitant to the production or end-of-life management of nanomaterials.

To summarize, the Panel agreed that:

- At present, caution should be taken when comparing different formulations of nanosilver products.
- It is important to test the active ingredient in nanocomposites.
- There is a need to differentiate between the pristine active ingredient nanosilver versus the end use product.
- Oxidative state of a pristine nanosilver should be analyzed.

Charge Question 3C. Please comment on the extent to which the scientific literature indicates that nanosilver physicochemical properties change under different environmental or physiological conditions, what those conditions are, and how this variation could be best addressed.

Panel Response: Reported research results suggest that chemical composition affects the suspension/resuspension and dissolution/aggregation dynamics of nanoparticles, the particle size distribution and nanoparticle surface composition, resulting in a wide range of biological responses depending on the type of toxicity test used (Gao et al., 2009; Choi et al., 2009; Zhang et al., 2009). In order to address these variations, test guidelines and standards approximating realistic environmental and physiological conditions should be developed or validated and such testing should be conducted as part of the registration application process.

Properties of virtually all nanoparticles, including silver, can change substantially depending on conditions. In the environment, nanoparticles will tend to adsorb other molecules and coagulate with other particles. A number of publications demonstrate that the ionic strength, natural organic matter content and pH affect particle size distribution and fate (Fabrega, et al., 2009). It should be recognized that nanoparticles in aqueous media are colloidal material and will behave as such. Much information is available about colloid fate and transport based on the Derjaguin, Landau, Verwey and Overbeek (DLVO) theory. In physiological media, particles will be rapidly coated with adsorbed proteins and other materials. The actual physicochemical properties of particles are likely to change as a particle moves from one physiological compartment to another (e.g., blood to tissue) due to changes in fluid composition. These changes are likely to have significant impact on cellular recognition and response and are important factors for interpretation of *in vitro* testing. The National Toxicology Program (NTP) has recognized this and decided not to utilize *in vitro* testing. If data on nanosilver are specifically desired, the recommended approach is that particle properties are measured as the surrounding matrix (i.e., solvent or embedding material) is varied.

In the literature, there is evidence to show that for some nanoparticles, the physicochemical properties do change when changing the suspension matrix, temperature, and pH. But for other nanoparticles, the physicochemical properties do not change. A more detailed

literature review is needed to accurately report which particles do what, how, and when. It is the Panel's opinion that each nanomaterial should be characterized in the primary phase (pristine, dry state), secondary phase (in aqueous suspension), and tertiary phase (using *in vitro* or *in vivo* fluids, cells, or tissues) and each material should be considered on a case-by-case basis. Further, environmental conditions such as organic matter content, redox potential, ionic strength and composition, pH, temperature and physical processes (photolysis, sorption to solids) are relevant. This is a function of any surface coating (e.g., thiol-carboxylate complexes with calcium – i.e., soft/hard water). Some of these conditions (e.g., ionic strength) may lead to aggregation while others (e.g., organic matter content) may lead to dispersion (Zhang 2008a; Zhang 2008b).

In conclusion, the Panel agreed that:

- Exposure to environmental conditions are important and specific physiological components will change the particles' physical properties. The functional group on particles will largely determine the nature and extent of their modification.
- Test guidelines and standards approximating realistic environmental and physiological conditions should be developed and they should incorporate measurement of changes in particle properties throughout the test.
- Coagulation is an important property to consider.

Charge Question 4. Research Needs.

A. In the next year, what types of new information on individual products would be most useful to EPA for assessing potential risks of antimicrobial pesticides containing nanosilver or nanosilver composites, such as toxicity studies, exposure studies, etc.

B. What types of long term research would be most helpful for improving the assessment of the potential risks of antimicrobial pesticide products containing nanosilver or nanosilver composites?

Panel Response: Knowledge gaps concerning potential exposures and risks related to nanosilver are broad. There is very little information about nanosilver releases during the product life cycle, including the environmental fate and transport, transformation of nanosilver in the environment, waste generation, etc. Environmental fate and transport studies, leaching studies, dispersion and agglomeration studies, and toxicity studies that do exist have provided some valuable but fragmented information. Toxicity studies that have been conducted to assess effects of nanosilver vary greatly with respect to control samples, particle characterization, composition, size, aggregation information, and solubility of particles. In addition, there is a lack of comparative toxicity studies addressing effects related to particle size, aggregation state, particle dissolution, etc., rendering it difficult to make regulatory decisions related to particle characteristics and potential hazards associated with nanosilver exposure. Finally, frameworks that could be used to integrate available fragmented data to assess product risk are not available.

In light of the lack of information currently available on nanosilver, there are a number of research needs to be considered. The Panel suggested a number of immediate and long-term research recommendations aimed at addressing data needs related to (1) environmental fate,

transport and transformation assessment and (2) toxicity studies. Further, the Panel suggested an overall recommendation for use of an integrated risk-based decision framework that could be used to support regulatory decision making. The lowest levels (0 and 1) address the immediate or priority research needs related to question 4A. High priority should be placed on evaluating the fate, transport, and transformation of nanosilver in the environment as it is released from products, and the potential ecotoxicological effects related to this release. Level 2 is aimed at addressing question 4B, long-term research needs, including testing whether nanosilver toxicity is due to release of free silver ion in a variety of model systems. In addition, it addresses apical mammalian toxicity and ADME studies, long-term ecotoxicity studies, and chronic fate and toxicity studies. These research needs are outlined in greater detail below.

Priority Research Needs - Level 0. Level 0 involves gathering information related to environmental fate, transport and transformation of nanosilver as it exists currently in the market relative to realistic use, and evaluating the current knowledge related to nanosilver toxicity. This information provides the background necessary to design and conduct research that will address knowledge gaps and allow for effective risk assessment.

Environmental Fate, Transport, and Transformation Needs

- Assess what products contain nanosilver in the market today and determine what form (powder, colloid, composite, etc) the nanosilver is. Determine the total amount of nanosilver that could potentially be released into the environment.
- Collect information on existing physicochemical properties for currently registered silver products containing nanosilver, particularly size distribution, so that a potential relationship between size (or other appropriate characteristics such as surface area) and hazard can be assessed in the future.
- Conduct studies on dissolution kinetics, rate constants, for different nanosilver sizes and coating permutations.
- Evaluate ion release kinetics related to physicochemical properties of nanosilver in static and dynamic equilibrium.
- Evaluate whether it is feasible to assess the fate of colloidal silver, which contains nanosilver, as it is currently available in products in the market to serve as a case-study relative to realistic use of the products.

Toxicity Assessment

- Begin a review, or meta-analysis, of current nanosilver toxicity studies related to addressing toxic effects of single nanoparticles, particle size ranges, aggregates, or silver ions. This information will aid in determining which studies show a unique effect of nanosilver compared to other forms of silver currently regulated, and will provide some basic information on what dose metric may be most relevant for evaluating nanosilver toxicity.

Level 1. Level 1 addresses research needs regarding characterization of nanosilver, which may be applicable to other nanomaterials as well. Characterization of materials in products and released from products relevant to manufacturing (powders, colloids, or master-batch) and use patterns of the product will provide data necessary for exposure assessment, assessing source apportionment, and conducting studies evaluating toxicity or hazards under realistic use scenarios. Evaluation of the toxicity of nanosilver as it relates to various physicochemical

properties of the material will aid in determining what dose metric and size ranges may be of interest from a regulatory perspective.

Environmental Fate, Transport, and Transformation Needs

- Gather information on how nanosilver is chemically or otherwise bound/incorporated into a substrate or composite, as this will likely dictate what form and how much of the material is released over time in a given realistic use scenario.
- Depending on the product and the use pattern for a product, establish what amount is released from the product and in what form (ion, nanoparticle, composite).
- Evaluate and model the lifecycle of products and the nanosilver material released from them. The evaluation should address the following questions: What mediums (soil, sediment, landfill, waste treatment water, seawater, fresh water, bodily fluids, biological fluids, etc.) will the material come into contact with in its lifecycle; what will be the physicochemical characteristics of the material in that medium (including, but not limited to, structure, size distribution, surface reactivity, adsorption characteristics, aggregation state, dissolution rate, speciation) and how will those characteristics change over time in that medium (physicochemical transformations); and what properties of that medium dictate transformation (eg. pH, temperature, presence of microorganisms)?
- Determine the size threshold at which quantum effects (physical and chemical) predominate for a specific form of nanosilver, relative to those forms used in manufacturing and bulk material, and those forms that are released during product use.

Toxicity Assessment

- Based on data related to nanosilver forms used in manufacturing and release of nanosilver from products, determine the appropriate controls and test samples for toxicity testing and modeling relative to the lifecycle of the product as described in the needs related to environmental fate.
- Evaluate potential dosing regimens relevant to the model system to be used and perform tests to ensure the accuracy of the delivered doses.
- Begin ecotoxicity studies in sentinel species relevant to the form and amount of nanosilver released from products in the mediums that pertain to the lifecycle of the product.
- Begin epidemiological studies to evaluate potential health effects related to exposure in human populations.
- Based on size threshold data related to the quantum effects of nanosilver, begin to assess whether the physical "nano" properties of nanosilver (size range, surface area, dissolution, etc) are related to adverse effects on biological processes across species in their ecologically relevant media. Determine what properties and concentrations of nanosilver render it toxic in a given model system.

Long Term Research Needs - Level 2. Using data gathered on environmental fate, transport, and transformation of nanosilver, as well as physicochemical characteristics that may contribute to nanosilver toxicity, (a) test the "Zero Hypothesis" (Wijnhoven et al., 2009) to determine if the

toxicity of nanosilver released into the environment is due solely to free silver ion, (b) conduct comparative toxicity studies to address which particle parameters are important in mode or mechanism of action of nanosilver, and (c) evaluate the long-term, chronic fate and toxicity of nanosilver.

Environmental Fate, Transport, and Transformation Needs

- As determined from the research described above, compare and contrast representative forms and amounts of nanosilver used in manufacturing and released from products with ionic silver using existing EPA exposure, fate and transport models and protocols that will be used to evaluate nanosilver.
- Assess differences in fate, transport, and transformation of nanosilver under environmentally relevant conditions, as compared to free ion, and as compared to bulk, micro-, or macro-silver compounds.
- Assess long-term environmental persistence/accumulation of the material released from the products.

Toxicity Assessment

- Test the "Zero hypothesis" in a number of different biologically and environmentally relevant toxicity models. Conduct comparative mammalian and ecological toxicity studies to evaluate toxicity related to the form and concentration of nanosilver released from products under realistic use scenarios relative to that of free silver ion. Where possible, mammalian toxicity studies should focus on *in vivo* systems, as *in vitro* studies may fail to predict hazards of nanomaterials under realistic exposure scenarios (Walker and Bucher, 2009). Studies should take into consideration the following points:
    - incorporate information obtained from environmental fate, transport, and transformation studies regarding exposure and form of the nanosilver in the physiological or environmentally relevant medium related to the exposure. Mimic the "real world" exposure to the greatest extent.
    - include a range of physicochemical characterization analyses that will facilitate cross-study comparisons, including aggregation/agglomeration information.
    - cover a range of doses that include the levels released after realistic use or exposure scenarios, as well as multiple exposure routes.
    - when possible, evaluate absorption, distribution, metabolism, and excretion (ADME) of silver nanoparticles relative to free ion, including mechanics of translocation across membrane barriers
    - conduct comparative toxicity studies that also evaluate differences in toxicity between bulk, micro-, or macro-silver compounds.
- Conduct toxicity studies to assess effects of long-term, chronic exposure to nanosilver as it exists at occupational, end-use, and environmentally relevant exposure levels, and in the relevant form as it is released. These studies are necessary to evaluate developmental, neurological, and immunological toxicity, as well as to assess potential carcinogenicity of the nanomaterial exposure. These studies should also be comparative in nature, taking into consideration multiple doses, sizes of silver and composition (free ion versus particles or composites). Studies should evaluate:
    - multiple routes of exposure.

- absorption, distribution, metabolism and excretion (ADME) of different forms of nanosilver and silver.
- effects of exposure across multiple generations.
- differences in toxicity related to age and sex.

- Conduct ecotoxicity studies in sentinel organisms and their progeny over time, relative to the life span of the organism, to assess the long term effects of chronic environmental exposure. Studies in this domain should also be comparative taking into account dose, size, and composition as it may pertain to the environmental medium where the organism may be exposed.

Even if significant information on exposure and toxicity of nanosilver is collected, assessing potential risks of antimicrobial pesticides containing nanosilver or nanosilver composites will be a significant challenge. Risk assessment has been proposed as the foundation for many regulatory frameworks for nanomaterials, but its application to nanotechnology requires not only a significant information base but also mechanistic dose-response and mode of action models. The Panel raised additional points that would aid in addressing risk assessment research needs. These included a need for an interagency regulatory definition of *nano* that goes beyond a single dimension which may also involve lower-end limit (e.g., less than or equal to 1 nm), and a clarification on how aggregates and agglomerates will be considered. A need for characterization standards of physicochemical properties of nanomaterials was also identified, as well as the need for a standard reference material for comparative purposes. In addition, the question was raised as to whether or not the novel "nano" properties of nanomaterials require new guidelines, testing procedures, or models for evaluating hazards, or can current evaluation models be validated. From a risk assessment perspective, it may be beneficial to develop a risk-informed decision framework that could be used for assessment based on uncertain and heterogeneous information characteristic of the current state of nanosilver exposure, toxicity and risk research (Linkov et al., 2009). In addition, decision models for nano-enabled pesticide risk assessments that utilize multiple criteria and metrics supported by experimental studies may also be valuable (Tervonen et al., 2009). For this task, multi-criteria decision analysis and quantitative life-cycle assessment tools could be appropriate.

In summary, the most useful short-term information would include the following:

1. Given the scientific knowledge in the field, assessing nanosilver risk in absolute numbers (e.g., as with cancer risk estimation) may not be possible. Alternative problem formulation (e.g., product classification in high/medium/low risk categories) could be essential to guide risk assessment research.
2. Develop framework that will be used to determine how physicochemical characteristics of nanomaterials will be integrated to assess risks. This framework needs to be operational in the immediate future while information on basic nanosilver properties and toxicity is collected.
3. Multiple products in current use contain nanosilver. Determining the total silver content (μg/g, μg/L or other units) of nano-silver, colloidal-silver, etc. in feedstocks, aggregates/mixtures and products containing nanosilver would be useful in assessing risks.

4. Where possible, nanosilver in suppliers products (large scale materials) should be characterized.
5. A critical issue that must be clarified is use of the terminology "nano". The common definition is one that often includes < 100 nm in one dimension and poses a unique property. For standardization, the unique property for nanosilver should be established, as well as for aggregates of nanosilver or nanosilver incorporated via binders.
6. Another critical issue that needs resolution for all nanomaterials is determining which dose metric should be used to assess exposures. For example, the appropriate unit mass concentration, surface area concentration, number and size distribution, etc. should be defined.

REFERENCES

Ahamed M., Karns M., Goodson M., Rowe J., Hussain S.M., Schlager J.J., and Hong Y. 2008. DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells. *Toxicol Appl Pharmacol*. 233(3):404-410.

Alkilany, A.M., Nagaria, P.K., Hexel, C.R., Shaw, T.J., Murphy, C.J., and Wyatt, M.D. 2009. Cellular uptake and cytotoxicity of gold nanorods: molecular origin of cytotoxicity and surface effects. *Small*. 5(6):701-8.

Anjilvel, S. and Asgharian, B. 1995. A multiple-path of particle deposition in the rat lung. Fundam Appl Toxicol. 28(1): 41-50.

AshaRani, P.V., Mun, G. L.K., Hande, M.P., and Valiyaveettil, S. 2009. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. *ACS Nano*. 3(2):279-290.

Auffan, M., Rose, J., Bottero, J.Y., Lowry, G.V., Jolivet, J.P., and Wiesner, M.R. 2009. Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. *Nat Nanotechnol.* DOI:10.1038/NNANO.2009.242.

Balogh, L. Nigavekar, S.S., Nair, B.M., Lesniak, W., Zhang, C., Sung, L.Y., Kariapper, M.S., El-Jawahri, A., Lianes, M., Bolton, B., Mamou, F., Tan, W., Hutson, A., Minc, L., and Khan, M.K. 2007. Significant effect of size on the in vivo biodistribution of gold composite nandevices in mouse tumor models. *Nanomedicine*. 4:281-296.

Bar-Ilan, O., Albrecht, R.M., Fako, V.E., and Furgeson, D.Y. 2009. Toxicity assessments of multisized gold and silver nanoparticles in zebrafish embryos. *Small*. 5: 1897-1910.

Baroli, B., Ennas, M.G., Loffredo, F., Isola, M., Pinna, R., and Lopez-Quintela, M.A. 2007. Penetration of metallic nanoparticles in human full-thickness skin. *J Invest Dermatol* 127:1701–1712.

Behrens, I., Pena, A.I., Alonso, M.J., and Kissel, T. 2002. Comparative uptake studies of bioadhesive and non-bioadhesive nanoparticles in human intestinal cell lines and rats: the effect of mucus on particle adsorption and transport. *Pharm Res*. 19(8): 1185-93.

Benn, T.M. and Westerhoff, P. 2008. Nanoparticle silver released into water from commercially available sock fabrics. *Environ Sci Technol* 42:4133–4139.

Bianchini, A., Grosell, M., Gregory, S.M., and Wood, C.M. 2002. Acute silver toxicity in aquatic animals is a function of sodium uptake rate. *Environ Sci Technol*. 36: 1763-1766.

Bielmyer, G.K., Grosell, M., Paquin, P.R., Mathews, R., and Wu, K.B. (2007) Validation study of the acute biotic ligand model for silver. *Environ Toxicol Chem*. 26: 2241-2246.

Brown, D.M., Wilson, M.R., MacNee, W., Stone,V., and Donaldson, K. 2001. Size-dependent proinflammatory effects of ultrafine polystyrene particles: A role for surface area and oxidative stress in the enhanced activity of ultrafines. *Toxicol Appl Pharmacol*. 175(3):191–199.

Carlson, C., Hussain, S.M., Schrand, A.M., Braydich-Stolle, L.K., Hess, K.L., Jones, R.L., and Schlager, J.J. 2008. Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. *J Phys Chem B*. 112(43):13608-13619.

Cha, K., Hong, H.W., Choi, Y.G., Lee, M.J., Park, J.H., Chae, H-K., Ryu, G., and Myung, H. 2008. Comparison of acute responses of mice livers to short-term exposure to nano-sized or micro-sized silver particles. *Biotechnol Lett*. 30(11):1893-1899.

Chappell, M. A., Miller, L. S., George, A. J., Price, C. L., Mao, J.-D., Bednar, A. J., Seiter, J. M., Kennedy, A. J., and Steevens, J. A. Simultaneous dispersion-dissolution of humic-formulated engineered silver nanoparticles. Submitted for publication.

Chen, K. L., Mylon, S. E. and Elimelech, M. 2006. Aggregation kinetics of alginate-coated hematite nanoparticles in monovalent and divalent electrolytes. *Environ Sci Technol* 40, 1516-1523.

Chen, K. L., and Elimelech, M. 2006. Aggregation and deposition kinetics of fullerene (C-60) nanoparticles. *Langmuir*. 22, (26), 10994-11001.

Chen, K. L., and Elimelech, M. 2007. Influence of humic acid on the aggregation kinetics of fullerene (C-60) nanoparticles in monovalent and divalent electrolyte solutions. *J. Colloid and Interface Sci*. 309, (1), 126-134.

Choi, O. and Hu, Z. 2008. Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. *Environ Sci Technol*. 42(12):4583–4588.

Choi, O., Clevenger, T.E., Deng, B., Surampalli, R.Y., Ross, L., and Hu, Z. 2009. Role of sulfide and ligand strength in controlling nanosilver toxicity. *Water Res*. 43(7):1879-1886.

Donaldson, K., and Li. X.Y. and MacNee, W. 1998. Ultrafine (nanometer) particle mediated lung injury. *J Aerosol Sci*. 29(5-6):553–560.

Driscoll, K.E. 1996. *Role of inflammation in the development of rat lung tumors in response to chronic particle exposure. Particle overload in the rat lung and lung cancer: implications for human risk assessment*. In: Mauderly JL, McCunney RJ, eds. Philadelphia, PA: Taylor & Francis, pp.139–152.

Duffin, R., Tran, C.L., Clouter, A., Brown, D.M., MacNee, W., Stone, V.and Donaldson, K. 2002. The importance of surface area and specific reactivity in the acute pulmonary inflammatory response to particles. *Ann Occup Hyg*. 46:242–245.

Elder, A., Lynch, I., Grieger, K., Chan-Remillard, S., Gatti, A., Gnewuch, H., Kenawy, E., Korenstein, R., Kuhlbusch, T., Linker, F., Matias, S., Monteiro-Riviere, N., Pinto, R.S., Rudnitsky, R., Savolainen, K., Shvedova, A. 2008. Human Health Risks of Engineered Nanomaterials; Critical Knowledge Gaps in Nanomaterials Risk Assessment. In I. Linkov and J Steevens (Eds.), *Nanomaterials: Risks and Benefits*. Netherlands: Springer.

Fabrega, J., Fawcett, S.R., Renshaw, J.C., and Lead, J.R. 2009. Silver nanoparticle impact on bacterial growth: Effect of pH, concentration, and organic matter. *Environ Sci Technol*. 43: 7285-7290.

Florence, A.T., Hillery, A.M., Hussain, N. and Jani, P.U. 1995. Factors affecting the oral uptake and translocation of polystyrene nanoparticles: Histological and Analytical Evidence. *J Drug Target*. 3(1)65-70.

Gajjar, P., Pettee, B., Britt, D.W., Huang, W., Johnson, W.P. and Anderson, A.J. 2009. Antimicrobial activities of commercial nanoparticles against an environmental soil microbe, *Pseudomonas putida* Kt2440. *J Biol Eng*. 3:9.

Gao, J., Youn, S., Hovsepyan, A., Llaneza, V.L., Wang, Y., Bitton, G. and Bonzongo, J-C.J. 2009. Dispersion and toxicity of selected manufactured nanomaterials in natural river water samples: effects of water chemical composition. *Environ Sci Technol*. 43(9):3322-3328.

Geranio, L., Heuberger, M. and Nowack, B. 2009. The behavior of silver nanotextiles during washing. *Environ Sci Technol*. DOI: 10.1021/es9018332.

Griffitt, R.J,. Luo, J., Gao, J., Bonzongo, J-C., Barber, D.S. 2008. Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms. *Environ Toxicol Chem*. 27(9):1972-1978.

Griffitt, R.J., Hyndman, K., Denslow, N.D., Barber, D.S. 2009. Comparison of molecular and histological changes in zebrafish gills exposed to metallic nanoparticles. *Toxicol Sci*. 107(2):404-415.

Heinlaan, M., Ivask, A., Blinova, I., Dubourguier, H.C. and Kahru, A. 2008. Toxicity of nanosized and bulk ZnO, CuO and TiO_2 to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. *Chemosphere*. 71(7):1308-16.

Hellstrand, E., I. Lynch, A. Andersson, T. Drakenberg, B. Dahlback, K.A. Dawson, S. Linse, and T. Cedervall. 2009. Complete high-density lipoproteins in nanoparticle corona. *Febs J*. 276, 3372-81.

Hussain, S.M., Hess, K.L., Gearhart, J.M., Geiss, K.T., Schlager, J.J. 2005. In vitro toxicity of nanoparticles to BRL rat liver cells. *Toxicol in Vitro*. 19(7):975-983.

Hwang, E.T., Lee, J.H., Chae, Y.J., Kim, Y.S., Kim, B.C., Sang, B. and Gu, M.B. 2008. Analysis of the toxic mode of action of silver nanoparticles using stress-specific bioluminescent bacteria. *Small*. 4:746-750.

Ispas, C., Andreescu, D., Patel, A., Goia, D.V., Andreescu, S. and Wallace, K.N. 2009. Toxicity and developmental defects of different sizes and shape nickel nanoparticles in zebrafish. *Environ Sci Technol*. 43(16): 6349-56.

Jeong, G.N., Jo, U.B., Kim, Y.S., Ryu, H.Y., and Yu, I.J. 2010. Histochemical study of intestinal mucins after administration of silver nanoparticles in Sprague-Dawley rats. *Arch Toxicol.* 84(1): 63-69.

Ji, J.H., Jung, J.H., Kim, S.S., Yoon, J.U., Park, J.D., Choi, B.S., Chung, Y.H., Kwon, I.H., Jeong, J., Han, B.S., Shin, J.H., Sung, J.H.., Song, K.S. and Yu, I.J. 2007. Twenty-eight-day inhalation toxicity study of silver nanoparticles in Sprague-Dawley rats. *Inhal Toxicol* 19:857-871.

Jiang, Z.J., Liu, C.Y. and Sun, L.W. 2005. Catalytic properties of silver nanoparticles supported on silica spheres. *J Phys Chem B*. 109(5):1730-5.

Jiang, W., H. Mashayekhi, and B. Xing. 2009. Bacterial toxicity comparison between nano- and micro-scaled oxide particles. *Environ Pollut*. 157, 1619-1625.

Johnston, C.J., Finkelstein, J.N., Mercer, P., Corson, N., Gelein, R. and Oberdörster G. 2000. Pulmonary effects induced by ultrafine PTFE particles. *Toxicol Appl Pharmacol*. 168:208-215.

Kaegi, R., Ulrich, A., Sinnet, B., Vonbank, R., Wichser, A., Zuleeg, S., Simmler, H., Brunner, S., Vonmont, H., Burkhardt, M., and Boller, M. 2009. Synthetic TiO_2 nanoparticle emission from exterior facades into the aquatic environment. *Environ Pollut*. 156(2):233-9.

Kasemets, K., Ivask, A., Dubourguier, H.C. and Kahru, A. 2009. Toxicity of nanoparticles of ZnO, CuO and TiO_2 to yeast Saccharomyces cerevisiae. *Toxicol In Vitro*. 23(6):1116-22.

Kawata, K., Osawa, M., and Okabe, S. 2009. In vitro toxicity of silver nanoparticles at noncytotoxic doses to HepG2 human hepatoma cells. *Environ Sci Technol*. 43: 6046-6051.

KEMTI. 2008. GLP Study reports submitted to KFDA: *90 day subchronic oral toxicity study of silver nanoparticles*. Unpublished.

Kim, C. S. 2000. Methods of calculating lung delivery and deposition of aerosol particles. *Respir Care* 45(6):695–710.

Kim, Y.S., Kim, J.S., Cho, H.S., Rha, D.S., Kim, J.M., Park, J.D., Choi, B.S., Lim, R., Chang, H.K., Chung, Y.H., Kwon, I.H., Jeong, J., Han, B.S. and Yu, I.J. 2008 Twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley Rats. *Inhal Toxicol.* 20(6):575-83.

Kim, W.Y., Kim, J., Park, J.D., Ryu, H.Y. and Yu, I.J. 2009 Histological study of gender difference in accumulation of silver nanoparticles in kidneys of Fisher 344 rats. *J Toxicol Environ Health, Part A.* 72: 1279–1284.

Kim, S., J.E. Choi, J. Choi, K.H. Chung, K. Park, J. Yi, and D.Y. Ryu. 2009. Oxidative stress-dependent toxicity of silver nanoparticles in human hepatoma cells. *Toxicol In Vitro.* 23, 1076-84.

Korea FDA (KFDA). 2008. *90 day inhalation toxicity study of gold nanoparticles.* Unpublished.

Kreyling, W.G., Semmler, M., Erbe, F., Mayer, P., Takenaka, S., Schulz, H., Oberdörster, G., Ziesenis, A., 2002. Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. *J Toxicol Environ Health A.* 65(20):1513-30.

Lansdown, A., Williams, A., Chandler, S. and Benfield, S. 2005. Silver dressings: absorption and antibacterial efficacy. *J Wound Care.* 14(4):155-60.

Larese, F.F., D'Agostin, F., Crosera, M., Adami, G., Renzi, N., Bovenzi, M. and Maina, G. 2009. Human skin penetration of silver nanoparticles through intact and damaged skin. *Toxicol.* 255(1-2):33-37.

Lawler, D.F. 1986. Removing particles in water and wastewater. *Environ Sci Technol.* 20:9:856-861.

Lee, H.Y., H.K. Park, Y.M. Lee, K. Kim, and S.B. Park. 2007. A practical procedure for producing silver nanocoated fabric and its antibacterial evaluation for biomedical applications. *Chem Commun (Camb)*, 2959-61.

Lee, J. H., Yu , I.J. 2009a. *Toxicity assessment in vivo using standard nanoparticles.* Final report submitted to the Korea Research Institute of Standard Science. Unpublished.

Lee, J.H., and Yu, I.J. 2009b. *Standardization of nanomaterial exposure assessment method and development on guidance to exposure management.* Progress report submitted to Korea Evaluation Institute of Industrial Technology. Unpublished.

Linkov, I., Satterstrom, K., Steevens, J., Ferguson, E., Pleus, R. 2007. Multi-criteria decision analysis and environmental risk assessment for nanomaterials. *J Nanoparticle Res.* 9:543-554.

Linkov, I., Satterstrom, F.K., Monica, J.C., Jr., Foss Hansen, S. and Davis, T.A. 2009. Nano Risk Governance: Current Developments and Future Perspectives. *Nanotechnology: Law and Business* 6:203.

Lison, D., Lardot, C., Huaux, F., Zanetti, G. and Fubini, B. 1997. Influence of particle surface area on the toxicity of insoluble manganese dioxide dusts. *Arch Toxicol.* 71(12):725–729.

Lok, C.N., Ho, C.M., Chen, R., He, Q.Y., Yu, W.Y., Sun, H., Tam, P.K., Chiu, J.F.and Che, C.M. 2006. Proteomic analysis of the mode of antibacterial action of silver nanoparticles. *J Proteome Res.* 5:916-924.

Lok, C.N., Ho, C.M., Chen, R., He, Q.Y., Yu, W.Y., Sun, H., Tam, P.K., Chiu, J.F. and Che, C.M. 2007. Silver nanoparticles: Partial oxidation and antibacterial activities. *J Biol Inorg Chem.* 12:527-534.

Lu, F., Wu, S.H., Hung, Y., and Mou, C.Y. 2009. Size effect on cell uptake in well-suspended, uniform mesoporous silica nanoparticles. *Small.* 5(12):1408-13.

Lundqvist, M., Stigler, J., Elia, G., Lynch, I., Cedervall, T., Dawson, K.A. 2008. Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. Proc Natl Acad Sci. 105(38): 14264-14270.

Luoma, S.M. 2008. Silver nanotechnologies and the environment: old problems or new challenges? Project on Emerging Technologies. Woodrow Wilson International Center for Scholars.

Mackay, M.E., Tuteja, A., Duxbury, P.M., Hawker, C.J., Van Horn, B., Guan, Z.B., Chen, G.H. and Krishnan, R.S. 2006. General strategies for nanoparticle dispersion. *Science.* 311 (5768):1740–1743.

Malik, N. Wiwattanapatapee, R., Klopsch, R., Lorenz, K., Frey, H., Weener, J.W., Meijer, E.W., Paulus, W. and Duncun, R. 2000. Dendrimers: relationship between structure and biocompatibility in vitro, and preliminary studies on the biodistribution of I-125-labelled poly(amidoamine) dendrimers in vivo. *J Control Release.* 65:133-148.

Massoudieh, A. and T.R. Ginn. 2008. Modeling Colloid-Enhanced Contaminant Transport in Stormwater Infiltration Basin Best Management Practices. *Vadose Zone J.* 7, 1215-1222.

Matsumura, Y., Yoshikata, K., Kunisaki, S., and Tsuchido, T. 2003 Mode of bactericidal action of silver zeolite and its comparison with that of silver nitrate. *Appl Environ Microbiol.* 69: 4278-4281.

Maynard A. 2002. Experimental determination of ultrafine TiO_2 deagglomeration in a surrogate pulmonary surfactant: Preliminary results. *Ann Occup Hyg.* 46(1)(Suppl):197–202.

McDaniels, T.L. and Gregory, R. 2004. Learning as an objective within a structured risk management decision process. *Environ Sci Technol.* 38(7):1921-6.

McNeil, S.E. 2009. Nanoparticle therapeutics: a personal perspective. Wiley Interdisciplinary Reviews: *Nanomedicine and Nanobiotechnology.* 1(3):264-271.

Mills, W.B., S. Liu, and F.K. Fong. 1992. Literature-Review and Model (Comet) for Colloid Metals Transport in Porous-Media - Reply. *Ground Water.* 30, 106-106.

Miura, N. and Y. Shinohara. 2009. Cytotoxic effect and apoptosis induction by silver nanoparticles in HeLa cells. *Biochem Biophys Res Commun.* 390, 733-7.

Morones, J.R., Elechiguerra, J.L., Camacho, A., Holt, K., Kouri, J.B., Ramirez, J.T., and Yacaman, M.J. 2005. The bactericidal effect of silver nanoparticles. *Nanotechnology.* 16: 2346-2353.

Mueller, N.C., and Nowack, B. 2008. Exposure modeling of engineered nanoparticles in the environment. *Environ Sci Technol.* 42: 4447-4453.

Nel, A., Xia, T., Madler, L., and Li, N. 2006. Toxic potential of materials at the nanolevel. *Science.* 311: 622-627

U. S. NIOSH. 2005. *NIOSH current intelligence bulletin: Evaluation of health hazard and recommendations for occupational exposure to titanium dioxide (draft).* Available at www.cdc.gov/niosh/review/public/TIo2/pdfs/TIO_2Draft.pdf.

U. S. NIOSH. 2009. *Approaches to Safe Nanotechnology: Managing the Health and Safety Concerns Associated with Engineered Nanomaterials.* Cincinnati, OH: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 2009–125. http://www.cdc.gov/niosh/docs/2009-125/.

Oberdörster, G., Ferin, J., Gelein, R., Soderholm, S.C. and Finkelstein, J. 1992. Role of the alveolar macrophage in lung injury–studies with ultrafine particles. *Environ Health Perspect.* 97:193–199.

Organization for Economic Cooperation and Development. 2009. *Emmision assessment for identification of sources and release of airborne manufactured nanomaterials in the workplace: compilation of existing guidance.* ENV/JM/MONO(2009)16. Available at https://www.oecd.org/dataoecd/15/60/43289645.pdf.

Paddle-Ledinek, J.E., Nasa, Z. and Cleland, H.J. 2006. Effect of different wound dressings on cell viability and proliferation. *Plast Reconstr Surg.* 117(7 Suppl):110S-118S.)

Pal, S., Tak, Y.K. and Song, J.M. 2007. Does antibacterial activity of silver nanoparticle depend on shape of nanoparticle? A study on gam-negative *E. coli. Appl Environ Microbiol.* 73:1712-1720.

Park, J., Kwak, B.K., Bae, E., Lee, J., Kim, Y., Choi, K., Yi, J. 2009. Characterization of exposure to silver nanoparticles in a manufacturing facility. *J Nanpart Res.* 11:1705-1712.

Ratte, H.T. 1999 Bioaccumulation and toxicity of silver compounds: A review. *Environ Toxicol Chem.* 18: 89-108.

Robichaud, C.O., Tanzil, D., Weilenmann, U. and Wiesner, M.R. 2005. Relative risk analysis of several manufactured nanomaterials: an insurance industry context. *Environ Sci Technol.* 39(22):8985-994.

Ryman-Rasmussen JP, Riviere JE, and Monteiro-Riviere NA. 2007. Surface coatings determine cytotoxicity and irritation potential of quantum dot nanoparticles in epidermal keratinocytes. *J Invest Dermatol.* 1:143–153.

Sambhy, V., MacBride, M.M., and Peterson, B.R. 2006. Silver bromide nanoparticle/polymer composites: Dual action tunable antimicrobial materials. *J American Chemical Society.* 128: 9798-9808.

Sayes, C.M., and Warheit, D.B. 2009. Characterization of nanomaterials for toxicity assessment. *Nanomedicine and Nanobiotechnology.* 1:660-670.

Seager, T. and Linkov, I. 2008. Coupling Multi-Criteria Decision Analysis and Life Cycle Assessment For Nanomaterials. *J. Industrial Ecology.* 12:282-285.

Seager, T.P., and Linkov, I. 2008. *Uncertainty in Life Cycle Assessment of Nanomaterials: Multi-criteria decision analysis framework for single wall carbon nanotubes in power applications.* ISSN: 1874-6519. Springer Netherlands.

Semmler, M., Seitz, J., Erbe, F., Meyer, P., Heyder, J., Oberdörster, G. and Kreyling, W.G. 2004. Long-term clearance kinetics of inhaled ultrafine insoluble iridium particles from the rat lung, including transient translocation into secondary organs. *Inhal Toxicol.* 16:453–459.

Sonavane, G., Tomoda, K., Sano, A., Ohshima, H., Terada, H., Makina, K. 2008. In vitro permeation of gold nanoparticles through rat skin and rat intestine: effect of particle size. *Colloid Surf B.* 65(1):1-10.

Sondi, I., and Salopek-Sondi, B. 2004. Silver nanoparticles as antimicrobial agent: a case study on E-coli as a model for Gram-negative bacteria. *Journal Coll Interface Sci.* 275: 177-182.

Sung, J.H., Choi, B.G., Maeng, S.H., Kim, S.J., Chung, Y.H., Han, J.H., Song, K.S., Lee, Y.H., Cho, Y.B., Cho, M.H., Kim, K.J., Hyun, J.S. and Yu, I.J. 2004. Recovery from welding-fume-exposure-induced lung fibrosis and pulmonary function changes in Sprague Dawley rats, *Toxicol Sci.* 82: 608-613.

Sung, J.H., Ji, J.H., Yun, J.U., Kim, D.S., Song, M.Y., Jeong, J., Han, B.S., Han, J.H., Chung, Y.H., Kim, J., Kim, T.S., Chang, H.K., Lee, E.J., Lee, J.H. and Yu, I.J. 2008. Lung function changes in Sprague-Dawley rats after prolonged inhalation exposure to silver nanoparticles, *Inhal Toxicol.* 20(6):567-74,

Sung, J.H., Ji, J.H., Park, J.D., Yoon, J.U., Kim, D.S., Jeon, K.S., Song, M.Y., Jeong, J., Han, B.S., Han,J.H., Chung, Y.H., Chang, H.K., Lee, J.H., Cho, M.H., Kelman, B.J. and Yu, I.J. 2009. Subchronic inhalation toxicity of silver nanoparticles. *Toxicol Sci.* 108 (2): 452-61.

Sweet, L. and Strohm, B. 2006. *Nanotechnology – Life-Cycle Risk Management. Human and Ecological Risk Assessment.* 12(3):528-551.

Takenaka, S., E. Karg, C. Roth, H. Schulz, A. Ziesenis, U. Heinzmann, P. Schramel, and J. Heyder. 2001. Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. *Environ Health Perspect.* 109 Suppl 4, 547-51.

Tervonen, T., Linkov, I. Figueira, J., Steevens, J., Chappell, M., Merad, M. 2009. Risk-based Classification System of Nanomaterials. *J. of Nanoparticle Res.* 11:757-766.

Tervonen, T., Figueira, J.R., Lahdelma, R., Dias, J.A. and Salminen, P., 2009. A stochastic method for robustness analysis in sorting problems. *Eur J Oper Res.* 192(1):236-242.

Tjalve, H., Henriksson, J., Tallkvist, J., Larsson, B.S. and Lindquist, N.G. 1996. Uptake of manganese and cadmium from the nasal mucosa into the central nervous system via olfactory pathways in rats. *Pharmacol Toxicol.* 79(6):347-56.

Tosco, T., Tiraferri, A., and Sethi, R. 2009. Ionic strength dependent transport of microparticles in saturated porous media: modeling mobilization and immobilization phenomena under transient chemical conditions. *Environ Sci Technol.* 43(12): 4425-31.

Trop, M., Novak, M., Rodl, S., Hellbom, B., Kroell, W. and Goessler, W. 2006. Silver-coated dressing acticoat caused raised liver enzymes and argyria-like symptoms in burn patient. *J Trauma.* 60(3):648-52.

Van Hoecke, K., Quik, J.T., Mankiewicz-Boczek, J., De Schamphelaere, K.A., Elsaesser, A., Van der Meeren, P., Barnes, C., McKerr, G., Howard, D.V., Van de Meent, D., Rydzynski, K., Dawson, D.A., Salvati, A., Lesniak, A., Lynch, I., Silversmit, G., De Samber, B., Vincze, L., Janssen, C.R. 2009. Fate and effects of CeO2 nanoparticles in aquatic ecotoxicity tests. *Environ Sci Technol.* 15;43(12):4537-46.

Verma, A., Uzun, O., Hu, Y., Hu, Y., Han, H-S., Watson, N., Chen, S., Irvine, D.J. and Stellacci, F. 2008. Surface-structure-regulated cell-membrane penetration by monolayer-protected nanoparticles. *Nat Mater*. 7:588-595.

Vlachou, E., Chipp, E., Shale, E., Wilson, Y.T., Papini, R. and Moiemen, N.S. 2007. The safety of nanocrystalline silver dressings on burns: a study of systemic silver absorption. *Burns*. 33(8): 979-85.

Walker, N.J. and Bucher, J.R. 2009. Forum Series, Part V. A 21st century paradigm for evaluating the health hazards of nanoscale materials. *Toxicol Sci*. 110 (2): 251-254

Wallace, W.E., Keane, M.J., Murray, D.K., Chisholm, W.P., Maynard, A.D., Ong, T-M. 2007. Phospholipid lung surfactant and nanoparticle surface toxicity: Lessons from diesel soot and silicate dusts. *J Nanopart Res*. 9:23-38.

Wang, Y.B. 2009. Differential Effects of Sodium Selenite and Nano-Se on Growth Performance, Tissue Se Distribution, and Glutathione Peroxidase Activity of Avian Broiler. *Biol Trace Elem Res*. 128, 184-190.

Wijnhoven, S.W.P., Peijnenburg, W.J.G.M., Herberts, C.A., Hagens, W.I., Oomen, A.G., Heugens, E.H.W., Roszek, B., Bisschops, J., Gosens, I., Van De Meent, D., Dekkers, S., De Jong, W.H., Van Zijverden, M., Sips, A.J.A.M., Geertsma, R.E., 2009. Nano-silver: a review of available data and knowledge gaps in human and environmental risk assessment. *Nanotoxicology*. 3(2): 109-138.

Yu, I.J., Park, J.D., Park, E.S., Song, K.S., Han, K.T., Han, J.H., Chung, Y.H., Choi, B.S., Chung, K.H., and Cho, M.H. 2003. Manganese distribution in brains of Sprague Dawley rats after 60 days of stainless steel welding-fume exposure. *Neurotoxicology*. 24(6): 777-785.

Zhang, Y., Chen, Y., Hristovski, K., Westerhoff, P. and Crittenden, J.C. 2008a. Stability of commercial metal oxide nanoparticles in water. *Water Res*. 42(8):9:2204-2212.

Zhang, Y., Chen, Y., Westerhoff, P. and Crittenden, J.C. 2008b. Stability and removal of water soluble CdTe quantum dots in water. *Environ. Sci. Technol*. 42(1) :321-325.

Zhang, Y., Chen, Y., Westerhoff, P. and Crittenden, J. 2009. Impact of natural organic matter and divalent cations on the stability of aqueous nanoparticles. *Water Res*. 43:4249-4257.

Zhang, L.W. and Monteiro-Riviere, N.A. 2009. Mechanism of quantum dot nanoparticle cellular uptake. *Toxicol Sci*. 110(1):138-55