NANOMATERIAL CASE STUDY WORKSHOP:

DEVELOPING A COMPREHENSIVE ENVIRONMENTAL ASSESSMENT RESEARCH STRATEGY FOR NANOSCALE SILVER

JANUARY 4–7, 2011

RESEARCH TRIANGLE PARK, NORTH CAROLINA

WORKSHOP REPORT

PREPARED FOR EPA/NCEA

DECEMBER 2011

PREPARED BY ICF INTERNATIONAL UNDER
EPA CONTRACT NUMBER EP-C-09-009,
WORK ASSIGNMENT NUMBER 2-12

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PREFACE

On January 4–7, 2011, ICF International (ICF) organized and coordinated a workshop on nanoscale silver (Nanomaterial Case Studies Workshop: Developing a Comprehensive Environmental Assessment Research Strategy for Nanoscale Silver) for the National Center for Environmental Assessment (NCEA) in the U.S. Environmental Protection Agency's (EPA) Office of Research and Development (ORD). The workshop was the second in a series that NCEA is conducting to further the development of a research strategy for completing comprehensive environmental assessments of nanomaterials. The basis of the workshop was the report *Nanomaterial Case Study: Nanoscale Silver in Disinfectant Spray*. Prior to and separate from the workshop, EPA convened a Public Information Exchange to explain the rationale for the case study approach, the choice of nanomaterials and applications, and the results from a previous workshop on nanoscale titanium dioxide. In compliance with requirements of the Federal Advisory Committee Act (5 U.S.C. Appendix 2; see http://www.gsa.gov/portal/content/100916), ICF conducted the workshop on nanoscale silver and prepared this summary independently of EPA, with EPA funding. Although this summary is an independent document, it links to and should be viewed in concert with, EPA's external review draft of *Nanomaterial Case Study: Nanoscale Silver in Disinfectant Spray* (U.S. EPA, 2010b).

The outcomes of this and future workshops in the series—prioritized information gaps and risk tradeoffs—will be used in developing and refining a long-term research strategy to assess potential human health and ecological risks of nanomaterials and to manage associated risks of specific nanomaterials.

DISCLAIMER

Mention of commercial or trade names does not constitute endorsement by the U.S. Environmental Protection Agency (EPA). The views expressed in this report are those of the workshop participants and do not reflect EPA opinions or policy.

ICF International, EPA's contractor, provided logistics and note-taking at the workshop and prepared this report. This work was conducted under EPA Contract Number EP-C-09-009, Work Assignment Number 2-12.

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1. Workshop Objectives and Design

Engineered nanoscale materials (nanomaterials) are conventionally described as having at least one dimension between 1 and 100 nanometers (nm) and possessing unusual, if not unique, properties that arise from their small size. Like all technological developments, nanomaterials offer the potential for both benefits and risks. Given the emerging state of nanotechnology, however, much remains to be learned about the characteristics and effects of nanomaterials before such assessments can be completed.

In its 2007 Nanotechnology White Paper (2007), the U.S. Environmental Protection Agency (EPA) included the recommendations shown in the text box at the right regarding the risk assessment of nanomaterials. The approach the National Center for Environmental Assessment (NCEA), in EPA's Office of Research and Development, adopted is to draft a case study that details the information currently available to complete a comprehensive environmental assessment (CEA) for a selected nanomaterial in a specific application. The CEA approach consists of both a framework and a process. The CEA framework provides a structure to develop a comprehensive view of what is known about the nanomaterial application beginning with the product life cycle; progressing to its environmental fate and transport, exposure-dose in ecological and human populations, and finally, ending with its human health, ecological, and other (aesthetic, climate, energy, sustainability, etc.) impacts. This approach enables identification of gaps in our knowledge and corresponding research topics that could help support a CEA of the nanomaterial. Compiling the information on what is known about the nanomaterial is the first step in the CEA process (Figure 1-1). Next, a collective judgment process is used to evaluate and then prioritize this information. Collective judgment, as has been applied in the CEA process to date, refers to a formal, structured procedure that enables a diverse group of individuals to be heard individually and represented in a transparent record of the collectively reached outcomes. In turn, it supports an essential feature of CEA: the inclusion of diverse technical and stakeholder perspectives to ensure that a holistic evaluation is achieved (U.S. EPA, 2010c).

Recommendations to Address Overarching Risk Assessment Needs – Case Study

One way to examine how a nanomaterial assessment would fit within EPA's overall risk assessment paradigm is to conduct a case study based on publicly available information on one or several intentionally produced nanomaterials. … From such case studies and other information, information gaps may be identified, which can then be used to map areas of research that are directly affiliated with the risk assessment process. This has been done in the past with research on airborne particulate matter.

Additionally, a series of workshops involving a substantial number of experts from several disciplines should be held to use available information and principles in identifying data gaps and research needs that will have to be met to carry out exposure, hazard, and risk assessments.

2007 Nanotechnology White Paper (2007) (p. 89)

The outcomes of the workshops—prioritized information gaps and risk tradeoffs—will be used in developing and refining a long-term research strategy to assess potential human health and ecological risks of nanomaterials and to manage associated risks of specific nanomaterials.

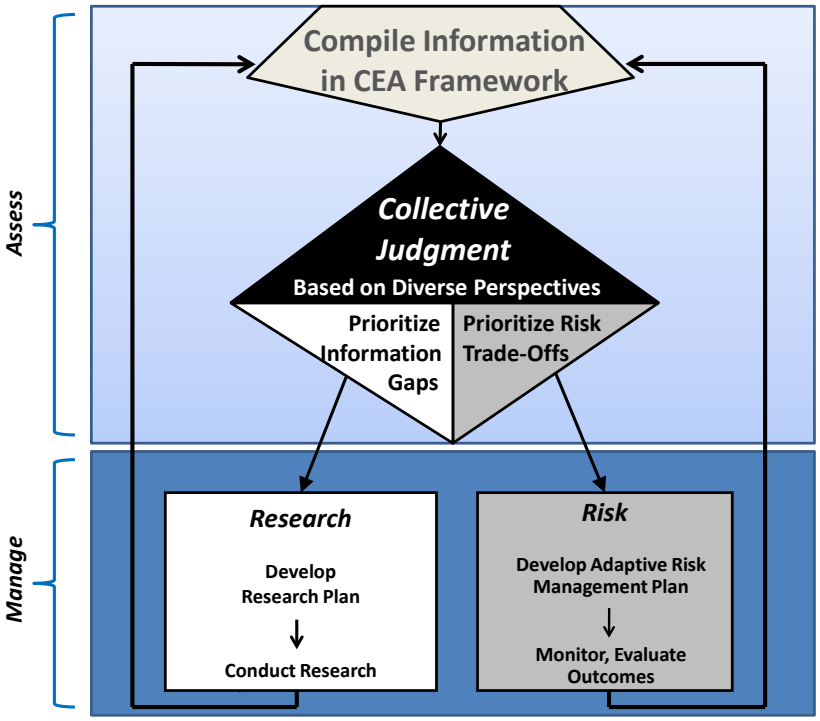


Figure 1-1. Steps in the CEA process.

The first workshop in this series, "Nanomaterial Case Study Workshop: Developing a Comprehensive Environmental Assessment Research Strategy for Nanoscale Titanium Dioxide," was held in 2009 and focused on EPA's *Nanomaterial Case Studies: Nanoscale Titanium Dioxide in Water Treatment and in Topical Sunscreen* (U.S. EPA, 2010a). The outcomes from that workshop are reported in EPA's *Workshop Summary for the EPA Board of Scientific Counselors* (U.S. EPA, 2010c). NCEA sponsored its second "Nanomaterial Case Study Workshop: Developing a Comprehensive Environmental Assessment Research Strategy for Nanoscale Silver" January 4–7, 2011, in Research Triangle Park, North Carolina. The starting point for the workshop was EPA's external review draft of *Nanomaterial Case Study: Nanoscale Silver in Disinfectant Spray* (U.S. EPA, 2010b).

Both workshops used nominal group technique (NGT) as the collective judgment tool to facilitate the discussion and prioritization of information needs among the group of diverse participants. NGT is a structured process whereby several individuals (nominally a group) are convened to identify and rank a number of choices and each person is afforded an equal opportunity to offer his or her view(s) about which choices are highest priority. More information regarding how this technique was put to use is provided in Section 1.4.2.

The most recent workshop on nanoscale silver improved on the format based on the lessons learned from the first workshop and the input of EPA's Board of Scientific Counselors. Fewer participants were selected for the 2011 workshop, reducing overall costs. In addition, one group of participants was used in the second workshop, instead of two, eliminating time required to consolidate the priority research needs they identified. The time saved was allotted to breakout group discussions, allowing for the participants to report on the top 13 rather than top 8 consolidated research needs. In fact, for the nanosilver workshop, less consolidation was encouraged, which resulted in more, and more distinct, categories of research needs.

An important point to emphasize is that none of the nanomaterial case study documents and workshops are intended to be ends in and of themselves, even though intrinsically they might have value or be of interest. They are primarily viewed as initial steps in the development and refinement of a long-range research strategy to support CEAs of selected nanomaterials. Full implementation of such a strategy requires preparing additional nanomaterial case studies, and the process is expected to evolve, reflecting adjustments and modifications as additional nanomaterials are considered and new information becomes available.

This report describes the outcomes of the 2011 nano-Ag workshop. Figure 1-2 illustrates the workshop design. Refer to the 2010 *Workshop Summary for the EPA Board of Scientific Counselors* for more information on the approach used in developing the case studies, the rationale in designing the workshops, and the outcomes of the 2009 workshop (U.S. EPA, 2010c).

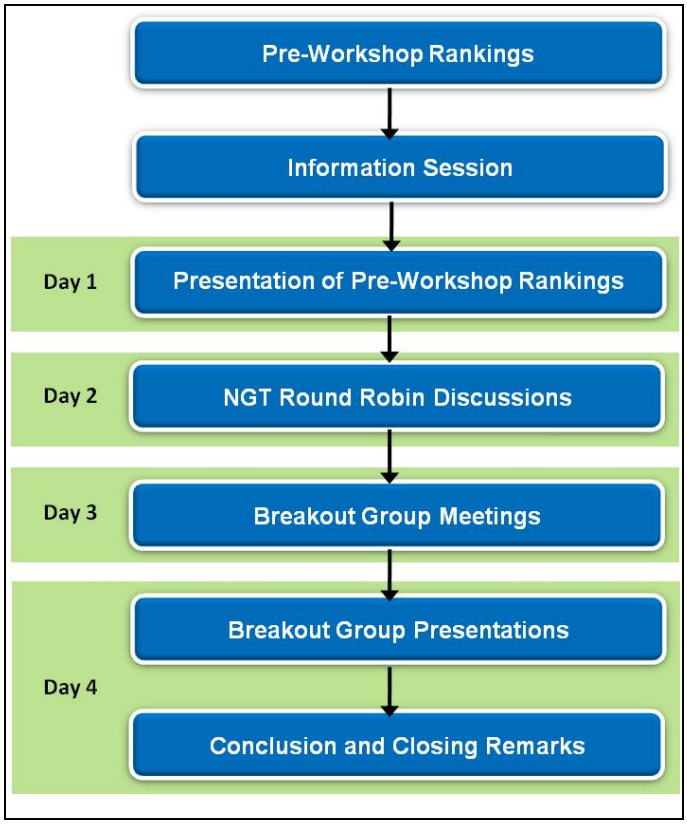


Figure 1-2. Nano-Ag Workshop Design.

1.1. Workshop Objectives

The goal of this workshop was to prioritize responses to the following question:

> What research or information is most needed to conduct a comprehensive environmental assessment of nano-Ag used in disinfectant spray?

As discussed in Section 1.4.2, participants were guided through the NGT process, which enabled them to identify and rank issues in response to the above question using their independent judgments and the collective judgment of the group. The sections that follow describe the selection of participants, pre-workshop activities, implementation of NGT for the workshop, and structure of the breakout group reports.

1.2. Selection of Participants

Securing a multidisciplinary and multistakeholder set of workshop participants involved several steps, with a goal of achieving a diverse array of technical and stakeholder perspectives to yield insights that would be useful in defining what is essential to complete a CEA of this emerging nanotechnology. EPA retained ICF International to help organize and facilitate the workshop.

First, a list of candidate participants was developed based on information EPA provided, Internet searches, and other investigation. From available biographical information, participants were assigned to categories based on their sector (academia, consulting, NGO, etc.) and subject matter expertise. Considerable attention was given to achieving, as much as possible, a balanced representation across sectors and areas of expertise.

A target number of 25 participants was set. Because the 49 participants in the 2009 nano-TiO_2 workshop, working in two NGT groups, independently identified similar research priorities, EPA decided that 25 participants, working in one NGT group were sufficient to accomplish the 2011 workshop goals.

To ensure a final total of 25 participants, 30 potential participants were initially invited. When a potential participant declined, an alternate was identified to maintain a balanced distribution of disciplines and stakeholders. Ultimately, 23 participants, listed in Table 1–1, attended the workshop. Table 1-2 lists the sector representation, and Section 4.2 presents the biographical sketches each participant provided.

Table 1–1. Workshop Participant Names and Affiliations

Participant	Affiliation	Sector
Mary Boudreau	U.S. Food and Drug Administration	Government
Mark Chappell	U.S. Army Engineer Research and Development Center	Government
Hongda Chen	USDA National Institute of Food and Agriculture	Government
Mary Jane Cunningham	Nanomics BioSciences	Industry
James Delattre	NanoHorizons	Industry
David Ensor	RTI International	Consulting
Michael Hansen	Consumer's Union	NGO, Labor, Journalism
Carol Henry	Independent Consultant	Consulting
Matthew Hull	NanoSafe Inc.	Industry
Ian Illuminato	Friends of the Earth	NGO, Labor, Journalism
Larry Kapustka	LK Consultancy	Consulting
Bojeong Kim	Virginia Tech University	Academia
Kristen Kulinowski	Rice University CBEN	Academia
Debbie Lander	DuPont	Industry
Paul Lioy	EOHSI / Rutgers University – UMDNJ-RWJMS	Academia
Brian O'Connor	FPInnovations – PAPRICAN	Industry
Maria Powell	Nanotechnology Citizen Engagement Organization	NGO, Labor, Journalism
Gurumurthy Ramachandran	University of Minnesota	Academia
Christie Sayes	Texas A&M University	Academia
Maria Sepulveda	Purdue University	Academia
Brian Strohmeier	RJ Lee Group	Consulting
Michael Tolocka	University of North Carolina – Chapel Hill	Academia
Dik van de Meent	RIVM Laboratory for Ecological Risk Assessment	Government

Table 1–2. Participant Sector Representation

Sector	Total	Participants
Academia	7	B, F, L, O, U, V, W
Industry	5	D, H, R, S, T
NGO, Labor, Journalism	3	A, K, N
Consulting	4	C, F, G, M
Government	4	I, J, P, O
Total	23	--

Upon acceptance of the invitation, each participant received a conflict of interest disclosure form to complete; no conflicts were identified. Generally, a written agreement was executed with each nonfederal-government participant for reimbursement of travel expenses and payment of an honorarium of $2,000 for services. A purchase order agreement and honorarium were used to help ensure that participants would understand that a commitment of their time and attention was expected and that their services were not being offered gratis.

1.3. Pre-Workshop Review and Rankings

Confirmed participants were asked to review the nano-Ag case study document in advance of the workshop and, using an Excel-based form, submit their preliminary rankings of research questions listed in the case study. They also were invited to submit review comments on the case study, to be considered in revising the case study document prior to final publication. The objective of having the participants rank the questions and review the draft case study document prior to the workshop was both to help ensure that participants actively read the document and to prepare them to provide final ranking of the issues in priority order. Essentially, this pre-workshop exercise was intended to substitute for the brainstorming aspect of NGT (Van de Ven and Delbecq, 1972).

Participants were asked to determine their rankings of research questions by selecting: (1) the 10 most important questions identified in the draft case study document in rank order from 1 to 10; (2) 15 additional questions—in no particular order—that were also of high but lesser importance; and (3) up to 10 questions of lowest priority in laying the foundation for a CEA of nano-Ag. Participants also were invited to submit modifications of existing questions from the case study and new questions not included in the document. All revised and new questions were compiled and distributed to the workshop participants via email one week before the workshop, and the questions were included in the folders of materials provided to the participants at the workshop. During the initial plenary session at the workshop, the facilitators presented the results of the pre-workshop ranking of the questions. Figure 1-3 presents the pre-workshop ranking results for the top ten ranked questions. The instructions to participants detailing the pre-workshop ranking procedure are presented in Section 4.3 of this report; lists of new and revised questions are included in Section 4.4; and the pre-workshop ranking results for all questions and the methodology used to analyze the results are presented in Section 4.5.

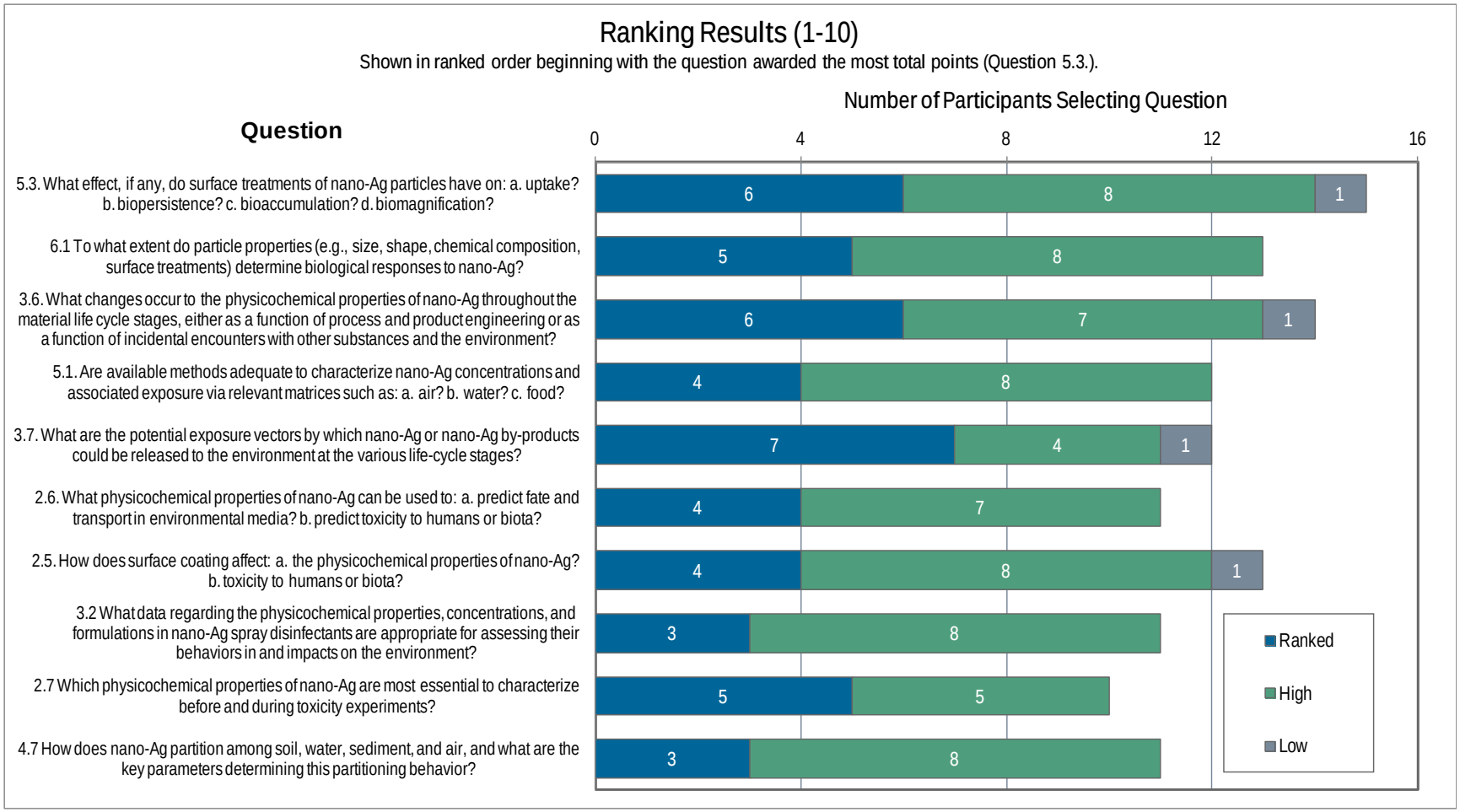


Figure 1-3. Pre-workshop ranking results for the top ten research questions posed in the nanosilver case study document.

1.4. Workshop Activities

This section describes several key workshop-related activities, including an EPA Public Information Exchange, the NGT process, and development of reports by participants.

1.4.1. Information Exchange and Introduction

Prior to and separate from the workshop, EPA convened a Public Information Exchange to explain the rationale for the case study approach, the choice of nanomaterials and applications, and the results from the 2009 workshop on nano-TiO_2. During the exchange, EPA clarified that ICF would conduct the Nanomaterial Case Study Workshop on Nano-Ag independently of EPA, with EPA funding, in compliance with requirements of the Federal Advisory Committee Act (5 U.S.C. Appendix 2; see http://www.gsa.gov/portal/content/100916).

1.4.2. Nominal Group Technique

A description of the NGT process was provided to participants in advance of the workshop (see text box on the following page). The workshop agenda (Section 4.1) provides further detail about how the meeting was conducted.

The workshop was structured to introduce participants to NGT in the opening plenary session, when the pre-workshop ranking results also were presented and discussed to stimulate further thought about the relative importance of the various questions. At the end of the session, participants were asked to carefully consider and then select the top research priorities, along with rationales, for presentation during the NGT round robin.

The round-robin procedure allowed individuals up to 3 minutes each to present to the group a single high priority research/information need and a rationale for selecting that issue in relation to conducting a CEA. Participants also were allowed to present an entirely new question or to modify the phrasing or content of an existing research question. Each high priority research need was written on a flip-chart to enable consideration by the group. After each participant had spoken, the procedure was repeated for two more rounds, until all research priorities were posted on the wall. Altogether, participants identified 78 research issues as information needs during this stage: 58 unique issues and 20 issues that more than one participant proposed (see Section 2).

The second part of the NGT process involved consolidating similar or overlapping research needs into related research topic areas. Participants were given the opportunity to propose consolidating two or more research needs into a research theme, subject to approval by those participants who had nominated the respective research needs in question. Participants indicated that consolidation into research themes would be easier if the research questions were grouped by topic. Participants then divided those topics into research themes according to instruction by the facilitator that the themes should be amenable to being addressed in a single request for proposals or applications. The facilitator also reiterated the need to consolidate individual research needs into research themes to allow the participants to develop breakout group reports to ultimately guide future research prioritization.

The individual research questions were retained for later reference. Altogether, 56 of the 58 unique research needs were categorized into 23 consolidated research needs. Ten of the unique research needs were assigned to two consolidated research needs: 2.5, 2.6, 4.6, 4.10, 5.17, 6.8, 6.10, 6.16, N.9, and N.12.

Description of Nominal Group Technique

This summary was distributed to workshop participants and observers prior to the workshop.

Nominal Group Technique (NGT) is a structured process for a set of individuals to identify and rank a number of choices. Typically, several individuals (nominally a group) are convened and each person is afforded an equal opportunity to offer his or her view(s) about which choices are highest priority. When a large number of choices are under consideration, they may be grouped or consolidated into a more manageable number. A multi-voting process is then used to rank the choices. In the January 2011 Nanomaterial Case Studies Workshop, the participants will form one NGT group of approximately 25 individuals. This brief document provides an overview of NGT as it will be implemented at this workshop.

Round Robin Discussions. Each participant will be asked to state and provide justification for the research question they believe embodies the most important research or information need with respect to nano-Ag. This brief oral presentation (to be conducted without visual aids) must be completed within a **3-minute period** (strictly enforced). Each presentation should include a statement or description of the research question and an explanation of why it is a high priority in relation to a comprehensive environmental assessment of nanoscale silver (nano-Ag). As time permits, additional priorities will be presented in subsequent rounds of presentations. If another participant precedes you and speaks to the issue you intended to present, you may use your time in support of the same issue or you may raise a different issue that you consider to also be a high priority.

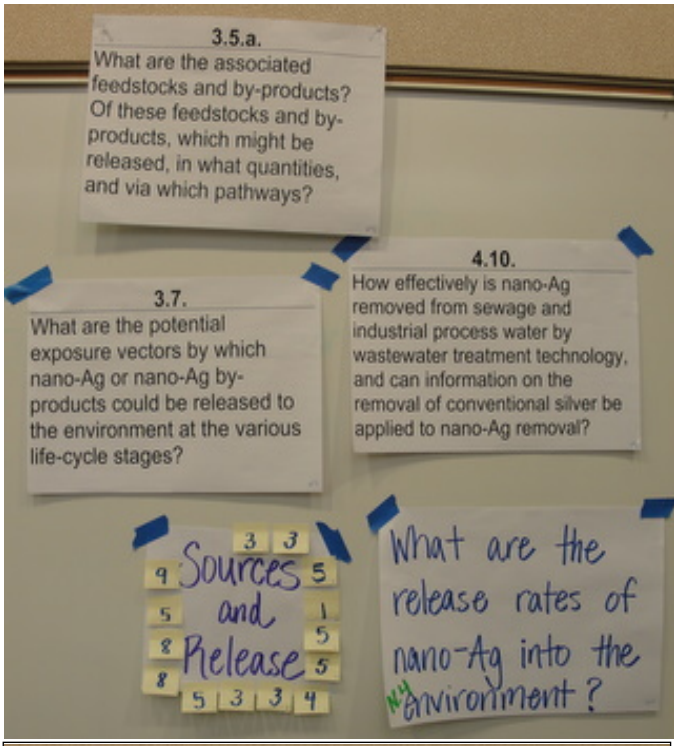
Consolidation and Multi-Voting. Each research question will be noted on a large sheet of paper and displayed for the group. A facilitator will work with the group to determine which questions can be consolidated into major research areas, thereby consolidating the total number of questions to around 20–30 themes. The consolidation process will be followed by multi-voting, during which participants will assign weighted votes to the research questions they deem most important for supporting a comprehensive environmental assessment of nano-Ag. The pre-workshop ranking process used multi-voting to develop a preliminary list of the top 10 questions, and essentially the same process will be used during the workshop.

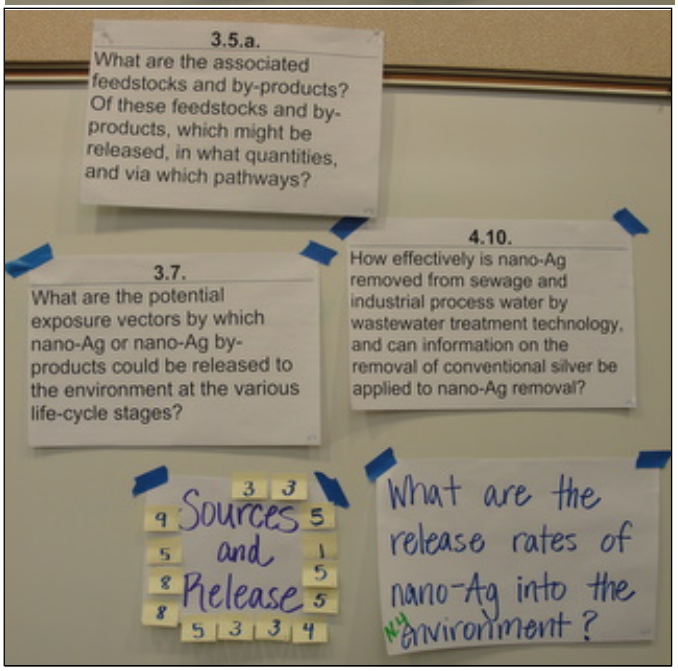
Breakout Group Discussions and Summaries. After the group has prioritized the research questions through multi-voting, the participants will convene to discuss the ranking results. The participants will then be divided into breakout groups (each comprising 3 to 4 individuals), with each group assigned one of the top priorities. The breakout groups will discuss their assigned areas and prepare short written summaries in a standardized format that describe the research question of interest, explain what additional data are needed and why, and present other related information (including, as appropriate, alternate viewpoints). Then, the group will reconvene, review the next set of research questions based on the multi-voting results, and divide into new breakout groups to discuss the next set of priorities and develop another set of written summaries.

Plenary Discussion. Finally, the participants will reconvene in plenary and each of the summaries from the two sets of breakout groups will be presented. A primary objective of this final session will be to identify linkages among most highly ranked research areas.

The result of the workshop will be the set of the research questions selected through the NGT process as most important by the group. These questions and the summary information developed by the breakout groups will be incorporated into a workshop report.

The third part of the NGT involved a multi-voting exercise to develop a ranking of the consolidated research needs in terms of their importance for conducting a CEA. Each participant was given 10 Sticky Notes, labeled 1 to 10 with an identifying letter. The participants were then asked to rank their top 10 research priorities by giving 10 points to the research need they deemed most important for conducting the CEA, 9 points to their next highest priority, and so on, down to 1 point. Only 10 research topics could be ranked by an individual, and each topic could receive only one ranking per individual. After the voting process, the results were tallied and the ranked research priorities were identified. The ranking of research priorities is listed in Section 3.1.





Example of four questions consolidated into a research theme with multi-vote results.

1.4.3. Breakout Group Reports

The agenda was structured to allow for two separate breakout group sessions so that participants joined one breakout group in the morning and the other in the afternoon. At the 2009 workshop, only one breakout session was included for the most highly ranked themes (ranked 1 through 8). By scheduling two separate breakout group sessions for the 2011 workshop, participants could create reports for the most highly ranked themes (ranked 1 through 7) and also for the middle tier themes (ranked 8 through 14). This approach enabled participants equal opportunity to contribute to one highly ranked theme and one middle tier theme, rather than some participants contributing to two highly ranked themes and other participants contributing to two middle tier themes. It also expanded the number of consolidated themes on which participants created reports from 8 in the 2009 workshop to 13 (with one research topic omitted) in the 2011 workshop.

Participants volunteered to work on an issue of their choice (with guidance to limit group sizes to 3 or 4 people), resulting in 13 breakout groups corresponding to the top 14 research topics. Participants omitted one research topic (human and mammalian test methods) for a breakout group presentation, but the 13 remaining breakout groups were instructed to reference this topic in their reports. The groups were given around 3 hours for each breakout group session (including lunch) to develop a short report fleshing out descriptions of the research topic areas using an MS Word document template

(Section 4.6). The facilitator observed the breakout groups, offering guidance when appropriate. The written reports are presented in full in Section 3.2 of this report. On the last day of the workshop, a spokesperson from each breakout group gave a 5-minute presentation to the plenary group, using a provided PowerPoint template (Section 5). These presentations were meant to summarize each breakout group's written report, with particular emphasis on the topic's connections to other priority areas. Time was allowed for the plenary group to respond to these presentations, especially for the purpose of pointing out additional connections or relationships among research topic areas. The presentations and discussion points are also presented in Section 3.2 of this report.

2. Research Questions Proposed During Nominal Group Technique

2.1. Round 1

2.7 Which physicochemical properties of nano-Ag are most essential to characterize before and during toxicity experiments?

Participant A[1]

- From the viewpoint of consumers, I want to represent what the personal health impact will be.
- There are both exposure and health questions; it was a toss-up for me.
- It is important to know what you are looking at and why before you conduct a health assessment.
- When you come to conclusions, you need to understand whether a certain harmful effect is due to a particular characteristic.
- You must understand what physicochemical properties are important vis-à-vis toxicity of nanoparticles.

2.10 Do adequate analytical methods exist to detect and characterize nano-Ag in environmental compartments and in biota?

Participant B

- Once nano-Ag is released to the environment, the biggest challenge we have is to distinguish nano-Ag from incidental and naturally occurring particles.
- The difference between nano-Ag and incidental or naturally occurring particles is not like quantum dots or nanotubes, which are obviously manmade.
- It is difficult to know the source of nano-Ag in environmental compartments.
- For a CEA framework, we need to have a methodology to identify what they are and how much they are in the environmental matrix, so we can address which questions are important.

6.1 To what extent do particle properties (e.g., size, shape, chemical composition, surface treatments) determine biological responses to nano-Ag?

Participant C

- There are several others questions that say the same thing in a different way, so it was difficult to pick this one.
- I agreed with everything Michael Hansen said about physicochemical properties determining effects.
- You must make sure you are measuring the right thing (e.g., single particles, clusters) if you want to find the best analytical method.

6.3 Are the effects observed for exposure to nano-Ag due to silver ion release or the presence of nanoparticles? Can this be distinguished?

Participant D

- There is a multitude of hazardous tests that can be done, but you need to know what the hazard is to begin with (e.g., nano-Ag or ions).
- We know silver ions are toxic, so that is important to address.
- It is important to know whether nano-Ag is acting as a vector for the ions.
- If nano-Ag is a vector, you make it a different threshold as it is providing ions at a point of exposure, rather than diffused.

[1] Participants' names were coded to so that individuals could openly express ideas during the workshop.

- We need to know whether silver nanoparticles act differently in people, due to the oxidation of nano-Ag.

2.10 Do adequate analytical methods exist to detect and characterize nano-Ag in environmental compartments and in biota?

Participant E

- I am coming from the public health and environmental policy perspective.
- New technologies are invented for benefits, but there are also dangers and opportunities.
- It is clear that these technologies (e.g., nano-Ag sprays) are going to be produced, marketed, and sold and will become part of the environment.
- Do we have rapid, routine, and cheap systems to detect nano-Ag in the environment? I do not think we are close.
- If we cannot detect it, we cannot measure it, and we cannot be sure what is happening.

6.3 Are the effects observed for exposure to nano-Ag due to silver ion release or the presence of nanoparticles? Can this be distinguished?

Participant F

- The physicochemical properties, route of exposure, and health effects are the main three themes.
- Is the function of the nano-Ag in this spray as an antimicrobial going to kill the bacteria?
- Nano-Ag kills bacteria over a longer time according to the literature.
- The literature also shows that particles act differently than ions.
- It all comes back to dose and the concentration. Will we be spraying it more often because it is not as effective as ions only?
- There is a fundamental difference in toxicology between a low dose and a high dose. The literature is filled with overdose, not low-dose exposures.
- We must determine if the spray is a low-dose or high-dose scenario.

N.3 What is the half-life of nano-Ag in the environment?

Participant G

- What are the basic mechanisms?
- If you released 20-nm particles, they would rapidly coagulate and ultimately be 0.5 micron. Will it last a week or a year?
- What is the reactivity in various media?
- There is a definitional problem that will be solved in the courts.
- Based on work done in Paul Lioy's lab, it is hard to determine how much nano-Ag in a particle makes it a nano-Ag particle.
- A nano-Ag particle has a very low mass compared to the whole particle.
- What makes it a nanomaterial? How many nanoparticles make a nanomaterial?

N.3 What is the half-life of nano-Ag in the environment?

Participant H

- If it has a short lifetime, it is much easier to understand the fate.
- If it becomes an ion, we can establish fate and transport. If it is a particle, we know what happens.
- If it keeps moving, releasing over time, we do not know how to think about it and it is more difficult to model.
- Persistence is vague. I prefer half-life.

N.4 What are the release rates of all sources of nano-Ag into the environment?

Participant I

- No effects, no exposure; no release, no exposure.
- Many questions ask how something influences nano-Ag. The important thing we need to understand is how readily something influences.

6.1 To what extent do particle properties (e.g., size, shape, chemical composition, surface treatments) determine biological responses to nano-Ag?

Participant J

- It is important to ensure safety of the product to the consumer and environment.
- Particle properties pose a threat to me as a consumer or to the environment.

6.16 Are the current tests for regulatory acceptance relevant to nano-Ag?

Participant K

- The consumer is our priority.
- As we settle into research priorities, I question whether we are allowing products on the market that should not be allowed.
- If human health is a priority, should we make a precautionary gesture toward the public and hold off on further market entry until we know more?

6.5 Is the available ecological effects evidence adequate to support ecological risk assessment for nano-Ag? If no, what research is needed to make an assessment possible?

Participant L

- Do we have the available information, which encompasses the rest of the Chapter 6 questions?

6.27 Are there sufficient data to develop concentration- or dose-response relationships instead of the current emphasis on point estimates or narratives of relative effects?

Participant M

- This would apply to ecological issues as well as human health issues.
- This is related directly to the behavior of the particles, so many other things get folded into it.
- Nanomaterials function as colloidal products producing an odd bathtub-shaped dose-response (partly due to different exposure routes).
- When we start at low concentrations, we get effects, but when we move to high concentrations, the effect goes away.
- Until we do good characterization of what is in the test system, we will fail.
- It is going to be difficult to tell the public how little we know.
- This is the way to improve eco-tox.

3.7 What are the potential exposure vectors by which nano-Ag or nano-Ag by-products could be released to the environment at the various life-cycle stages?

Participant N

- I work with citizens and state government agencies, and we work to understand and prevent health and environmental exposures.
- I want to call attention to the top of the CEA diagram.
- We tend to find out something is bad after it is released and then try to go back and determine the sources, but I would like to see us avoid that with nano-Ag.
- As we know, nano-Ag is already in products and silver is the second most toxic to aquatic organisms.

- We need to understand the potential pathways, where the releases will be, what types of releases, what organisms and humans will be exposed, etc.
- From the standpoint of risk management, if we do not understand where the releases are, we cannot shut off the valve.

5.21 What information exists on the temporal changes in the release of ionic silver by nano-Ag in relation to particle physicochemical and environmental characteristics?

Participant O

- We know these particles release ionic silver, but in my opinion, we do not understand how different environmental chemistry conditions (e.g., pH, ionic strength) affect the particles, especially over time.

4.1 Do the properties of nano-Ag that differ from those of well-characterized colloidal and bulk silver, if any, cause them to behave differently in aquatic, terrestrial, and atmospheric environmental compartments?
a. If they do differ, how do they differ?
b. Can information about how colloidal silver behaves in these environments be used to understand how nano-Ag behaves?

Participant P

- Is there any real difference between nano-Ag and bulk silver in the environment over time?
- Soils are mostly rich in calcium, so you have a strong flocculent there.
- Electrostabilized nanoparticles are not thermodynamically stable.
- Those sterically stable, coated by organics are typically consumable by microorganisms.
- There is not a good chance of persistence after digestion.
- I do not think there is a lot of chance for nano-Ag to stay dispersed (high van der Waals force).
- Because we are moving through a solid, we are talking about filtration. Clay particles do not have enough separation to move these small particles.

2.1 What information could be provided about the nano-Ag contained in spray disinfectants to enable adequate characterization of exposure routes and toxic effects?

Participant Q

- This probably encompasses everything we have discussed so far.
- In studies we have conducted looking at toxicity in vivo in rats, we found that particle size when injected (i.v. or by gavage), in relation to silver acetate, the area under the curve decreased as particle size increased.
- We saw an inverse relationship and sex differences.
- We need to know what sized particles are actually contained in the disinfectant sprays.
- Do manufacturers have sufficient data to provide to EPA?
- Are these particles are going to agglomerate like bulk particles? If they agglomerate, they are no longer nano-sized.
- Will the agglomerates fall out of the spray onto surfaces?
- What surface treatments are done to the nano-Ag included in the sprays?
- How are they held in dispersion?

2.6 What physicochemical properties of nano-Ag can be used to:
a. predict fate and transport in environmental media?
b. predict toxicity to humans or biota?

Participant R

- Want to combine with 2.5; what physicochemical properties can be used to predict fate and transport and effects and what are the surface effects?
- The manufacturers are not concerned because they put the nanomaterial in a matrix or composite, and it will never break down from their perspective.
- Will the use cause it to break down?
- Physicochemical properties and surface effects are important to this question.

6.14 What are the biological responses observed at current nano-Ag occupational exposure levels?

Participant S

- All of the questions so far require somebody to test the material in a lab, and we can be somewhat reckless win the lab with how we handle them.
- I do not think the material is a huge concern for consumers at this point.
- It is a concern to those who are manufacturing and handling the material in the lab.
- We should measure nano-Ag levels in facilities or total silver levels in production facilities and focus on acute and chronic exposure at those levels.

N.7 What are the phys-chem properties of currently available and historic silver products?

Participant T

- The evolution of terminology has been obscured.
- The case study mentions the historical uses of silver but does not integrate them into the discussion.
- Cary Lee particles (chosen by OECD as reference materials for nano-Ag studies underway which tells us something about the value of historical data) were used in the 1800s.
- In the 1950s, the first product registered by FIFRA was a silver product.
- Registration under FIFRA is important because there are production data and an incidence database.
- If you go to the conventional silver data, there are nano-Ag data there that should be leveraged for risk assessment.
- For the avian toxicity studies referenced in the case study, it is 7-nm algaecide particles.
- These data have been applied to nano-Ag incorrectly.

N.3 What is the half-life of nano-Ag in the environment?

Participant U

- Kinetics, or what happens when nano-Ag when it is released into the environment, matter most when determining properties.
- This ties in to exposure and ultimately toxicity.
- If you release the 7-nm particles into the atmosphere, and data show 5-nm particles are toxic, there will eventually be solubilization that will change the 7-nm particles to 5-nm particles. At what point this happens is important.
- This is also dependent on how quickly the surface coating dissolves.
- Temperature is crucial in kinetics; there is an exponential dependence.
- This is also important for wastewater.

5.18 What is the distribution of exposure intensities and frequencies of such exposures among workers, homemakers, children, and maintenance personnel, and are these of concern for acute and or chronic health effects?

Participant V

- This is supported by 5.17 and is a legally logical question.
- There is no need to have a question as to whether people are exposed (there are releases into the environment), but what are the intensities of exposure and do these exposures lead to harmful effects?
- You cannot do a meaningful risk assessment without this information.
- Determining the chemical, physical, and particle-size characteristics at the time of exposure is critical.
- There is an ecological question and I can frame it in the same way: What exposures occur to fish and other benthic and nonbenthic organisms?
- Without exposure, there is no dose, with no dose, there are no effects.
- We need to know quantitatively whether particles should be taken off the market and whether new products should be on the market.
- Understanding exposure is the bottom line and all of the rest can be derived from that.
- This question should be expanded to include occupational setting, rather than just the consumer, although the most highly exposed might not be the most sensitive.
- Companies choose the material they are spraying, but the user has no choice. The various contexts for exposure are relevant.

5.17 How should dose and exposure be characterized for human exposures and how do the following parameters affect it: (1) physiological characteristics, (2) behavior, (3) lifestage, (4) susceptibility factors?

Participant W

- We need to determine who is exposed, at what levels, and in what contexts to assess exposure.
- If you look at 6.14, the response depends on what exposure metric is being used. Depending on the metric, you may or may not observe a dose-response relationship.
- Is it mass, surface area, surface chemistry, or number of particles?
- With population and stage of their lifecycle, it is difficult to determine which subgroup of the population is most highly exposed.
- Occupational populations might be most highly exposed, but once you look at consumers, it is not easy to determine which parts of the population will be most sensitive or susceptible.
- Which populations are we talking about and how do we measure exposures?

2.2. Round 2

5.14 Many effects of emerging substances are not known until many years after their introduction and use in commerce. What are the chronic and subchronic effects of nano-Ag, and how can we accelerate our understanding of them? Can nano-Ag have an impact on F-1 generation via changes in gene expression patterns?

Participant A

- For regulatory purposes, we have a set group of toxicity studies that should be done for various acute, subchronic, and chronic exposures, but for all of the products on the market, we do not think about if it would affect the next generation behaviorally or in another way.
- We now have the technology to start screening and looking at gene expression arrays to determine effects on the next generation.
- Are there things that current toxicity tests might not pick up that would be important?

- If there is not enough exposure the effect will not be there, but we need to look at it.

3.6 What changes occur to the physicochemical properties of nano-Ag throughout the material lifecycle stages, either as a function of process and product engineering or as a function of incidental encounters with other substances and the environment?

Participant B

- Transformation after release to the environment is not available because we still need data on the parent compound.
- This is in line with the half-life of nano-Ag particles, but is it losing antibacterial properties or nano characteristics?

2.12 Do adequate analytical methods exist to detect and characterize exposure to nano-Ag via soil, water, and air?

Participant C

- Methods is the key word, because it implies more than instrumentation.
- We have the instrumentation in existence (e.g., SEM, TEM, STEM, to 1–2 million times).
- The problem is determining which method to use with the instrumentation.
- If I have a product with nano-Ag at some small percentage, we can know a little about the nanoparticles, but in a gallon of river water, it becomes problematic.
- I do not know how to approach characterization in water, soil, and air samples.

6.10 At a minimum, what assays could be considered in a harmonized test guideline for determination of the human health effects of nano-Ag?

Participant D

- As a company that is producing a new nanomaterial, it is up to us to make sure there is no hazard to exposure.
- When it comes to human tests, it would be nice to have some guidelines from EPA and Environment Canada.
- You have to take into account what needs to be done and what has to be done.
- We need to know which procedures should be used to test what things from regulators.
- What needs to be done is more important than what can be done.

4.10 How effectively is nano-Ag removed from sewage and industrial process water by wastewater treatment technology, and can information on the removal of conventional silver be applied to nano-Ag removal? Is the nano-Ag harmful to the beneficial organisms in wastewater treatment?

Participant E

- This is part of characterizing the ecological impacts. Yes, it is out there but how can we detect it?
- The potential of nano-Ag particles is to do the beneficial antimicrobial activities but also they could knock out the beneficial things.
- What is the bacterial resistance?

4.1 Do the properties of nano-Ag that differ from those of well-characterized colloidal silver, if any, cause them to behave differently in aquatic, terrestrial, and atmospheric environmental compartments?
a. If they do differ, how do they differ?
b. Can information about how colloidal silver behaves in these environments be used to understand how nano-Ag behaves?

Participant F

- "Is nano-Ag different from colloidal silver?" is a research question that needs to be addressed.
- The literature has a debate as to whether or not people are seeing that nano-Ag particles are more efficient than any other antimicrobials we have out there (including metal salts).
- Can we characterize efficacy for killing bacteria and understand that a little bit more?
- If it does, what about the beneficial bacteria in our gut. If it is indeed killing bacteria, either by slow release or by ionic silver, are we killing the bacteria in our gut? What is the bacterial resistance?

2.9 Are there standard nano-Ag reference materials that can be used in exposure and effects testing to aid in comparison of results among investigators?

Participant G

- The methods that we have talked about need validation.
- We need round-robin testing and references for that.
- It depends on the media the particles are dispersed in (e.g., surfactant, powder, aerosol).
- Mary Boudreau mentioned OECD has reference materials and two different coatings in liquid form.

4.12 How could existing models applicable to conventional silver be used to adequately predict the transport and fate of nano-Ag through environmental compartments, or how could they be modified to do so?

Participant H

- I do not see a great concern for humans, but rather a focus on the environment.
- We are going to need modeling, because these questions will take years to answer.
- We think a lot can be done with models we have used for REACH and we can modify them to get in the ballpark.
- We can use a tiered approach using the worst case to hone in on where we need to go and what tests we will need to get the answer.
- For nano-Ag, I am going to do a different risk approach for the spray than I am for an industrial use.
- You can follow up with what is really going on after you get the guidance from the models.

N.11 What are the rates of dissolution of nano-Ag into the environment?

Participant I

- I am talking about the dissolution of nano-Ag to silver ions.
- From an environmental risk assessment and ecosystem exposure, the answer is going to be it is the ions that do the job.
- I need to ask for release rates of silver ions from materials, specifically, the rate of dissolution of nano-Ag.
- If dissolution occurs on a short timescale, we might not be interested in how rapidly it occurs from an ecological perspective.
- We can assume it is being transformed into ionic silver all at once.

- If dissolution is slow, we would then need to know at what rate this dissolution occurs.
- From the perspective of the nanoparticle, this may not be different from half life.
- From the perspective of toxicity of silver ions in water, it would mean that you ask for a rate of entry into the environment.

5.1 Are available methods adequate to characterize nano-Ag concentrations and associated exposure via relevant matrices such as:

a. air?

b. water?

c. food?

Participant J

- Safety implementation to industry, consumer, and regulation are important.
- This is a daunting task.
- How do you isolate and characterize the nanoparticles?

N.1 Does the release of nano-Ag contribute to climate change?

Participant K

- This is an interdisciplinary question.
- Nano-Ag when exposed to sludge releases 4x the amount of nitrous oxide, which is a potent GHG.

N. How can we incentivize researchers to focus in on the most critical questions and best methods for CEA?

Participant L

- There are a lot of data out there on nano-Ag, but applicability of existing data to risk assessors is a problem.
- Anything we can do to improve research quality needs to be done, such as reference materials.
- If we design a good research experiment, we need pristine materials varied in a very precise way, but this might have little relevance to the state of nano-Ag in life-cycle stages associated with actual exposures.

6.26 Is there evidence of adaptive tolerance developing in microorganisms to Ag and to nano-Ag that would render the products useless, especially as the products gain widespread use?

Participant M

- When we get to the point of regulation, there will be an efficacy component to the final decision.
- If we do not anticipate the possibility of resistant strains developing and becoming infective, we will overestimate the benefits the product might give us.
- This has a ripple effect, especially through ecological consequences.
- We do not what happens when we stir the pot in a complex ecological system. It could trigger an unknown response.
- We need to know whether resistance is a concern.

5.18 What is the distribution of exposure intensities and frequencies of such exposures among homemakers, children, and maintenance personnel, and are these of concern for acute and or chronic health effects?

Participant N

- These products are already on the market and people are using them and being exposed.
- At the State level, consumers and public health workers are concerned about whether these products are safe.
- Ethically, the most important thing is to understand the exposures right now as soon as possible.
- Methods need to be worked out, but we have most of the instrumentation we need.
- People are exposed before it is released into the environment, so we need to focus our attention there.
- In the past, we have waited until people become sick or die before we do anything, but we need to reverse that.

2.10 Do adequate analytical methods exist to detect and characterize nano-Ag in environmental compartments and in biota?

Participant O

- A lot of us have addressed methodological issues, especially related to exposure, in the second round.
- It is easy to measure these particles in simple matrices, but if you have a whole organism, it is difficult to determine how much exposure has taken place.
- We can measure total silver, but we do not know how relevant that is to nano-Ag.

4.6 Does nano-Ag form the same strong complexes with anions as conventional <u>and dissolved</u> silver, and if so, is it also effectively immobilized in aquatic environments?

Participant P

- Environmental fate is the low-hanging fruit in terms of nano-Ag research.
- We know nano-Ag is not inherently soluble and the surface must be oxidized.
- What are the release kinetics of nano-Ag in the environment?
- Do humics increase or decrease dissolution?
- Iron oxides are where all the heavy metals are. Do we expect iron oxides to accumulate all of the silver in the soil?
- What is the differential activity of iron oxides or free moving silver?
- There is the question of species and distribution with respect to the complexes silver can form in the environment.
- Do these complexes have the same toxicity and antimicrobial activity? Typically, no.

4.2 Does the particle size <u>or source or agglomeration state</u> of nano-Ag affect the rate of release of silver ions in environmental compartments?

Participant Q

- Silver was characteristically used as an antibiotic in history.
- Does the particle size affect the release of ions into the environment?
- We need to talk about what the toxic substance is.

2.5 How does surface coating affect:
a. the physicochemical properties of nano-Ag?
b. toxicity to humans or biota?

Participant R

- There are a lot of surface coatings that are biocompatible.
- Are these coatings adequate enough to neutralize adverse effect?

N.6 How urgent is the need for the benefits offered by the candidate application/material?

Participant S

- If you look at the use of silver in wound creams, it addresses a need that is not addressed by other products.
- Do we have alternatives to using nano-Ag?
- This may be a philosophical question. We do not want to intrude on the free market or inhibit entrepreneurs.
- We should compare efficacy of nano-enhanced products to other products.

N.3 What is the half-life of nano-Ag in the environment?

Participant T

- There have been new findings since the case study (e.g., publications by Bojeong Kim and Bernd Nowack).
- If half-lives are sufficiently short, we have a massively simplified risk assessment in terms of environmental fate.
- If so, consumer products might be indistinguishable from other naturally occurring or known (e.g., mining, photography) silver releases.
- Understanding speciation that follows half-life is important.
- How does the particle size of silver sulfides change?
- How likely is it that we will see silver sulfides? Publications by Choi and Hu discuss this.
- Entrained in municipal waste stream, there are four orders of magnitude more silver sulfide; excess silver sulfide is very stable.
- Historically, these silver sulfides are the reason that silver algaecides have been deemed less of an environmental risk.

5.1 Are available methods adequate to characterize nano-Ag concentrations and associated exposure via relevant matrices such as:
a. air?
b. water?
c. food?

Participant U

- It might be easy to determine the dose, because there is so much material in the environment.
- Nanomaterial, in general, might be considered at the pictogram level and smaller and what type of health effects that small amount might cause.
- For some techniques, I am uncertain about efficacy toward unraveling surface coating that might be carbonaceous.
- There have been issues with microscopy and ICP agreement.

N.7 What are the phys-chem properties of currently available and historic silver products? <u>What are the properties of those chemicals in terms of exposure</u>?

Participant V

- We are dealing with near-field, intermediate, or far-field inhalation or ingestions.
- We will have to deal with agglomerates.
- The toxicity tests that will be necessary to determine whether the efficacy in terms of product remain the same.
- Does the transformed product have the same potential for human health effects?

5.16 What effect, if any, do surface treatments of nano-Ag particles have on human exposures and uptake?

Participant W

- This is a rewording of 5.3.
- It affects half-life, exposure, and if you unbundle those properties, the most important part is surface properties.
- The surface effects are the most important in my opinion.
- We need to know how it affects human exposure.

2.3. Round 3

N.9 Do nano-Ag products actually offer more efficacy than products on the market?

Participant A

- EPA does not require efficacy data.
- What is the real antimicrobial effect of the silver that is in the sprays or in some of the other products?
- Does it really have an effect? You do not need efficacy information to get it on the market.
- There should be a requirement of efficacy of these products and there should be a benefit analysis compared to something already out there.

6.8 What are the most sensitive ecological endpoints to nano-Ag exposure? Are there sufficient data/analytical techniques to determine how sensitive specific endpoints and organisms are to nano-Ag exposure, including:
a. benthic invertebrates;
b. marine invertebrates; and
c. freshwater invertebrates?

Participant B

- This is related to 6.22.
- From a regulatory standpoint, it is important to know what the human and environmental risks are for these materials.
- Developing a protocol to measure ecological toxicity is very important.

5.3 What effect, if any, do surface compositions of nano-Ag particles have on:
a. uptake?
b. biopersistence?
c. bioaccumulation?
d. biomagnification?

Participant C

- The treatment that the manufacturers put on nano-Ag might be completely gone.
- The surface is where the interaction with tissue, bacteria, biota, and people occurs, not the bulk of the material.
- I have never seen a material where the surface is the same as the bulk material, even in small particles.

- It is important to understand the surface composition of the particles as they are manufactured as well as once they are in the environment.

6.15 Do current publications describing the health effects of nano-Ag particles and laboratory-generated nano-Ag particles accurately depict the toxicity of commercially available nano-Ag materials?

Participant D

- In doing a CEA, it is critical to look at the literature, but sometimes bad apples get in there.
- We need to determine how the materials in the lab compare to the hundreds of products on the market.
- What you start with sometimes dictates what you end with, so we need to test the actual products.

6.33 Can we predict whether widespread resistance to silver ions may develop and if so, are silver and/or nanosilver likely to be useful antimicrobials in the future?

Participant E

- How we used silver through the 40s and 50s is different from how we use it now.
- The current approach is some is good, more is better.
- Historical medicinal use and FIFRA-approved products have been limited and will be different from how we use them now.
- Information in the literature or from FDA might demonstrate that there is no antibiotic resistance.
- This is an important issue as nano-Ag products penetrate markets.

2.16 Do nanoparticles react with materials (i.e. organic matter, other metals, polymers) and alter properties such as REDOX potential or leached metal ion rates?

Participant F

- We recently published a series of papers on redox chemistries of nano mixtures.
- We need to think about it being reduced, as well as the reducing agent, and if it changes.
- We need to determine whether the reducing agent goes from a passive to an active material.
- Redox properties need to be added to the minimal characterization data reported for nanomaterials.

5.22 Which sources, pathways, and routes offer the greatest exposure potential to nano-Ag for humans?

Participant G

- We are primarily talking about consumer chemicals.
- If a carpet is treated with nano-Ag, dust might be resuspended with nano-Ag, which we might inhale.
- We have to look for unexplained and unintended consequences.
- Could there be synergy between previous chemical applications and nano-Ag?

4.10 How effectively is nano-Ag removed from sewage and industrial process water by wastewater treatment technology, and can information on the removal of conventional silver be applied to nano-Ag removal?

Participant H

- Ions are bad, particularly to fish.
- Where does nano-Ag go? If it does not go the aqueous route, but settles out, it will not affect the fish.

- We have a lot of history from colloidal silver used in the photographic industry.
- There is probably a lot of removal going on, based on this historic data.
- We need to look into this from a nano-Ag perspective.
- Effective removal reduces releases to aqueous environments.
- If we have effective removal through wastewater treatment, the nano-Ag should settle out, which would cut out a route of exposure.

N.3 What is the half-life of nano-Ag in the environment?

Participant I

- This combines all of the other questions.
- We can work out sedimentation that removes nano-Ag from water.
- This more effectively gets to the knowledge we need to know rather than going through all of the research.

N.12 What are the relevant susceptibility factors in terms of exposure?

Participant J

- Each person will have a different degree of susceptibility.
- We need to address human nutrition. New genomic study on the future of foods is needed.
- We need a map of the susceptibility in order to map it to populations of interest.

2.8 What standardized test methods or characterization protocols are necessary to ensure that research results generated in multiple laboratories are consistent, reproducible, and reliable?

Participant K

- With 6.15.
- Studies I might use would differ from others. It would be interesting to work together to identify those priorities in terms of studies that would be used in reports.
- When we work at this scale, we are tapping into a new wavelength.
- We need to think beyond our own experience and take a big-picture perspective.
- From a quantum perspective, we do not understand the effects on the future.

5.22 Which sources, pathways, and routes offer the greatest exposure potential to nano-Ag for humans?

Participant L

- We are overweighted on hazard studies and underweighted on exposure studies.
- This leads to a skewed perception of risk without understanding of exposure.
- Hazard papers outweigh exposure papers by about 5:1.
- Mining the publications is not a substitute for a CEA.
- We need more information about sources and potential exposures to enable a CEA.

N.5 We need an integrated holistic approach to nano risk assessment. How can we do this?

Participant M

- The CEA process can be more than just workshops.
- I want to call for the use of integrative holistic assessments.
- In 1946, the WHO talked about health as being more than the absence of disease and toxic materials.
- Nanomaterials are going to expand into ethics and bioethics.
- I see the framework as a complex matrix with a lot of empty boxes that need to be filled so that somebody later can do a proper risk assessment.

- We need to make a dedicated effort to develop conceptual models and focus on exposure in order to improve efficiency.
- Exposure is currently the most ignored aspect. An integrated holistic approach would break down the artificial barrier between human and ecological effects.
- We need one coherent message that can be communicated to the general public.

5.5 Do particular species of biota and particular human populations have greater potential for exposure to nano-Ag?

Participant N

- What humans will be exposed to is closely tied to what ends up in the environment and accumulated in animals.
- There will be particular populations that are more likely to be exposed.
- This will help us to prioritize where we will go first for exposure assessments.
- The most exposed (e.g., janitors, maintenance workers) are often the least thought of. This introduces social science questions.
- There needs to be a greater focus on exposure.

6.13 What are the fundamental biological responses to and associated mechanisms of nano-Ag exposure at the cell, organ, and whole-animal levels?

Participant O

- We have not talked about mechanisms of toxicity at the cellular level.
- We need to relate this to the physicochemical properties as well.

2.7 Which physicochemical properties of nano-Ag are most essential to characterize before and during toxicity experiments and post-exposure?

Participant P

- Most of the papers do not show post-exposure characterization data.
- The ones that do are highly aggregated, indicating that exposures are not associated with nanomaterials at all.
- If we are talking about chronic exposures, this is very important.

5.5 Do particular species of biota and particular human populations have greater potential for exposure to nano-Ag?

Participant Q

- We need to know which human or environmental groups are exposed.
- Antibacterial sprays might be used more around children (e.g., daycare centers spraying toys and food trays).
- Even if the particles settle out in the water, we need to think about ground feeders.

3.5A What are the associated feedstocks and by-products; of these feedstocks and by-products, which might be released, in what quantities, and via which pathways?

Participant R

- We need to know the starting material we are working with.
- Several toxicity case studies show that people measured a contaminant instead of the test product.
- There are earlier studies where people used investigative nanomaterials, but they were actually investigating the byproducts.
- Some of the results were released to media.

3.3 What are realistic strategies for collecting data on production quantities and product characteristics given that much of this information is proprietary, and how can this information help prioritize research needs?

Participant S

- Interesting assumptions are made in the White Paper about global production.
- This is extremely difficult to get, but we can do a bit more to get more information on these materials.
- It is good to make estimates in the absence of hard data, but we do need more data.
- What is possible to conceive as being a nanoparticle effect in the environment cannot necessarily be realized given current manufacturing techniques?
- We need to distinguish between what is possible and what can actually be realized.
- Different surface chemistries or article characterizations might cause health effects, but manufacturers can only test so many.
- It is important to link health effects to what is actually possible by manufacturers.

0.4 Have the database and risk assessment methodology used by FDA during approval of nano-Ag medical devices been integrated with EPA's database and risk assessment processes?

Participant T

- We need to make sure we know what we know before we set research priorities.
- There are areas of deep knowledge of silver in medical devices and at EPA.
- We might accept a greater risk in a clinical setting than in a consumer population.
- Nano-Ag is standard care for infected wounds and we might be able to get more information about dermal risk from this.
- FDA's view on antibiotic resistance is that it is of great concern.
- Based on the frequency with which they are approving new nano-Ag devices, we can assume FDA thinks it is a manageable risk in the medical devices realm.
- FDA might be able to say something about growing risk of staph infections and application on the consumer market.
- What happens in the consumer world can affect what happens in the medical world.
- There may be a potential to integrate materials for public health applications, such as treatment of food supplies (e.g., eggs).
- EPA disinfectant testing on commercially available disinfectants found that a number of them are inadequate and do not meet their marketing claims.
- We cannot assume that products currently on the market live up to their claims.

N.8 What kinds of exposure do these populations have, including physicochemical characteristics?

Participant U

- What kinds of exposures do these populations have?
- The exposure leads to dose.
- The type of exposure will guide the toxicology studies to focus on the right materials and the right properties.

N.13 How can the CEA framework be improved to ensure passive or active consumer/occupational exposure research is completed for nano-Ag and for other nanomaterials?

Participant V

- This encompasses what we have all been saying.

6.14 What are the short-term and long-term biological responses observed at current nano-Ag occupational exposure levels as well as consumer exposure levels?

Participant W

- We are not just looking at occupational exposure and its health effects, but rather all exposures.
- Biological effects capture more subtle effects as well.

2.4. Additional Rounds

The round robin was repeated for additional rounds until all research priorities that participants valued were posted on the wall.

5.5 Do particular species of biota and particular human populations have greater potential for exposure to nano-Ag throughout the entire lifecycle?

Participant A

- Recycling and disposal workers might be exposed.

6.33 Can we predict whether widespread resistance to silver ions may develop and if so, are silver and/or nanosilver likely to be useful antimicrobials in the future?

Participant E

- There are advanced methods that should be easily used, readably available, portable, and cheap.
- For the carpet example mentioned earlier, we could have a sniffer that monitors.
- Equipment, detection, and monitoring all get back to exposure.
- How we measure this outside of the laboratory is really going to be important.
- Exposure is the wasteland of risk assessment partly because it is so expensive.
- One of the issues I have heard is exposure to academic lab workers.
- There is an educational component to the nano issue, and the safety of the researchers is important.

6.6 At a minimum, what assays could be considered in a harmonized test guideline for determination of the ecological effects of nano-Ag?

Participant D

- This is the same as 6.10, except it is ecological instead of human.

N.14 How do we effectively communicate risk/benefit information for nano-Ag to the general public?

Participant J

- The knowledge gap between this group and the general public who will use this knowledge is huge.
- If the public has the perception that ingestion of nano-Ag only leads to discoloration of the skin, we are not doing our job.

N.5 We need an integrated holistic approach to nano risk assessment. How can we do this?

Participant K

- We have plenty of knowledge to pull from (e.g., Chinese traditional medicine).
- Experimenting with a holistic approach could be useful.
- Western science is a minority in this world, so we could learn from other perspectives.

2.21 For the purpose of assessing potential risk, what metrics are most informative for quantifying exposure and dose of nano-Ag?

Participant L

- There might be a misunderstanding that CEA does not encompass exposure.
- CEA takes a broad perspective on exposure.
- Everyone's exposure needs to be considered and controlled.

N.15 How do we engage citizens and workers in discussions about how nano-Ag sprays are being used?

Participant N

- There is a lack of social science here.
- How can we engage citizens and consumers in discussions and decisions about why they are buying the products and how they are actually using them, which is important to exposure?
- Use is often not as intended.
- Consumers are a valuable source of information.
- The only way to figure that out is to communicate with these people who actually use them.

N.10 How do we educate people about the risks, benefits, and safety related to nano products?

Participant F

- Communication, engagement, and education are critical.
- When I talk to students in engineering about toxicology, I get resistance.
- We need to integrate education and exposure students to safety at the college level.

Comment

Participant M

- The term exposure should be clarified, unless exposure is bringing a dose or concentration that is biologically relevant.
- We often tend to speak in shorthand, and we get into trouble with the public.
- If there is exposure, that is problematic. Unless that exposure is giving us a dose or exposure that is biologically relevant, we should avoid scaring people. People already think that because there is exposure, there is a risk.

Comment

Participant E

- Safety in academic laboratories is a big deal.
- If a document from EPA to send a message to the research community existed, it would be helpful.
- If we cannot detect it, then we do not know what we are doing.

3.9 Do explosion risks exist for dried nano-Ag powders or nano-Ag powders modified with certain types of surface coatings?

Participant S

- I am concerned about the explosive potential of nano-Ag.
- It is a potential acute risk.

Comment

Participant V

- The way we treat products is pedestrian. People use products and they do not know anything about them.
- We need to get back to the basics and educate our audience. The audience is the consumer and many are ignorant to the dangers posed.

3. Workshop Outcomes

3.1. Prioritized Research Questions

The questions presented in this section are the prioritized research themes resulting from the multi-voting at the end of the NGT session. These questions were presented in the round robin sessions and subsumed during the consolidation process. The research/information needs for conducting a CEA of nano-Ag in disinfectant spray are posed as questions at the end of Chapters 2–6 in the draft case study (U.S. EPA, 2010b). New questions posed by participants before the workshop were numbered either sequentially in each chapter following the last original question or, for new questions corresponding to multiple issues, sequentially beginning with 0.1. New questions posed by participants at the workshop were numbered sequentially beginning with N.1, based on the position of the flip-chart paper on the wall, not based on the order in which they were posed. Modifications to existing questions were designated by underlining edited text. Participants divided into breakout groups prepared 13 reports (presented in Section 3.2) on the research themes in the list below.

1 – Analytical Methods (120 points, 19 votes)

2.12. Do adequate analytical methods exist to detect and characterize exposure to nano-Ag via soil, water, and air?

2.10. Do adequate analytical methods exist to detect and characterize nano-Ag in environmental compartments and in biota?

2.9. Are there standard nano-Ag reference materials that can be used in exposure and effects testing to aid in comparison of results among investigators?

5.1. Are available methods adequate to characterize nano-Ag concentrations and associated exposure via relevant matrices such as:
a. air?
b. water?
c. food?

6.10. At a minimum, what assays could be considered in a harmonized test guideline for determination of the human health effects of nano-Ag?

2 – Exposure and Susceptibility (120 points, 17 votes)

5.17. How do the following parameters affect (1) physiological characteristics, (2) behavior, (3) lifestages, and (4) susceptibility factors?

N.12. What are the relevant susceptibility factors in terms of exposure?

N.8. What kinds of exposure do these populations have, including physicochemical characteristics?

5.5. Do particular species of biota and particular human populations have greater potential for exposure to nano-Ag through the life cycle?

5.22. Which source, pathways, and routes offer the greatest exposure potential to nano-Ag for humans?

5.18. What is the distribution of exposure intensities and frequencies of such exposures among homemakers, children, and maintenance personnel, and are these of concern for acute and or chronic health effects?

3 – Physical and Chemical Toxicity (115 points, 16 votes)

2.6.b. What physicochemical properties of nano-Ag can be used to: predict toxicity to humans or biota?

2.5.b. How does surface coating affect toxicity to humans or biota?

6.1. To what extent do particle properties (e.g., size, shape, chemical composition, surface treatments) determine biological responses to nano-Ag?

2.7. Which physicochemical properties of nano-Ag are most essential to characterize before, during, and after toxicity experiments?

4 – Kinetics and Dissolution (98 points, 15 votes)

N.3. What is the half life of nano-Ag in the environment?

5 – Surface Characteristics (81 points, 14 votes)

2.5.a. How does surface coating affect: the physicochemical properties of nano-Ag?

3.9. Do explosion risks exist for dried nano-Ag powders or nano-Ag powders modified with certain types of surface coatings?

5.3. What effect, if any, do surface treatments of nano-Ag particles have on:
a. uptake?
b. biopersistence?
c. bioaccumulation?
d. biomagnification?

5.16. What effect, if any, do surface treatments of nano-Ag particles have on human exposures and uptake?

6 – Sources and Release (76 points, 15 votes)

4.10. How effectively is nano-Ag removed from sewage and industrial process water by wastewater treatment technology, and can information on the removal of conventional silver be applied to nano-Ag removal?

3.7. What are the potential exposure vectors by which nano-Ag or nano-Ag by-products could be released to the environment at the various life-cycle stages?

3.5.a. What are the associated feedstocks and by-products; of these feedstocks and by-products, which might be released, in what quantities, and via which pathways?

N.4. What are the release rates of all sources of nano-Ag into the environment?

7 – Mechanisms of Nanoscale Silver Toxicity (72 points, 11 votes)

6.13. What are the fundamental biological responses to and associated mechanisms of nano-Ag exposure at the cell, organ, and whole-animal levels?

6.3. Are the effects observed for exposure to nano-Ag due to silver ion release or the presence of nanoparticles? Can this be distinguished?

8 – Test Methods – Mammals/Humans (67 points, 11 votes)

6.10. At a minimum, what assays could be considered in a harmonized test guideline for determination of the human health effects of nano-Ag?

2.8. What standardized test methods or characterization protocols are necessary to ensure that research results generated in multiple laboratories are consistent, reproducible, and reliable?

6.16. Are the current tests for regulatory acceptance relevant to nano-Ag?

Can nano-Ag have impacts on the F-1 (next) generation via changes in gene expression patterns?

9 – Ecotoxicity Test Methods (59 points, 10 votes)

6.6. At a minimum, what assays could be considered in a harmonized test guideline for determination of the ecological effects of nano-Ag?

2.8. What standardized test methods or characterization protocols are necessary to ensure that research results generated in multiple laboratories are consistent, reproducible, and reliable?

6.16. Are the current tests for regulatory acceptance relevant to nano-Ag?

Can nano-Ag have impacts on the F-1 (next) generation via changes in gene expression patterns?

10 – Is New Nano Unique? (59 points, 10 votes)

4.6. Does nano-Ag form the same strong complexes with anions as conventional silver, and if so, is it also effectively mobilized in aquatic environments?

N.7. What are the phys-chem properties of currently available and historic silver products?

N.9. Do nano-Ag products actually offer more efficacy than products currently on the market?

4.1. Do the properties of nano-Ag that differ from those of well-characterized colloidal silver, if any, cause them to behave differently in aquatic, terrestrial, and atmospheric environmental compartments?
a. If they do differ, how do they differ?
b. Can information about how colloidal silver behaves in these environments be used to understand how nano-Ag behaves?

11 – Biological Effects (56 points, 10 votes)

6.8. What are the most sensitive ecological endpoints to nano-Ag exposure?

N.12. What are relevant susceptibility factors (for biological response)?

6.14. What are the short-term and long-term biological responses observed at current nano-Ag occupational exposure levels as well as consumer exposure levels?

5.14. Many effects of emerging substances are not known until many years after their introduction and use in commerce. What are the chronic and subchronic effects of nano-Ag, and how can we accelerate our understanding of them? Can nano-Ag have impact on F-1 (next) generation via changes in gene expression patterns?

12– Ecological Effects Required for Risk Assessment (43 points, 9 votes)

6.8. What are the most sensitive ecological endpoints to nano-Ag exposure? Are there sufficient data/analytical techniques to determine how sensitive specific endpoints and organisms are to nano-Ag exposure, including:
a. Benthic invertebrates;
b. Marine invertebrates; and
c. Freshwater invertebrates?

6.5. Is the available ecological effects evidence adequate to support ecological risk assessment for nano-Ag? If no, what research is needed to make an assessment possible?

12 – Communication, Engagement, and Education (43 points, 9 votes)

N.14. How do we effectively communicate risk/benefit information for nano-Ag to the general public?

N.15. How do we engage citizens and workers in discussions about how nano-Ag sprays are being used?

N.10. How do we educate people about the risks, benefits, and safety related to nano products?

N.5. We need an integrated holistic approach to nano risk assessment. How can we do this?

14 – Fate and Transport of Nano-Ag (39 points, 12 votes)

2.6.a. What physicochemical properties of nano-Ag can be used to predict fate and transport in environmental media?

4.12. How could existing models applicable to conventional silver be used to adequately predict the transport and fate of nano-Ag through environmental compartments, or how could they be modified to do so?

14 – Adequacy of Current Data (39 points, 6 votes)

6.15. Do current publications describing the health effects of nano-Ag particles and laboratory-generated nano-Ag particles accurately depict the toxicity of commercially available nano-Ag materials?

6.27. Are there any parallels between health effects of conventional silver and those in emerging studies on nanosilver?

16 – Dissolution (36 points, 9 votes)

5.2. What information exists on the temporal changes in the release of ionic silver by nanoparticles physicochemical and environmental characteristics?

N.11. What are the rates of dissolution of nano-Ag into the environment?

4.2. Does particle size of nano-Ag affect the rate of release of silver ions in environmental compartments?

17 – Information from Manufacturers (35 points, 10 votes)

0.4. Has the database and risk assessment methodology used by FDA during approval of nano-Ag medical devices been integrated with EPA's database and risk assessment processes?

3.3. What are realistic strategies for collecting data on production quantities and product characteristics given that much of this information is proprietary?

17 – Adaptive Tolerance / Resistance (35 points, 8 votes)

6.33. The majority of toxicity studies with conventional silver were conducted over a decade ago. Are more studies needed that utilize state-of-the-art technology for comparing its mode of toxicity to that of nano-Ag? In other words, can we accurately say that nano-Ag and conventional silver have different modes of toxicity if most of the studies available for conventional silver were not conducted using current methods?

4.10. Is the nano-Ag harmful to the beneficial organisms in wastewater treatment?

19 – Metrics (33 points, 7 votes)

5.17. How should dose and exposure be characterized for human exposures?

2.21. For the purpose of assessing potential risk, what metrics are most informative for quantifying exposure and dose of nano-Ag?

20 – Kinetics II (22 points, 5 votes)

2.16. Does nano-Ag react with materials (i.e., organic matter, other metals, polymers) and alter properties such as REDOX potential or leached metal ion rates?

3.6. What changes occur to the physicochemical properties of nano-Ag throughout the life-cycle stages, either as a function of process and product engineering or as a function of incidental encounters with other substances and the environment?

N.1. Does the release of nano-Ag contribute to climate change?

21 – Benefits (9 points, 5 votes)

N.9. Do nano-Ag products actually offer more efficacy than products on the market?

22 – Incentivize Research for CEA (8 points, 1 vote)

N.2. How can we incentivize researchers to focus in on the most critical questions and best methods for CEA?

N.6. How urgent is the need for the benefits offered by the candidate application/material?

23 – CEA Framework (1 point, 1 vote)

N.13. How can CEA framework be improved to ensure passive or active consumer/occupational exposure research is completed for nano-Ag and for other nanomaterials?

3.2. Breakout Group Reports

Each section below includes the breakout group report for each of the top 13 ranked research priorities, followed by the group's PowerPoint presentation, and a summary of the plenary discussion that followed the presentation. Note that the plenary discussion took place after the collective judgment portion of the workshop and thus served strictly to clarify information or allow observers of the workshop to express their individual viewpoints. The views expressed in this discussion are those of each individual and do not necessarily represent the views or policies of the U.S. Environmental Protection. In addition, because participants incorporated the research priority on human and mammalian test methods (ranked 8) into the reports for the other priorities, a separate report is not presented below.

3.2.1. Analytical Methods

Group Members: Participants B, C, and S

3.2.1.1 *Group Summary*

Original Questions

- 2.9. Are there standard nano-Ag reference materials that can be used in exposure and effects testing to aid in comparison of results among investigators?
- 2.10. Do adequate analytical methods exist to detect and characterize nano-Ag in environmental compartments and in biota?
- 2.12. Do adequate analytical methods exist to detect and characterize exposure to nano-Ag via soil, water, and air?
- 5.1. Are available methods adequate to characterize nano-Ag concentrations and associated exposure via relevant matrices such as: a) air?, b) water?, and c) food?

Ideas Discussed

- Instrumentation vs. methods vs. ensemble techniques.
- Methods to measure exposure vs. concentration in tissue.
- Stress the need for broad availability of "reference materials."
- Nowhere in the above questions is the word "properties" mentioned (need to know what the properties are to develop analytical methods).
- Analytical methods for surfaces of nanoparticles are very limited; this is worrisome given the importance of the surface in so many of the other areas discussed; current methods focus on characterization of bulk surfaces or sufficiently large "clusters" of nanoparticles.
- Reference materials—availability of standard methods for analysis of reference materials (tolerance levels have not been not established)

Other questions to consider:

- 2.6. Which physicochemical properties of nano-Ag can be used to:

  a. predict fate and transport in environmental media?

  b. predict toxicity to humans or biota?

- 2.7. Which physicochemical properties of nano-Ag are most essential to characterize before and during toxicity experiments?
- 2.8. What standardized test methods or characterization protocols are necessary to ensure that research results generated in multiple laboratories are consistent, reproducible, and reliable?
- 2.13. What new analytical methods would enhance characterization of nano-Ag particles?
- 2.17. How can engineered nano-Ag particles be routinely, inexpensively detected, monitored, or distinguished from incidental, background, or naturally occurring nano-Ag particles?

Viewpoints:

- We need to know the metrics in order to focus on developing/refining the analytical methods.
- Near-term – integration of existing techniques and instrumentation into standardized/verifiable/reproducible methods for characterization of nanoparticles in diverse media (e.g., air, suspension, complex waters, biological tissue, soils/sediments).
- Long-term/high-risk research area – continue focus on developing rapid, inexpensive, routine analysis.
- Quantitative vs. qualitative for all metrics, not just concentration.
- General techniques applicable to a range of materials are possible, but will require tuning for specific materials.

Why is this research theme of high importance?

Without analytical methods, one cannot:

- Measure/determine exposure.
- Determine sources of release.
- Establish linkages between properties and toxicological or other effects.
- Evaluate fate/transport.
- Adequately characterize products (maybe more on the manufacturer side?).
- Accurately track/compare historic, current, and future levels in various biological/environmental compartments.

Where does this research theme fit within the CEA process?

Note: Measurement and Characterization bar spans all sections (vertically)

- Lifecycle Stages – for example, comparison of product changes (e.g., leaching) throughout life cycle.

- External Factors – for example, changes in properties under varying environmental, biological conditions; changes in surface characteristics attributable to co-occurring substances.
- Environmental Compartments and Gateways – enables determination of presence and form in varying compartments including wastewater.
- Organisms – evaluation of uptake, toxicity, partitioning.
- Ecosystems – partitioning in aquatic/terrestrial compartments, potential for bioaccumulation, etc.
- Effects – there is no way to arrive at accurate evaluation of effects without the above.

How would answering the research questions under this theme directly support or relate to a future CEA of nanomaterials?

- Enable the generation and verification of accurate data on, for example, lifecycle effects and interactions of nanoparticles with external environmental factors, organisms, and ecosystems, to inform the determination of effects and ultimately the estimation of risks.
- Need analytical methods to link properties to outcomes.

For each of the research questions under this theme, indicate whether it is relevant to (1) a specific application of nano-Ag, (2) all applications of nano-Ag, or (3) nanomaterials in general (not only nano-Ag).

In general, each question under this theme is relevant to 1, 2, and 3. One major area of distinction, however, is that the adequacy of existing analytical techniques varies based on the composition of the particular material:

1. Metal-based nanoparticles (e.g., certain quantum dots, Ag, Au, rare metallic)
2. Ubiquitous metal-based nanoparticles (e.g., iron oxide, zinc oxide)
3. Carbon-based nanoparticles (e.g., fullerenes, tubes)

Applications dictate the extent to which multiple techniques and analytical approaches must be integrated. For example, additional extraction/preparation techniques might be required depending on the nature of the matrix in which nano-Ag is incorporated.

Major question: How do preparation techniques alter nano-Ag properties? For example, preparation of biological tissue for transmission electron microscopic analysis requires many steps, chemical reagents, that can interact/alter nanoparticle properties.

Major need: techniques to characterize particles in their native state and minimize artifacts.

What challenges might arise in answering the research questions under this theme (e.g., from a technical, policy, or social perspective)?

- Limitations of instrumentation
  - Surface analysis of nanoparticles
  - "Rapid, field-portable" is nice, but not really in the lexicon right now (or in the near future).
- Cost of equipment, analysis, availability of funding for under-represented groups (e.g., social groups)

- Training of personnel to operate equipment, perform measurements
- Agreement on properties to measure
- Maintenance expense – "$1M dollar paperweight" (non-functioning equipment)
- Need for grants to provide:
  - Analytical equipment/training to those who need them
  - Maintenance/support of analytical equipment
  - Training/support of personnel
- Accessibility of instruments/time
- Availability of reference materials
  - Cost
  - Dissemination
  - Objectivity (when given commercial suppliers)
  - Long-term availability of reference material
- Chicken/egg scenario – need field studies to inform analytical methods; need analytical methods to conduct field studies
- Practicality of regulations – Policy decisions, threshold limit values, and water quality criteria will be developed based on the capabilities and results of analytical methods
  - Asbestos example – "no particles." Can we measure that? (no analytical method to reach 0%)
  - Perchlorate – measurements are more sensitive than where effects have been shown

How are the research questions under this theme related to other top priority themes or questions?

Theme No.	Theme/Question	Does Theme Relate to Analytical Methods?	Example of How Analytical Methods Relate to Other Theme
2	**Exposure and Susceptibility**	yes	e.g., allows us to quantify exposure
3	**Phys Chem Tox**	yes	e.g., allows the establishment of linkages between properties and tox
4	**Kinetics**	yes	e.g., permits determination of time-dependent changes in particle properties
5	**Surface Characteristics**	yes	e.g., evaluate changes in surface composition
6	**Sources and Release**	yes	e.g., identify sources and chemical form, and transformations of chemical form
7	**Mechanisms**	yes	e.g., particle vs. ionic species
8	**Test Methods (Humans)**	no	
9	**Test Methods (Ecological)**	no	
10	**Is new nano unique?**	yes	e.g., determination of novel properties for nano-Ag relative to bulk or ionic forms

Theme No.	Theme/Question	Does Theme Relate to Analytical Methods?	Example of How Analytical Methods Relate to Other Theme
11	**Biological Effects**	yes	e.g., determination of bio exposure levels; quantification of concentration
12	**Eco Tox**	yes	e.g., determination of eco exposure levels; quantification of concentration
13	**Communication, Engagement, and Education**	yes	e.g., training personnel to perform a methods appropriately
14	**Fate and Transport**	yes	e.g., determine changes in form, chemical interactions
15	**Adequacy of Current Data**	yes	e.g., must be able to compare the appropriateness of methods used
16	**Dissolution**	yes	e.g., evaluate release of ions, changes in particle structure
17	**Info from Manufacturers**	yes	e.g., must know what properties to measure and how to measure them appropriately; could reduce disconnect between supplier and independently determined specs
18	**Adaptive Tolerance/Resistance**	no	
19	**Metrics**	yes	e.g., quantify dosimetry
20	**Kinetics II**	yes	e.g., evaluate interactions and changes occurring at various stages of the product lifecycle
21	**Benefit**	no	
22	**Incentivize research for CEA**	no	
23	**CEA framework**	no	

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

- Allow us to identify and quantify what is present in a particular compartment
- Quantify exposure
- Permit monitoring over time
- Identify potential sources of release
- Facilitate management (e.g., wastewater treatment, recovery from certain processes)
- Improve accuracy of risk assessments
- Identify byproducts and transformation products (if we know how it looks now, we can better identify how it has changed through a particular process).

3.2.1.2 *Group Presentation Slides*

Short Description of Priority Theme:

- Accurate, reliable, and verifiable analytical methods are needed for the determination of physicochemical properties of nano-Ag in a range of environmental matrices and at varying points throughout the lifecycle of nano-Ag.
- These methods are essential to investigate all of the themes/elements of the CEA framework and their linkages (Exposure/Dose → Effects).

Why is this research theme of high importance?

- Without accurate, reliable, and verifiable analytical methods, we cannot:
  - Characterize products (e.g., manufacturer specifications)
  - Make comparisons among different investigators, labs
  - Measure/determine exposure
  - Determine sources of release
  - Establish linkages between properties and effects
  - Evaluate fate/transport
  - Accurately track and compare historic, current, and future levels in various biological/environmental compartments.

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

- Allow us to identify and quantify what is present in a particular compartment
- Quantify exposure
- Permit monitoring over time
- Enable identification of potential sources of release
- Facilitate management (e.g., wastewater treatment, recovery)
- Improve accuracy of risk assessments
- Identify byproducts and transformation products.

3.2.1.3 *Presentation Notes*

Presenter: Participant S

- Establish linkages between different aspects of the CEA and physicochemical properties
- Establish accuracy of physicochemical characterization
- Without these methods, cannot characterize products
- Determine sources of release
  - Need well developed methods

- Evaluate fate/transport
  - Need to characterize particles at one point in transport chain or else we do not know how it might transform
- Developing analytical methods allows us to go back to previous studies where methods were not as robust
- Monitoring over time – so we know how levels are changing over time

3.2.1.4 *Questions and Answers*

- Participant G: Reference materials fell off the table – even though OECD has some reference materials, there is a role for reference materials in different matrices.
  - Answer: Comes back to verifiability. NIST provides some reference materials, but availability and awareness of those materials needs to improve.
- Participant E: Routine availability of these systems and methods and mobilizing them into the field is critical. If we don't have that, we won't be able to test these materials.
  - Answer: I wonder if these techniques or devices will be available in my lifetime because geochemistry has been using TEM and EDX for many years and they've never progressed to where they can be used in the field. A couple can, like Raman scattering, are hand held.
- Question: Geochemistry has a timeline that doesn't suit commercially available products. Consumer products push the technology. If we don't explicitly ask for cheaper, more reliable, portable technologies, we won't get it.
  - Answer: We feel there is value in ensemble techniques. We laid out near- and long-term priorities. Long term would be innovative development for small research grants.
- Participant G: There may be someone who can develop something affordable for silver, but until that need is articulated, we will not get it.
  - Participant B: Our plans and projects have a timeline. Technical difficulties of analyzing and estimating nano-Ag in consumer products are that there is the matrix. We need a method of simple preparation to remove the nano-Ag from the matrix. We need less about the instrumentation and more about the preparation of samples. Accessibility to instrumentation is one thing, but we also need a protocol of what we want to see in those products.
- Participant R: The equipment to do this would take up a quarter of the room and they want it to be much smaller. There has to be another revolution in technology to do this for nanomaterials and we are not ready to do that. They can do it on a large scale, but smaller scale, no.
  - Participant P: I work a lot in this. It is not always necessary to have portable technologies. This large technology is very effective. You can successfully transport samples to the lab and to these instruments. We have the technology to do this stuff, but they are not field portable.

3.2.2. Exposure and Susceptibility

Group Members: Participants L, N, V, and W

3.2.2.1 *Group Summary*

Short Description of Priority Theme:

Based on our theme, exposure and susceptibility, the following questions were deemed necessary to answer before completing a CEA:

- What is the distribution of exposure intensities and frequencies of such exposures among occupational and consumer populations and susceptible groups such as women of childbearing age, children, maintenance workers, and other groups for acute and chronic health effects?
- How do the parameters of physiological characteristics, behavior, lifestage, and genetic factors influence the design and the hypotheses to be tested for nano-Ag exposure?
- How do the physical and chemical properties of nano-Ag influence the kinds of exposures experienced by individuals or populations?
- What are the appropriate measurement and modeling metrics that should be used to characterize exposure?
- Which sources, pathways, and routes throughout the lifecycle offer the greatest exposure potential to nano-Ag for humans?

Why is this research theme of high importance?

- Production and use of more than 500,000 kg of nano-Ag-containing materials per year can lead to human contact with nano-Ag across lifecycle stages. Thus, many human populations will be exposed to nano-Ag throughout their lifetimes.
- Because few research studies and occupational measurements have been conducted and published, little is known about which populations are exposed, the magnitudes of these exposures, and the factors that affect the exposures.
- The lack of exposure information, especially in light of the growing hazard knowledge base, is limiting the ability of the Agency to complete a balanced CEA for eventual application to risk assessment.

Where does this research theme fit within the CEA process?

At the present time, the characterization of human environmental and occupational exposure is not explicitly mentioned within the CEA framework. This must change because the topic of exposure and the routes of exposure (inhalation, dermal, and ingestion) are central to the CEA. To accomplish this, the current CEA Framework needs a box below environmental compartments and gateways, and above organisms that explicitly define exposure characterization.

How would answering the research questions under this theme directly support or relate to a future CEA of nanomaterials?

Answering these questions will provide information for half of the equation required to complete a quantitative assessment of human risk. Because risk, in its simplest terms, is equal to hazard × exposure, and most of the currently available knowledge relates to hazard, the quantitative

characterization of exposure among susceptible populations and the general public is required immediately.

For each of the research questions under this theme, indicate whether it is relevant to (1) a specific application of nano-Ag, (2) all applications of nano-Ag, or (3) nanomaterials in general (not only nano-Ag).

As we now envision the uses of nano-Ag and other nanomaterials within commerce and manufacturing and the remaining components of a product's lifecycle, the characterization of human exposure is required. For all nanomaterials, however, the spectrum of potential human exposure can change based on application, use, and lifecycle stage. Thus, the characterization of the distribution and intensity of exposure is required for nanomaterials in general.

What challenges might arise in answering the research questions under this theme (e.g., from a technical, policy, or social perspective)?

Technical challenges:

- Personal sampling (i.e., inhalation, dermal, and ingestion) and microenvironmental sampling tools are needed for appropriate metrics for nano-Ag (e.g., size, number, shape, surface, mass).
- Tools are needed for conducting biological marker measurements of exposure to the skin and eye, and for bioaccumulation or transport in the blood and urine. These markers should include, where necessary, the inhalation, dermal, or ingestion routes of exposure.
- The susceptible and user populations that would make up the pool of subjects for a quantitative exposure characterization need to be defined.

Social:

- Gaining access to workplaces to conduct workforce identification and exposure measurements.
- Gaining access to information on parent and product nano-Ag characteristics to design an exposure study properly.
- Focusing the manufacturers and the commercial interests on the value and benefits of completing quantitative exposure assessments for products that contain nano-Ag before they are:
  - generally distributed to the public (consumer and users)
  - produced, that is, during the design of a production process
  - released and then placed in a waste stream.

Policy:

- The lack of clear regulatory guidance on workplace or personal product exposure limits for exposure management.
- Provide public health and environmental agencies with the resources and capacity to assess consumer and environment exposures to nano-Ag.
- Provide occupational health agencies with the resources and capacity to assess consumer and environment exposures to nano-Ag.
- Provide the mechanisms for funding exposure characterization projects on nano-Ag.

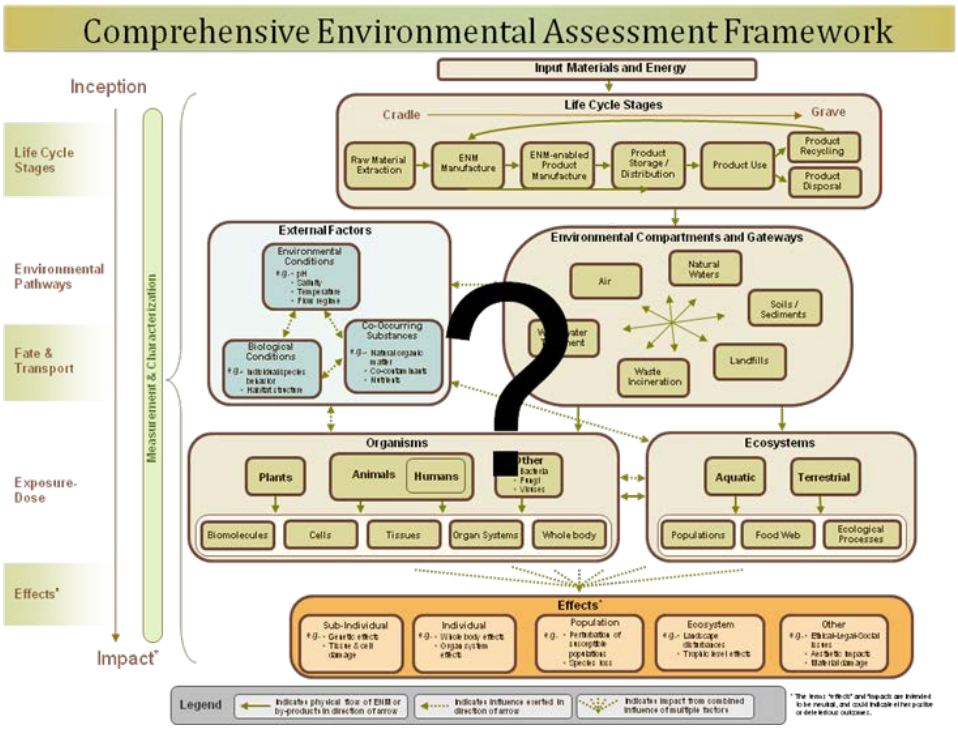
How are the research questions under this theme related to other top priority themes or questions?

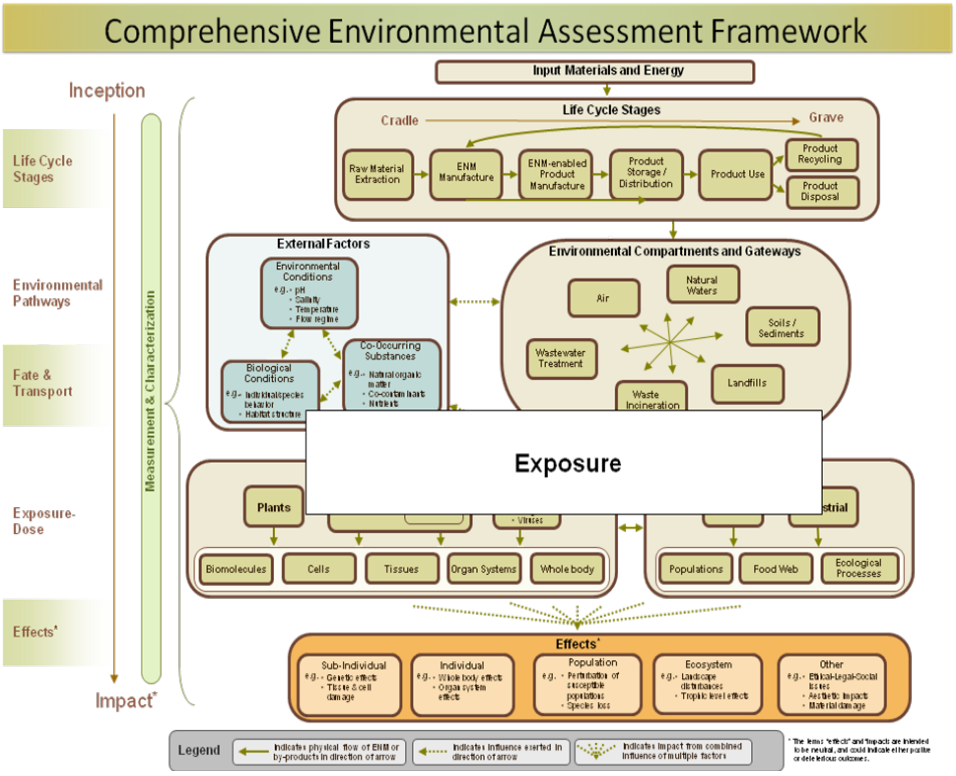
- For Highest priority Topics 1–7: Analytical Methods, Surface Characteristics, Sources and Release.
- Biological Effect, Communication, Engagement and Education, Fate and Transport.

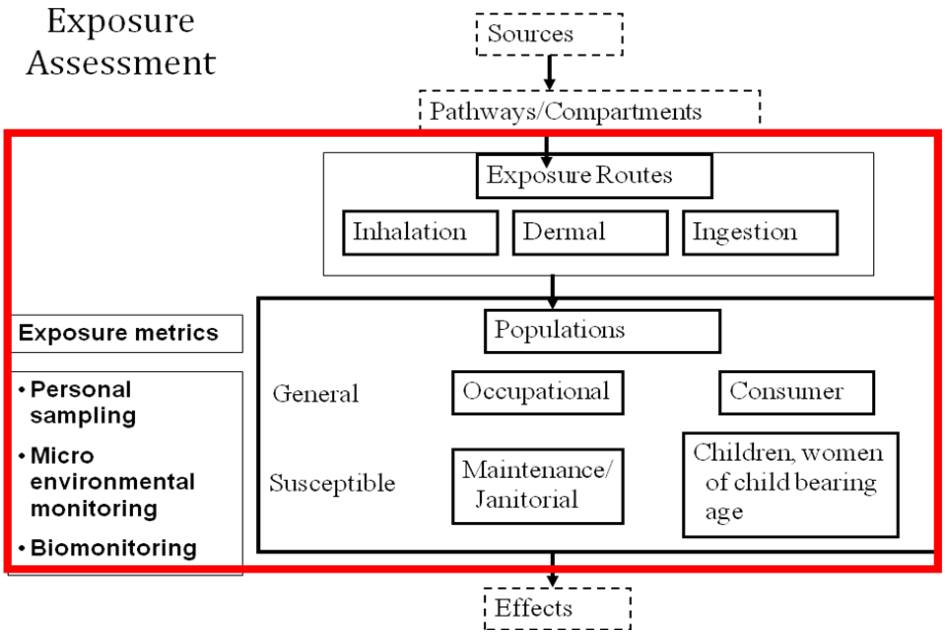
How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

The characterization and assessment of exposure are necessary prerequisites to the implementation of exposure controls or application of techniques to prevent consumer contact with nano-Ag that could result in relevant health effects. Once workers are handling nano-Ag-containing materials and products are released into commerce, exposures will occur. If exposures are high in intensity, duration, or frequency, they could lead to health effects that are not traditionally covered in conventional toxicology studies. Thus, minimizing the potential for exposures early in product development will minimize the potential for unintended consequences.

3.2.2.2 *Group Presentation Slides*







Key Research Questions

- Exposure distributions among occupational and consumer populations and susceptible groups
- Effects of physiological characteristics, behavior, lifestage, and genetic factors.

- Effects of physical and chemical properties of nano-Ag on exposure.
- Appropriate measurement and modeling metrics.
- Sources, pathways, and routes throughout the lifecycle that lead to the greatest exposure potential?

Importance of Research Theme

- Many human populations will be exposed to nano-Ag-containing materials throughout their lifetimes.
- Because few research studies and occupational measurements have been conducted and published, little is known about which populations are exposed, the exposure magnitudes, and the factors that affect exposures.
- While hazard knowledge base is LARGE, exposure knowledge is **small**. This limits the ability to complete a balanced CEA and risk assessment.

Reducing Chances of Unintended Consequences

- Identifying populations of interest and key exposure routes can help identify appropriate management and control measures.
- This will help minimize exposures and thus, health impacts.

3.2.2.3 *Presentation Notes*

Presenter: Participant W

- Exposure is on the left-hand column of the CEA framework, but we couldn't find it in the boxes. It is perhaps implicit in the arrows, but it should be explicit.
- We created a box for exposure.
  - Start with sources, pathways, various compartments and media, exposure routes, populations and susceptible groups within those populations, the effects.
- Exposure metrics – challenges
  - What are the appropriate metrics?
  - How to do personal sampling?
  - What biomarkers are relevant?
- Key research areas
  - Exposure distributions, frequency, magnitude in occupational and nonoccupational and susceptible groups
  - Relevant matrices
  - Effects of physiological characteristics
  - Effects of physical/chemical properties on magnitude of exposure
  - Modeling is essential part of exposure/susceptibility assessment

- Many human populations will be exposed to nano-Ag throughout their life and lifecycle of the materials.
- There are few studies of compliance and noncompliance, so we know little about exposure and exposure distribution.
- There is about a 5:1 ratio of hazard to exposure research.
- To balance CEA, we need to correct above imbalance.
- To reduce the chances of unintended consequences, we must identify the populations of interest and the key exposure routes. We can then identify appropriate ways to minimize exposure and impacts.

3.2.2.4 *Questions and Answers*

- Participant L: We want to emphasize that we did not consider nonhuman populations.
- Participant G: For exposure, is it best to focus on the 1- to 100-nm size range?
  - Participant W: No, I don't think so, personally. Particles can agglomerate to form larger entities, but they might still retain some nano-sized features. Or they can be inhaled in agglomerated form and they could disaggregate. I think we should also look at larger particles up to a few nanometers.
  - Participant N: This is sort of a chicken/egg question. If there is a suspicion that there are nanomaterials, what size are people exposed to? We do not even know the size of the particles in the product. We couldn't say whether to concentrate on 1–100 until we look and see what is actually in the product.
- Observer 1: Looking at women of childbearing age – are they more exposed or are you thinking of it from an effects standpoint?
  - Answer: These products are designed to be used in the home, so we thought their exposure patterns might be different from a working adult.
  - Participant L: One member felt susceptibility could not be teased out from exposure.
- Observer 2: You focused on humans – is it an intentional prioritization that it was more important to focus on humans or just based on the people in your group?
  - Participant L: It was definitional. Exposure is a term you use for humans, whereas you use concentration for other organisms, so I could not rebut that from a technical standpoint.
  - Participant N: There was some controversy. I think nonhuman exposures are important and I don't see human exposures as separate from those in the environment. It was somewhat an issue of time, so we decided that exposure to humans via fish and biota would be covered in our pathways.
  - Participant W: It was definitional – the way the discussion had progressed up to that point. When we come to human populations, we are talking exposure. When we talk about biota we are talking about concentrations in them (tissue concentrations), and we felt that would be covered in ecotox.
- Participant M: That really is naïve – exposure is used all across the ecotox arena. It is true there are tissue concentrations, but how do they get there?

- Participant A: I agree it is not just humans. I think you missed a susceptible population – immunocompromised individuals. They might use these products more often and use them in the home.
  - Participant W: I think we will add that – that is a great point.
  - Participant W: One more point on exposure to biota. One group member used to be editor of Exposure Science Journal, and that is all human, not eco.
- Participant A: I did not see much on dosimetry. One of the big issues is what is our dose metric? Traditionally we look as mass, which is not appropriate for nano. For immunocompromised individuals, it might be particle numbers that affect them – based on mass, there is a huge difference in particle number and surface area.
  - Participant W: We talked about exposure metrics, which include those issues. It was more in the modeling part – modeling metrics – and no one has specifically talked about doses of nanomaterials in humans – it is a modeled entity. That will be captured in biomarkers where we will be monitoring something.
- Participant Q: Assessing exactly what people will be coming in contact with – FDA did a survey on 300 products on the market. We contacted suppliers and asked what the form of silver in the product was. About 75% of the companies said it is a powder and looks like dust. I don't think the suppliers of applications going to consumers even know what they are using. We will need to go to source of the suppliers and then make sure the suppliers are using the materials in the way it is intended.
  - Participant W: Access to parent material or product is a challenge – social challenge.

3.2.3. Physical and Chemical Toxicity

Group Members: Participants F, P, and Q

3.2.3.1 *Group Summary*

Short Description of Priority Theme:

These areas focus on linkages between specific physicochemical properties and toxicological effects. This theme represents a cross-section among different disciplines seeking to integrate different levels of theory into a comprehensive environmental assessment. Physicochemical properties (e.g., surface coatings, surface charge, size, shape, chemical composition) of nano-Ag (and other nanoparticles) can be used to predict toxicity to humans and biota. This theme also captures specific endpoints in which nanoparticle characterization is important before, during, and after toxicity experiments.

Why is this research theme of high importance?

Physicochemical characterization:

- Allows for an interdisciplinary and multidisciplinary approach
  - Requires competencies in biology, chemistry, physics, ecology, geochemistry, and toxicology
- Represents a transitional and influential field. It is similar to the principles that govern technology transfer.
- Improves particle biocompatibility through an iterative material design process

  - Demonstrates details in the interactions (e.g., effects, uptake, transport) between particles and biological systems, representing opportunities to optimize particle design for novel application

- Provides an opportunity to improve on past research and development
  - Compels the environmental and toxicology communities to deal with solid-phase chemistry
  - Encourages chemists and physicists to consider the impact of engineered materials and products

Where does this research theme fit within the CEA process?

The CEA should reflect the chemical and physical properties of the particles at every stage lifecycle. The CEA should capture the physical and chemical properties of the particle at each stage of the lifecycle and should correlate with the route of exposure and dosing concentration. The CEA should link the physical and chemical properties of the particle to each potential toxic effect at the sub-individual, individual, population, and ecosystem levels.

How would answering the research questions under this theme directly support or relate to a future CEA of nanomaterials?

Using models to predict toxicity:

- *Knowledge Gap*: Marry fundamental physical/chemical models with the empirical toxicology models and decision-based sciences.
- *Expected Results*: The resultant model or models must ultimately talk to, feed, and relate to each other.

Effects of Surface Coating:

- *Knowledge Gap*: Surface coating represents a modification of the behavior of the pristine particle and might represent a further modification of the properties and a potentially different particle in CEA.
- *Expected Results*: Potential surface modification of nanoparticles can have an effect on the physiological and biochemical responses to the particle.

Effects of Other Particle Properties:

- *Knowledge Gap*: There is a need to tease out the weighted contributions of each feature, e.g., size, shape, chemical composition, and surface charge, to their biological response and the integration of these properties.
- *Expected Result:* Effect = A(size)+B(shape)+C(chemical composition)+D(surface charge).

Characterization and re-characterization in the experiment design:

- *Knowledge Gap*: Some of the best published papers that focus on the fate of a particle are when the researchers track the nanoparticle throughout the toxicity study and map the results in terms of accumulation and speciation.
- *Expected Result:* Inclusion of particle characterization before and after the toxicity study will enhance the accuracy within the assessment.

For each of the research questions under this theme, indicate whether it is relevant to (1) a specific application of nano-Ag, (2) all applications of nano-Ag, or (3) nanomaterials in general (not only nano-Ag).

For all four research questions, physicochemical characterization is necessary to specific applications of nano-Ag, all applications of nano-Ag, and all applications of nanomaterials in general.

What challenges might arise in answering the research questions under this theme (e.g., from a technical, policy, or social perspective)?

Technical challenge: separating the dissolved silver from particle silver and what the effects of the different species are.

Policy challenge: at the different stages of experimentation, complete chemical/physical characterization is prohibitively expensive.

Social challenge: explaining how the different methods and instrumentation work and the information they provide.

How are the research questions under this theme related to other top priority themes or questions?

Physical/chemical characterization provides a fundamental understanding of the nanomaterial. A fundamental understanding of the nanomaterial is necessary for all of these other top priority themes:

- Test methods development for human and mammalian systems
- Test methods development for ecological systems
- Test methods development for analytical methods
- Surface characteristics
- Ecological toxicity
- Fate and transport
- Mechanisms
- Biological Effects
- Is nano unique
- Kinetics I
- Kinetics II
- Exposure/Dose
- Exposure/Susceptibility

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

Physicochemical characterization strengthens the nanomaterial iterative design process that represents an inherent proactive approach designed to reduce the chance of unintended consequences through guided, environmentally sustainable nanotechnologies. Coupling physical/chemical properties with ecotoxicological and human health effects provides specific information to developers with respect to needed modifications of the materials.

3.2.3.2 *Group Presentation Slides*

Short Description of Priority Theme:

- These areas focus on linkages between specific physicochemical properties and toxicological effects.
- This theme represents a cross-section among different disciplines seeking to integrate different levels of theory into a comprehensive environmental assessment.
- Physicochemical properties (e.g., surface coatings, surface charge, size, shape, chemical composition) of nano-Ag (and other nanoparticles) can be used to predict toxicity to humans and biota.
- This theme also captures specific endpoints in which nanoparticle characterization is important before, during, and after toxicity experiments.

Why is this research theme of high importance?

Physicochemical Characterization

- Allows for an interdisciplinary and multidisciplinary approach
  - Requires competencies in biology, chemistry, physics, ecology, geochemistry, and toxicology
- Represents a transitional and influential field. It is similar to the principles that govern technology transfer
- Improves particle biocompatibility through an iterative material design process
  - Demonstrates details in the interactions (e.g., effects, uptake, transport) between particles and biological systems, representing opportunities to optimize particle design for novel application
- Provides an opportunity to improve upon past research and development
  - Compels the environmental and toxicology communities to deal with solid-phase chemistry
  - Encourages chemists and physicists to consider the impact of engineered materials and products

Using Models to Predict Toxicity

- *Knowledge Gap*: Marry fundamental physical/chemical models with the empirical toxicology models and decision-based sciences.
- *Expected Results:* The resultant model or models must ultimately talk to, feed, and relate to each other.

Effects of Surface Coating:

- *Knowledge Gap:* Surface coating represents a modification of the behavior of the pristine particle and might represent a further modification of the properties and a potentially different particle in CEA.
- *Expected Results:* Potential surface modification of nanoparticles can have an effect on the physiological and biochemical response to the particle.

Effects of Other Particle Properties:

- *Knowledge Gap*: There is a need to tease out the weighted contributions of each feature, e.g., size, shape, chemical composition, and surface charge, to their biological response and the integration of these properties.
- *Expected Result*: Effect = A(size)+B(shape)+C(chemical composition)+D(surface charge).

Characterization and Re-characterization in the Experiment Design:

- *Knowledge Gap*: Some of the best published papers that focus on the fate of a particle are when the researchers track the nanoparticle throughout the toxicity study and map the results in terms of accumulation and speciation.
- *Expected Result*: Inclusion of particle characterization before and after the toxicity study will enhance the accuracy within the assessment.

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

- Physicochemical characterization strengthens the nanomaterial iterative design process that represents an inherent proactive approach designed to reduce the chance of unintended consequences through guided environmentally sustainable nanotechnologies.
- Coupling physical/chemical properties with ecotoxicological and human health effects provides specific information to developers with respect to needed modifications of the materials.

3.2.3.3 *Presentation Notes*

Presenter: Participant F

- I wonder if we can predict potential toxicities by measuring physical/chemical properties and using them in modeling.
- Focus on the link between physical/chemical properties and biological effects.
- This has been an issue since the beginning.
- It is a cross-section among several disciplines, integrating several levels of theory.
- Let us characterize nanomaterials and let us characterize the cells before exposure, during exposure, and after exposure. Can we move toward recapturing the nanomaterials?
- Physical/chemical properties are the key in toxicological research, allowing for a multidisciplinary approach.
- It is a transitional field, an iterative process.
- It forces the environmental scientist and toxicologist to learn solid-state physics and engineers to consider impacts.
- Using models – mathematical or experimental – to predict toxicity: We would expect results of the models to "talk" to each other. There are existing models, but the research opportunity is in integrating models from different disciplines. How can some model data influence upstream or downstream models?
- Effects of surface coating – modification of behavior of particle and possible modification of properties: This might make it a different particle in terms of the CEA – ions, particle, or composite material?

- Other particle properties – weighted contributions of physical/chemical properties, properties from what we know from the literature: Weight size heavily if it is more important, but don't exclude other properties because some of it is synergy.
- Characterization and re-characterization are issues.
- We can reduce unintended consequences through guided technologies.

3.2.3.4 *Questions and Answers*

- Participant G: Your list of properties did not include surface area.
  - Participant F: For what we were talking about, we might be most used to looking at particles in a suspension or a complex matrix, and it is difficult for us to measure surface area – we can calculate it – but I rely on other properties that I can measure.
  - Participant P: We normalize surface charge by surface area, called specific surface charge.

3.2.4. Kinetics and Dissolution

Group Members: Participants G, I, T, and U

3.2.4.1 *Group Summary*

Short Description of Priority Theme:

The research questions are the following:

- What is the half-life of nano-Ag in the environment?
- What are the rates of dissolution of nano-Ag in the environment?
- Do nanoparticles react with other materials (e.g., organic matter, other metals polymers) and alter properties such as redox potential or leached metal ion rates?
- What information exists on the temporal changes in the release of ionic silver by nano-Ag in relation to particle physicochemical and environmental characteristics?
- Does the particle size, source, or agglomeration state affect the rate of release of silver ions in environmental compartments?

Why is this research theme of high importance?

The question we must answer is: What is the persistence of nano-Ag in the environment? Environmental persistence is a function of the physical and chemical characteristics of nano-Ag. In this research area, we must consider all the possible mechanisms of transformation in soil, water, and air:

- Dissolutions – Redox chemistry
- Passivation
- Coagulation
- Growth
- Binding to humic substances in soils and water
- Deposition onto surfaces

- Secondary effects of nano-Ag reactivity.

The speciation that occurs on these physical and chemical transformations establishes relevant test materials, exposure, toxicology, and measurement methods.

Where does this research theme fit within the CEA process?

This theme fits within the environmental pathways and fate and transport. The pollutant is partitioned to the environmental compartments and is affected by external factors. This, in turn, affects its physical and chemical transformations and ultimately its toxicological and ecological effects.

How would answering the research questions under this theme directly support or relate to a future CEA of nanomaterials?

For this CEA, we need to accurately predict concentrations of nano-Ag and its reaction products. Failure to accurately identify relevant silver species in the environment will result in unproductive research activities in exposure, toxicology, development of test methods, and risk assessment.

For each of the research questions under this theme, indicate whether it is relevant to (1) a specific application of nano-Ag, (2) all applications of nano-Ag, or (3) nanomaterials in general (not only nano-Ag).

Knowledge of the chemical and physical transformation mechanisms of nanomaterials is key for understanding the exposure, dose, and toxicity of all engineered nanomaterials in the environment.

What challenges might arise in answering the research questions under this theme (e.g., from a technical, policy, or social perspective)?

One of the most important technical challenges is measurement technology to measure nano-Ag as it is transformed. Although a mass spectrometric method has been developed for air studies and microscopy has been utilized for condensed-phase studies, better methodologies might be needed to accurately measure chemical and physical transformations.

A resulting policy challenge is the emphasis from federal agencies to prioritize chemical and physical transformations of nano-Ag and other nanomaterials. Of particular concern is the timing of the research programs. Because the relevancy of other programs depends on the results of this and the measurements research, positioning the research is a particular challenge.

Again, because the other research questions of exposure, dose, toxicology, and effect depend on testing the correct materials in the proper physicochemical form, it is imperative that the chemistry community communicate these results to the researchers undertaking these endeavors. What is more, EPA should be proactive in their call for research as to what is known about the physicochemical properties of nano-Ag and its products as they exist in the environment.

How are the research questions under this theme related to other top priority themes or questions?

- Analytical methods – Analytical methods need to be developed for proper identification of nano-Ag and its transformation products.
- Exposure and susceptibility – The kinetics determine what the exposure concentration of all nano-Ag species will be.
- The physical and chemical properties on toxicity – The kinetics determine what the physical and chemical properties of nano-Ag species will be.

- Surface characteristics – The kinetics determine what the physical and chemical properties of nanomaterials and the daughter species will be.
- Sources and release – Oxidation and dissolution are sources of active silver species in environment compartments.
- Biological Effects, Ecological Toxicity, and Mechanisms of Toxicity – The kinetics determine the oxidation state of the silver which can then act as a toxicant.
- Human and Ecology Test Methodologies – Researchers should be aware of the transformations of nano-Ag to design proper test methodologies.
- Is new nano unique? – Data and risk assessments for existing and historical "colloidal" silver can inform our understanding of chemical and physical transformations of nano-Ag.
- Communication – Researchers in other fields should have an appreciation of the time factor between sources and exposure.
- Fate and Transport – A feedback needs to be established between modelers and experimentalists on the chemical and physical transformations of nanomaterials.

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

Answering these research questions will inform proper experimental conditions for exposure and toxicity studies. There are connections between the persistence of nano-Ag and the type of coating of these particles. Thus, lifetimes of nano-Ag might be extended from seconds or minutes to months or years.

3.2.4.2 *Group Presentation Slides*

Short Description of Priority Theme:

- Knowledge of the physical and chemical transformations of nano-Ag is crucial for productive research activities in exposure, toxicology, development of test methods, and risk assessment.

Why is this research theme of high importance?

- What is the persistence of nano-Ag in the environment?
- Environmental persistence is a function of the physical and chemical characteristics of nano-Ag.
- We must consider the possible mechanisms of transformation
  - Dissolutions – Redox chemistry
  - Passivation
  - Coagulation
  - Growth
  - Binding to humic substances in soils and water
  - Deposition onto surfaces
  - Secondary effects of nano-Ag reactivity

In air, water, and soil:

- The speciation that occurs as a result of these physical and chemical transformations establishes relevant test materials, exposure, toxicology, and measurement methods.

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

- For this CEA, we need to accurately predict concentrations of nano-Ag and its reaction products.
- Failure to accurately identify relevant silver species in the environment will result in unproductive research activities in exposure, toxicology, development of test methods, and risk assessment.

3.2.4.3 *Presentation Notes*

Presenter: Participant U

- Nanomaterials are designed to be more reactive – quantum effect, increased surface defects, etc.
- When released to atmosphere, water, or soil, they will react.
- Knowledge of reaction pathways is crucial for productive research activities.
- Exposure, tox, test methods, and risk assessment – all need a kinetics base.
- Persistence
  - Size, surface coatings, and physical/chemical properties are important.
  - Need to consider all reaction pathways in all compartments.
  - Dissolution – Take silver0 and put it in water, it will be a long time before it dissolves. If you have nano-Ag in water with some ionic strength, some material will be adsorbed onto the surface of the nano-Ag and will take an electron away in a redox reaction. One question was: Does size make a difference for dissolution chemistry? Yes. As they get smaller, atoms are mostly on the surface.
  - Complication – if you start with 20-nm nano-Ag, it starts to dissolve; now you have more atoms on the surface and the reaction or dissolution rate will increase.
  - There are many different pathways in which it can react – it can form silver sulfide, can grow, can bind to humic substances (making it more bioavailable), deposit onto surfaces, etc.
- We need to know what happens between sources and exposure.
- Plot: y-axis is nano-Ag concentration and x-axis is time. Over time, nano-Ag starts to react away. Over time, reaction products increase. This is just reaction time independent of when a system is exposed. On a short time scale, you want to measure the properties of one mixture, and at the other, you want to measure the properties of another mixture.

- It is crucial to understand what happens between sources and receptors.
- Failure to assess kinetics will result in unproductive research.

3.2.4.4 *Questions and Answers*

- Participant F: Playing the Devil's advocate – for risk assessments of things we feel comfortable understanding, these molecules change over time. So certainly established risk assessment methods are used to look at, say, PFOA. The effects of time and transformation have been considered to the extent that they have already occurred. If these established risk assessments are not taking this transformation into consideration already, then we have a bigger problem than nano.

  For risk assessors, they run these pollution models to understand how these chemicals change over time and how people will be exposed, based on thousands of kinetics studies.

  - Participant I: In my opinion, that perspective is right. In ordinary assessment of chemicals, the daughter products should be considered.

- Participant A: It is true for many pesticides; they do include metabolites, so that is considered for many compounds going into the human body. This is not considered as a must for environment, but it is taken into account for some chemicals. If it goes into food, you look at compounds and metabolites. It might mostly be an issue for environmental effects.

  - Participant U: You look at metabolites, but the rates are not there, so that is important.

3.2.5. Surface Characteristics

Group Members: Participants C, G, S, and V

3.2.5.1 *Group Summary*

Short Description of Theme:

- How does the surface coating affect the physicochemical properties of nano-Ag?
- What effect, if any, do surface composition and surface treatments of nano-Ag particles have on a) human exposures and uptake, b) biopersistence, c) bioaccumulation, d) biomagnification, and e) other biological and environmental processes?

Why is this research theme of high importance?

- Surface characteristics, for example, composition, coatings, treatments, passivation, morphology, surface charge, surface area, can affect the behavior of nano-Ag as it moves away from the source towards some receptor.
- Surface characteristics can affect uptake and biological response.

Where does this research theme fit within the CEA process?

Surface characteristics can modulate the distribution, transport, and fate of nano-Ag as it moves through the lifecycle stages.

How would answering the research questions under this theme directly support or relate to a future CEA of nanomaterials?

A better understanding of the surface characteristics of nanomaterials will reduce the uncertainty in establishing the relationship of how the nano-Ag product will affect or be affected by the various processes associated with the CEA.

For each of the research questions under this theme, indicate whether it is relevant to (1) a specific application of nano-Ag, (2) all applications of nano-Ag, or (3) nanomaterials in general (not only nano-Ag).

Research questions regarding surface characteristics are relevant to nanomaterials in general.

What challenges might arise in answering the research questions under this theme (e.g., from a technical, policy, or social perspective)?

Surface characterization of nanoparticles in their native state is a challenge because of current instrumental limitations with respect to spatial resolution, sensitivity, detection limits, etc., and a lack of standardized methods for sample preparation and characterization.

How are the research questions under this theme related to other top priority themes or questions?

- Top 7 Priorities: Analytical Methods, Exposure and Susceptibility, Chem-Phys-Tox, Kinetics, Biological Mechanisms.
- Second 7 Priorities: Is New Nano Unique, Biological Effects, Fate and Transport.

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

Products having poorly understood surface characteristics can lead to exposures that were unanticipated with consequential unintended effects on ecosystems. A better understanding of surface characteristics can force reconsideration of how nano-Ag is applied in a product.

3.2.5.2 *Group Presentation Slides*

Short Description of Priority Theme:

- How does the surface coating affect the physicochemical properties of nano-Ag?
- What effect, if any, do surface composition and surface treatments of nano-Ag particles have on a) human exposures and uptake, b) biopersistence, c) bioaccumulation, d) biomagnification, and e) other biological and environmental processes?

Why is this research theme of high importance?

- Surface characteristics, for example, composition, coatings, treatments, passivation, morphology, surface charge, surface area, can affect the behavior of nano-Ag as it moves away from the source towards some receptor.
- Surface characteristics can affect uptake and biological response.

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

- Products having poorly understood surface characteristics can lead to exposures that were unanticipated with consequential unintended effects on ecosystems. A better understanding of surface characteristics can force reconsideration of how nano-Ag is applied in a product.

3.2.5.3 *Presentation Notes*

Presenter: Participant C

- X-ray photoelectron spectroscopy is of utmost importance.
- Surface is where particles will interact with things in the environment and anything else they come into contact with.
- Surface properties will affect many things in CEA.
- Surface properties are determined with analytical techniques. Twenty years ago, there were over 100 techniques to do this. Some measure different depths – "surface" is subjective.
- Many techniques don't have high spatial resolution, but there has been a lot of improvement.
- There are tabletop scanning electron microscopes, and you can get great images at great resolution to get elemental information, but surface chemistry analytical techniques need a lot of development.
- Most techniques still limited to aggregates of other accumulations.

3.2.5.4 *Questions and Answers*

- Participant P: Surfaces are so hard to deal with. We crank up the energy to hit them with high-flux X-rays, but then they permeate the surface and you pick up signals from the bulk. This highlights the challenge of doing surface spectroscopy. The more technology we throw at it, the more it increases curve balls.
  - Participant S: There is difficulty in characterizing the native surface; the preparation techniques required to allow us to look at the surface affect the surface. Particles in suspensions – are there things available to look at surfaces?

3.2.6. Sources and Releases

Group Members: Participants A, E, and J

3.2.6.1 *Group Summary*

Short Description of Priority Theme:

- Characterize the release rates of nano-Ag into the environment, including manufacturing and research workplaces, for all sources where relevant.
- Identify the associated feedstocks and by-products of nano-Ag spray disinfectants. Of these feedstocks and by-products, characterize those that could be released, in what quantities, and via which pathways.
- Identify, characterize, and where possible, quantify potential exposure vectors by which nano-Ag or nano-Ag by-products could be released to the environment at all lifecycle stages, including nano-Ag "life after death," e.g., use of sludges for agriculture and gardening.
- Characterize the effectiveness of nano-Ag removal from sewage and industrial process water by waste-waste treatment technology.
  - Characterize and quantify amounts and sizes of Ag in the sludge.

All stages of the lifecycle have potential intended and unintended releases. Lifecycle stages of nano-Ag products are not well understood. Limited data have been identified on specific points of release or the quantity of nano-Ag released as a result of the manufacturing, including feedstocks.

Validation of current information and additional information about specific points of release and the quantities of the releases are needed for the CEA. At this time, focusing on the potential releases from feedstocks and manufacturing processes would be important to characterize before commercial production increases.

Why is this research theme of high importance?

Without the basic information about sources and releases during all stages of the lifecycle, especially for the early stages of feedstocks and manufacturing process, the CEA cannot be completed. We need to know where is it and how much. This information is critical.

Where does this research theme fit within the CEA process?

This theme fits into the entire spectrum of life-cycle stages, including feedstocks, manufacturing, distribution, storage, use, disposal, and "life after death."

How would answering the research questions under this theme directly support or relate to a future CEA of nanomaterials?

Finding answers to this research question will lay a solid foundation for the nano-Ag spray CEA, as well for all other nano-material CEAs.

For each of the research questions under this theme, indicate whether it is relevant to (1) a specific application of nano-Ag, (2) all applications of nano-Ag, or (3) nanomaterials in general (not only nano-Ag).

- Characterize the release rates of nano-Ag into the environment, including manufacturing and research workplaces, for all sources where relevant.
  - All three
- Identify the associated feedstocks and by-products of nano-Ag spray disinfectants. Of these feedstocks and by-products, characterize those that could be released, in what quantities and via which pathways.
  - All three
- Identify, characterize, and where possible, quantify potential exposure vectors by which nano-Ag or nano-Ag by-products could be released to the environment at all lifecycle stages, including nano-Ag "life after death," e.g., use of sludges for agriculture and gardening.
  - All three
- Characterize the effectiveness of nano-Ag removal from sewage and industrial process water by waste-waste treatment technology.
  - Characterize and quantify amounts and sizes of Ag in the sludge.
  - Relevant to 1. For 2, relevant to "free" nano-Ag, such as hand sanitizers, cosmetics, or food contact substances.

What challenges might arise in answering the research questions under this theme (e.g., from a technical, policy, or social perspective)?

- Requiring this information to be collected as it is unlikely to be voluntarily offered.
- Collecting "proprietary" information on feedstocks and manufacturing processes, geographical information, and quantities. Who is producing what and where?
- Lack of readily available, robust, field-tested detection and monitoring devices and technologies.
  - Wide variety of complex matrices that could contain nano-Ag will pose significant challenges.
- Lack of labeling requirements will make identifying products containing nano-Ag difficult; therefore we cannot identify products to track releases that could result in consumer exposures.

How are the research questions under this theme related to other top priority themes or questions?

- Analytical methods
  - 2.10 – Do adequate analytical methods exist to detect and characterize nano-Ag in environmental compartments and biota?
  - 2.12 – Do adequate analytical methods exist to detect and characterize exposure to nano-Ag via soil, water, and air?
- Lifecycle Stages
  - 3.3 – What are realistic strategies for collecting data on production quantities and product characteristics given that much of this information is proprietary?
  - 3.6 – What changes occur to the physicochemical properties of nano-Ag throughout the materials lifecycle stages, either as a function of process and product engineering or as a function of incidental encounters with other substances and the environment? May be particularly applicable to the nano-Ag sludge issue.
- Fate and Transport
  - 4.11 – To what extent does nano-Ag bind to wastewater sludge and settle out or remain with treated water and enter downstream aquatic environments?
  - 4.14 – Leaching and runoff are two terms mentioned frequently as a means for introducing nano-Ag to the natural environment.
- Exposure
  - Sources and releases are critical to exposure.
  - 5.1 and 5.20 – Are available methods adequate to characterize nano-Ag concentrations and associated exposure via relevant matrices such as air, water, food, surface dust?
  - 5.7—Ecologically, is nano-Ag a point source or regional exposure problem? If it is a regional distribution issue, what are the exposure concentrations and concentration gradients in key media (e.g., air water, soil)?
- Surface characteristics
  - 5.3 – What effect if any does surface treatment of nano-Ag particles have on uptake, biopersistence, bioaccumulation, or biomagnification?

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

- For ecological and human health, potential disruption of microflora and fauna populations through biological actions of the nano-Ag.
  - Examples: Disruption of wastewater treatment organisms; human dermal or gut organisms
- Widespread use of nano-Ag sprays will select for microbial adaptation and resistance.
- If the research questions are answered and the sources and releases of nano-Ag spray are known, the focus can be on high-release geographic sites and the potential consequences of dispersing nano-Ag-containing sludge (via agriculture or gardening) can be reduced.
- From the limited data available, a holistic approach to nano-Ag spray production is needed, as other potentially hazardous and toxic materials are involved in the processes or product. These other ingredients within the disinfectant spray could also have impacts on the environment and human health.

3.2.6.2 *Group Presentation Slides*

Short Description of Priority Theme:

- Characterize the release rates of nano-Ag into the environment, including manufacturing and research workplaces, for all sources where relevant.
- Identify the associated feedstocks and by-products of nano-Ag spray disinfectants. Of these feedstocks and by-products, characterize those that could be released, in what quantities, and via which pathways.
- Identify, characterize, and where possible, quantify potential exposure vectors by which nano-Ag or nano-Ag by-products could be released to the environment at all lifecycle stages, including nano-Ag "life after death," for example, use of sludges for agriculture and gardening.
- Characterize the effectiveness of nano-Ag removal from sewage and industrial process water by waste-waste treatment technology.
  - Characterize and quantify amounts and sizes of Ag in the sludge.

Why is this research theme of high importance?

- Without the basic information about sources and releases during all stages of the lifecycle, especially for the early stages of feedstocks and the manufacturing process, the CEA cannot be completed.
- We need to know where is it and how much. This information is critical.

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

- For ecological and human health, potential disruption of microflora and fauna populations through biological actions of the nano-Ag.
  - Examples: Disruption of wastewater treatment organisms; human dermal or gut organisms
- Widespread use of nano-Ag sprays will select for microbial adaptation and resistance.

- If the research questions are answered and the sources and releases of nano-Ag spray are known, the focus can be on high-release geographic sites and the potential consequences of dispersing nano-Ag containing sludge (via agriculture or gardening) can be reduced.
- From the limited data available, a holistic approach to nano-Ag spray production is needed, as other potentially hazardous and toxic materials are involved in the processes or product. These other ingredients within the disinfectant spray could also have impacts on the environment and human health.

3.2.6.3 *Presentation Notes*

Presenter: Participant A

- Need to look at feedstocks and by-products, and need to characterize quantities and pathways.
- Missing from CEA – issue of life after death for these products. For nano-Ag in other products, if they get into wastewater, and then onto sludges that get used agriculturally, etc. Some of these sources could be regional and high
- All stages of life cycle have intended and unintended releases.
- Points and quantities of release are not well understood.
- Often we don't even know where the releasing facilities are located.
- Without basic information about where it comes from and how much is released through the lifecycle, specifically for feedstocks and by-products, you can't do a CEA.
- Earlier stages will result in larger exposures, but more they are limited in geographical space.
- Once you go down to the product level, exposures might be lower, but be more widespread.
- Because nano-Ag is antimicrobial, there might be disruption of wastewater treatment bacteria or human gut or skin microflora.
- A lot is confidential business information. This should not be proprietary. We need the information so we can focus on high-release areas and dispersing nano-Ag sludges.
- Exposure through local wastewater treatment plants could be high and might be used locally. WE need to know that so we can take action to reduce impact. People are often using these in their gardens and they are labeled as organic.
- We need a holistic approach to spray production because there are potentially other hazardous materials involved in the production and incorporated into the spray. This issue needs to be looked at collectively.

3.2.6.4 *Questions and Answers*

- None.

3.2.7. Mechanisms of Nanoscale Silver Toxicity

Group Members: Participants D, O, and R

3.2.7.1 *Group Summary*

Short Description of Theme:

A wealth of information exists on the mechanisms of toxicity of silver ions (Ag^+). However, little is known about the mechanisms by which nano-Ag induces toxicity in living organisms. This is of great importance due to the increase in the commercial and industrial use of nano-Ag in various products and the potential for increased exposure risks. Based on the current state of knowledge, there is a need for studies that address the following research topics related to mechanisms of toxicity of nano-Ag:

- Role of Ag^+ on nano-Ag toxicity
  - Are the effects observed for exposure to nano-Ag due to Ag^+ desorbed from the nanoparticles or the nanoparticles themselves?
  - Can nano-Ag generate Ag^+ in vivo?
  - Are the mechanisms of toxicity similar among conventional Ag, Ag^+, and nano-Ag?
- Role of physicochemical properties of nano-Ag on toxicity
  - How do different physicochemical properties (size, surface coating, surface area, etc.) affect the mechanisms of toxicity of nano-Ag at the cell, organ, and whole-animal levels?

Why is this research theme of high importance?

The need to understand the mechanisms and factors governing the toxicity of nano-Ag is of prime importance for assessing risk, decreasing the impact to non-target species, and proposing remedial actions when required.

Where does this research theme fit within the CEA process?

Mechanisms of toxicity of nano-Ag should be an integral component of any CEA framework process. For instance, during the lifecycle stages, the mechanism of action of the particles will impact how the product is manufactured to minimize adverse human exposure an environmental effects. Mechanisms of toxicity will also be impacted by external factors, such as water chemistry and presence of other contaminants. Finally, knowing the mechanism of toxicity of nano-Ag will help us understand the biological effects from the subcellular organism to the population levels.

How would answering the research questions under this theme directly support or relate to a future CEA of nanomaterials?

Conducting research on the mechanisms of toxicity of nano-Ag will help develop new and improved laboratory assays that could be applied to other types of metal-based nanoparticles. It will also help determine key issues related to toxicity that should be considered when testing other types of nanoparticles.

For each of the research questions under this theme, indicate whether it is relevant to (1) a specific application of nano-Ag, (2) all applications of nano-Ag, or (3) nanomaterials in general (not only nano-Ag).

- Role of Ag^+ in Ag-nanoparticle toxicity: Bullets 1 and 2 apply. And Bullet 3 applies only to metallic-based nanomaterials.
- Role of physicochemical properties of nano-Ag in toxicity: Bullets 1, 2, and 3 apply.

What challenges might arise in answering the research questions under this theme (e.g., from a technical, policy, or social perspective)?

The major challenge is the current inability to quantify the total amount of Ag^+ absorbed to the particles in nano-Ag products or formulations and in biological/environmental matrices. Another challenge is the inability to quantify the amount of nano-Ag that gets oxidized to Ag^+ inside the cell.

How are the research questions under this theme related to other top priority themes or questions?

Our research questions are related to the following themes: analytical methods, test methods, exposure and susceptibility, physicochemical toxicity, kinetics, surface characterization, sources and release, comparison between conventional and nanoscale silver, biological effects, ecotoxicity, communication, and fate and transport.

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

- If the mechanism of toxicity is primarily due to absorption of Ag^+ during the production phase, this could be addressed through changes/modifications to the manufacturing process.
- If the physicochemical properties of nano-Ag are found to affect the mechanisms of toxicity, without influencing the efficacy of the spray disinfectants, then steps could be taken to ensure that a safer nano-Ag product is generated.
- An understanding of the mechanisms of toxicity could lead to the development of manufacturing standards and exposure limits.

3.2.7.2 *Group Presentation Slides*

Short Description of Priority Theme:

Based on the current state of knowledge, studies are needed to address the following research topics related to mechanisms of toxicity of nano-Ag:

- Role of Ag^+ on nano-Ag toxicity
  - Are the effects observed for exposure to nano-Ag due to Ag^+ desorbed from the nanoparticles or the nanoparticles themselves?
  - Can nano-Ag generate Ag^+ in vivo?
  - Are the mechanisms of toxicity similar among conventional Ag, Ag^+, and nano-Ag?
- Role of physicochemical properties of nano-Ag on toxicity
  - How do different physicochemical properties (size, surface coating, surface area, etc.) affect the mechanisms of toxicity of nano-Ag at the cell, organ, and whole-animal levels?

Why is this research theme of high importance?

The need to understand the mechanisms and factors governing the toxicity of nano-Ag is of prime importance for:

- Assessing risk
  - Identifying critical components related to the CEA framework
  - Concentrate on most sensitive populations for exposure

- Minimizing the potential impact to non-target species
  - Eliminating unintentional effects to consumers and product users
  - Assuring product effectiveness
- Proposing remedial actions when required
  - Changes in manufacturing protocols
  - Worker protection guidelines
  - Recommendations for consumer use

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

- If the mechanism of toxicity is primarily due to absorption of Ag^+ during the production phase, this could be addressed through changes/modifications to the manufacturing process.
- If the physicochemical properties of nano-Ag are found to affect the mechanisms of toxicity, without influencing the efficacy of the spray disinfectants, then steps could be taken to ensure that a safer nano-Ag product is generated.
- An understanding of the mechanisms of toxicity could lead to the development of manufacturing standards and exposure limits.

3.2.7.3 *Presentation Notes*

Presenter: Participant D

- Role of silver ions on nano-Ag toxicity (e.g., toxicity of nano-Ag may be largely due to release of silver ions)
- Concerned at cell versus organ versus whole animal levels
- Had broad discussion incorporating both human and eco
- Intended to be toxic to bacteria, but what are off-target effects?
- Mechanism would affect worker guidelines, etc.
- Mechanism of toxicity – if due to silver ions during production phase, that would impact manufacturing process

3.2.7.4 *Questions and Answers*

- Participant F: Did your group talk about dosimetry at all? Is there a different MOA at lower/higher concentrations?
  - Participant R: This is not captured here, but it is discussed in the biological effects questions.

3.2.8. Ecotoxicity Test Methods

Group Members: Participants H, K, and M

3.2.8.1 *Group Summary*

Short Description of Priority Theme:

We considered questions 6.16 and 6.6.[2]

- Are the current tests for evaluating ecological receptors scientifically relevant and acceptable for regulation of nano-Ag substances/products? (6.16)
- What assays should be incorporated into a harmonized test battery for determining ecological effects of nano-Ag? (6.6)

Why is this research theme of high importance?

Releases of nano-Ag to the environment have occurred historically, are occurring, and are expected to increase as production expands.

There are novel properties of nano-Ag products, notwithstanding century-old use of nano-Ag, and these pose unknowns with respect to impacts to ecological receptors. Early studies reveal toxic responses following exposure to aquatic and terrestrial receptors. However, the methods have been challenged because they were designed for testing bulk substances and might not capture the unique behaviors of nano-Ag.

The critical physical-chemical properties that must be considered in toxicity tests methods include those pertaining to kinetics of dissolution, bioavailability, etc. Perhaps the most unique feature is the possibility that the nano-particle might function as a proximal delivery source of free ions at the surface of or in the cell, and not just as a concentration of free ions distributed in the test matrix.

There are gaps in conventional standardized test methods that can be picked up for nanomaterials. In particular, there are opportunities to move to population-, community-, and system-level methods. Candidates include mesocosms to explore secondary or cascade effects, multigeneration tests to explore transition to F1 and F2 generations, and system processes (e.g., nutrient cycling).

Where does this research theme fit within the CEA process?

Toxicity testing is used to measure the capacity of a test substance to have an adverse effect on organisms. It is used to obtain a hazard classification. The most important function pertaining to the CEA matrix is that surrogate organisms (the test subjects) integrate the exposure parameters to test substances (i.e., bio-accessibility, bioavailability) and effects. These data are the basis for completing the finals stages in assessing risk.

Contemporary toxicity test methods are typically limited to organism-level effects. The emergent properties of populations, communities, and ecological systems are not captured in the current tests.

[2] Question 2.8 was in this initial group, but the Breakout Group elected to delete it as it was functionally equivalent to the other two questions; the aspect of F1 impacts offered to 6.16 was incorporated in the response to question 1; N1 pertaining to climate change was also addressed in the response to Question 1.

How would answering the research questions under this theme directly support or relate to a future CEA of nanomaterials?

Test methods will be required for any substance and therefore will be useful for all future CEA efforts. The details of any test will likely require modifications that consider unique behaviors of test substances, not unlike what is needed in conventional testing of volatiles, semi-volatiles, organics, and metals.

Having consensus-based test methods improves the confidence end users will have and generates acceptance of the results among affected stakeholders.

For each of the research questions under this theme, indicate whether it is relevant to (1) a specific application of nano-Ag, (2) all applications of nano-Ag, or (3) nanomaterials in general (not only nano-Ag).

The basic methods will be applicable to all nanomaterials. Nevertheless, the specific modifications needed for characterization of particles before, during, and after the exposure period in the test will likely differ for each class of nanomaterial. Importantly, guidance can be developed as to which parameters should be modified to maintain the integrity of test protocols.

What challenges might arise in answering the research questions under this theme (e.g., from a technical, policy, or social perspective)?

There are several major challenges in the design of test methods for nanomaterials. These include:

- Selecting the test volume required for nominal performance of test organisms (conventional test procedures might be prohibitively expensive because of the cost of the test substance);
- Selecting the appropriate test concentrations that capture both biological response and realistic, possible environmental concentrations;
- Maintaining a homogeneous distribution of the test substance for the duration of the test;
- Characterizing the relevant physical and chemical parameters of the test substance;
- Choosing the most relevant response endpoints (e.g., are the conventional endpoints of growth, survival, reproduction sufficient to capture relevant impacts from nanomaterials); and
- Ensuring safety of laboratory technicians and others coming into contact with the test substances.

One emerging theme of responses seen in toxicity tests is the “bathtub” response surface. As the concentration of the test substance increases, aggregation/agglomeration effectively removes the test substance from the test matrix. This means that traditional range-finding approaches that test high concentrations are prone to giving false negative results. This also introduces challenges for chronic exposures as the aggregated/agglomerated material can subsequently revert to behaving merely as bulk material or be retrained into the media as nanomaterial at some future time.

How are the research questions under this theme related to other top priority themes or questions?

- Prelude to understanding mechanisms of action
- Relevance for and understanding of the importance of environmental concentrations
- Biological effects that might occur in the environment (useful in risk assessment)
- Communication and engagement – tied back to acceptance of standardized terminology

- Acquired/adaptive tolerance

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

Relevant data will be developed through these tests. There will be more evidence to take regulatory action. The development of population-, community-, and system-level test methods will make possible answering the higher level policy concerns that occur at these levels of ecological organization (i.e., not relying on inappropriate extrapolations from organism-level to systems impacts).

3.2.8.2 *Group Presentation Slides*

Short Description of Priority Theme:

- Are the current tests for evaluating ecological receptors scientifically relevant and acceptable for regulation of nano-Ag substances/products? (6.16)
- What assays should be incorporated into a harmonized test battery for determining ecological effects of nano-Ag? (6.6)
- (2.8 deleted because it merely restates what is in 6.6 and 6.16; N1 Climate Change link addressed in response to Question 1.)

Why is this research theme of high importance?

- Releases of nano-Ag to the environment have occurred historically, are occurring, and are expected to increase as production expands.
- Early studies reveal toxic responses following exposure to aquatic and terrestrial receptors. However, the methods have been challenged because they were designed for testing bulk substances and might not capture the unique behaviors of nano-Ag.
- There is a possibility that the nanoparticle might function as a proximal delivery source of free ions at the surface of or in the cell, and not just as a concentration of free ions distributed in the test matrix.
- There are gaps in conventional standardized test methods that can be picked up for nanomaterials. In particular, there are opportunities to move to population-, community-, and system-level methods.
- Candidates include mesocosms to explore secondary or cascade effects, multigeneration tests to explore transition to F1 and F2 generations, and system processes (e.g., nutrient cycling).

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

- Relevant data will be developed through these tests.
- There will be more evidence to take regulatory action.
- The development of population-, community-, and system-level test methods will make possible answering the higher level policy concerns that occur at these levels of ecological organization; (i.e., not relying on inappropriate extrapolations from organism-level to systems impacts.

3.2.8.3 *Presentation Notes*

Presenter: Participant M

- Humans are another set of the ecosystem, so we create artificial silos by separating them.
- Considering these questions is a lot of work. There was a workshop at Clemson last year, and Rick Cannady led one group. They generated about a 40-page submission that should be out soon.
- There needs to be some focus on making sure there is an added level of ensuring clean glassware and eliminating contaminants. Also consideration of different endpoints, including 'omics.
- Current methods are being challenged because of inability to measure what is there and we don't capture the unique behavior of nano-Ag.
- Trojan Horse – it's possible that there is a mechanism that is unique to nano and possibly nano-Ag.
- A postulated additional function is that the nano-Ag gets out through the membrane or internally and then becomes a concentrated source of free ions that would have a disproportionate effect if one were to normalize concentration across the matrix.
- Tox tests largely focused on individual organisms and because there are emergent properties of communities and ecosystems, we make an error in assuming that we can multiply the number of organisms and have a population.
- With respect to animal systems, we are getting fewer numbers of test organisms, so the power of tests is going down, making it increasingly difficult, especially for vertebrates.
- There are some candidate methods – standardized mesocosm tests. But also there are some problems – we don't always get same result, which is not surprising. That is the nature of an ecological system. Also, they are expensive, and we often have to minimize effort and cost, which minimizes results.
- Also looking at 'omics – we should look at F1 and F2. Modifications can translate into second and third generations.
- One thing we are learning more about in nanotox – concentration on x-axis is response only. If we challenge, we get a reduction in growth or survival, but once we get that response where everything falls out biologically, it should get worse. But what we find with chemistry is that, at a certain point, we get agglomeration/aggregation and settling out, and the response goes down as concentration increases, that is, a bathtub curve.
- Try to do a limit test. If you go to the far ends and test 0 and a high concentration, you will get a false negative. If you look at lower concentrations, though, you will get a response.
- The problem is: This relationship is not locked down. The nano-Ag that settles out or reacts to become unavailable might become available again at some point, but in what form?
- There is information to take regulatory action, but this would provide more evidence.

3.2.8.4 *Questions and Answers*

- Participant F: I like the representation of dose-response for nano. A traditional dose-response doesn't happen all the time – it's sometimes an interesting curve. Some nanomaterials do follow a linear or exponential curve, so we can't make assumptions.
- Observer 3: I want to follow up on testing at environmentally relevant doses. There is also an issue of extrapolating for human health and for stress at high levels. Did you think about low-dose testing?
  - Participant M: Bioassays were developed because we could not measure in environmental media, but we could elicit responses in the assay. Also without the bioassay, we don't know what it means to have any particular concentration in the environment. The way we arrive at reference values is to find a point that is biologically relevant, which is not necessarily something you could detect. I think we can do a lot with nominal concentrations, but the need for detection and characterization is also great, but we don't need to wait for it.
- Observer 4: Dispersion – the way we prepare the test system has a big impact, but I have not seen this issue addressed. We normally buy a powder and sonicate or stir, which can affect the results.
  - Participant M: Excellent point, we should add it in.
  - Participant S: The levels we see it toxic at are down in the ppb range. We can detect it, but only as silver, so we don't know much about the particle characteristics. For gold, we can see that the nano aspects are preserved throughout the assays, but we are using much higher doses.
- Question: What kind of test methods do you use to look at algae? Population dynamics or growth? Seems very broad.
  - Participant M: Standardized methods traditionally look at growth and cell size. In effect, that is population, not like other things that are single organism. One can measure chlorophyll content or effectiveness. There are five or six major groups of algae used in standard tests.

3.2.9. Is New Nano Unique?

Group Members: Participants B, F, Q, and T

3.2.9.1 *Group Summary*

Short Description of Priority Theme:

This theme addresses the fundamental question of size, morphology, and surface. Are new nanoparticles unique from bulk, ionic, and colloidal particles that have been commercially available for some time? For some nanomaterials, we should recognize that there are differences, for others there might not be. By using modern analytical tools and bioassays we can determine systematically which nanomaterials might pose a new risk.

Why is this research theme of high importance?

The historical information about colloidal silver can be applied to developing the CEA of nano-Ag. However, there is a need to resolve terminology issues. In addition, because there is already established data and risk assessments, research priorities and available funding can be used in a more efficient way,

while not minimizing efforts in validating or confirming results. This is a relatively financially inexpensive and time saving way to relate historical data and risk assessments to nano-Ag. For example, materials used in a previously conducted study that involved the avian toxicity effects could be characterized today with modern techniques and instrumentation in an effort to bridge results to fill knowledge gaps.

Specific to the theme of this group, that is, is new nano-Ag unique, silver nanoparticles can be produced into highly monodispersed suspensions, with unique surface coatings and treatments, and in different morphologies and shapes.

Where does this research theme fit within the CEA process?

The CEA should capture the historical database. Although new investigations can and should be initiated, it is important to identify specific experiments that address the question: Are we investigating a new material that is fundamentally different or the same materials with unique properties?

A supplemental research question is: Are new nano-Ag applications unique? This fits into the CEA lifecycle stages. New applications can be in a different form, format, or use pattern than historical applications of colloidal silver.

How would answering the research questions under this theme directly support or relate to a future CEA of nanomaterials?

A cross comparison of already established CEAs of:

- Similarly functioning material to the new nanomaterial
- Colloidal particles to nanomaterials
- Ions, including salts, to new nanomaterials
- Bulk materials to the new nanomaterials

However, EPA acknowledges that there are no complete CEAs. There is an urgent need to complete a comprehensive environmental assessment if EPA insists that this should be done for nanomaterials. Otherwise, EPA should support the concept of simply requiring a more traditional risk assessment.

For each of the research questions under this theme, indicate whether it is relevant to (1) a specific application of nano-Ag, (2) all applications of nano-Ag, or (3) nanomaterials in general (not only nano-Ag).

- For comparisons between other antimicrobial materials and new nano-Ag, it is specific to an application of nano-Ag.
- For comparisons between ionic silver and new nano-Ag, this is applicable to all soluble metal-based nanomaterials.
- For comparisons between bulk silver and new nano-Ag, this is applicable to all nanomaterials.

What challenges might arise in answering the research questions under this theme (e.g., from a technical, policy, or social perspective)?

- Technical challenge: Engagement of investigators to do a thorough literature review that compares their new materials with existing materials in an effort to put into context what is known and where the knowledge gaps are.

- Technical challenge: Overcoming terminology misconceptions. For example, the definition of colloids is different to not only different scientific disciplines, it is often misconceived in communication (i.e., between scientists and from science to general).
- Technical challenge: Resolving the poor characterization of the historical data relative to the better characterization of today's materials.
- Social challenge: If new nano is unique, and we pursue nanotechnologies, it is important to establish manufacturing methods and processes that are environmentally friendly.
- Policy challenge: Because there is an outstanding question of whether new nano-Ag is unique from other forms of silver, a debate in regulatory actions is ongoing.
- Social challenge: How do scientists begin a conversation of cost-benefit analyses with the public? For example, the health effects of salmonella vs. the health effects of nano-Ag.

How are the research questions under this theme related to other top priority themes or questions?

- Test methods development for human and mammalian systems
- Test methods development for ecological systems
- Test methods development for analytical methods
- Surface characteristics
- Ecological toxicity
- Fate and transport
- Mechanisms
- Biological Effects
- Physicochemical & toxicity
- Kinetics I
- Kinetics II
- Exposure/Dose
- Exposure/Susceptibility.

The historical data and risk assessments do inform all of these other themes. However, emerging surface coating technologies have the potential to influence these themes.

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

Emerging surface coating technologies can influence toxicity, mechanisms of action, persistence, fate and transport, half-life, and lifecycle stages.

Another consequence that could be avoided is the misapplication of resources (e.g., funding, time, reagents, efforts, animals).

3.2.9.2 *Group Presentation Slides*

Short description:

- This theme addresses the fundamental question of size, morphology, and surface. Are new nanoparticles unique from bulk, ionic, and colloidal particles that have been commercially available for some time? For some nanomaterials, we should recognize that there are differences, for others there might not be. By using modern analytical tools and bioassays, we can determine systematically which nanomaterials could pose a new risk.

Why is this research theme of high importance?

- The historical information about colloidal silver can be applied to developing the CEA of nano-Ag. However, there is a need to resolve terminology issues. In addition, because there is already established data and risk assessments, research priorities and available funding can be used in a more efficient way, while not minimizing efforts in validating or confirming results.
- This is a relatively financially inexpensive and time saving way to relate historical data and risk assessments to nano-Ag.
- Specific to the theme of this group, that is, is new nano-Ag unique, silver nanoparticles can be produced into highly monodispersed suspensions, with unique surface coatings and treatments, and in different morphologies and shapes.

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

- Emerging surface coating technologies have can influence toxicity, mechanisms of action, persistence, fate and transport, half-life, and lifecycle stages.
- Another consequence that could be avoided is the misapplication of resources (e.g., funding, time, regents, efforts, animals).

3.2.9.3 *Presentation Notes*

Presenter: Participant T

- I was not able to find any historical discussion in the TiO_2 document.
- This particular case offers a unique history that lets us ask this question.
- As described, you need to pull away some of the definitional soup that exists when you look at fields touching nano.
- Focus on size, morphology, and surface.
- Are new nanomaterials unique compared to colloidal, bulk, and ionic?
- Colloidal definition here is very different for eco and material scientists.
- There will be differences between historical and now – need to be well past using MSDS (material safety data sheets) for graphite for carbon nanotubes.
- Can't sideline relevant data that can inform research activities and priorities.
- Need to establish if new materials pose new risk.
- Historical data can be applied to CEA; however, there is a need to resolve terminology.
- Get a better sense of size ranges of historical products – what was once called colloidal might very well be nano.

- Strong database for silver –important with limited funds and resources to use it efficiently. Need to tease out new things to apply new dollars.
- Existence of historical materials does not substitute for current and future assessments. They can augment and inform them, though.
- Avian toxicity data – electron microscopy on test material, 7 to 10 nm, commercially available for a long time.
- Need to look at what is being commercialized.
- What are the things that were understood and weren't unique. Citrate-capped particles have been around for some time.
- Availability of unique surface coatings can change properties where we've made advancements, and we may not be able to draw from historical data to understand new morphologies.
- What are emerging surface coatings? They might have highest probability of unintended consequences.
- Opportunity cost – if information is available, we can bridge historical data and populate box in terms of what is known.
- Animal testing – if we can go back and characterize what previous studies have been tested for, we can bridge more easily and reduce costs.

3.2.9.4 *Questions and Answers*

- Participant F: One thing this group was passionate about – apples to oranges comparison of using historical data – there is no historical CEA to compare to new CEA.
  - Participant T: As you look at risk/benefit, it is difficult to compare two materials that have had risks assessed in two different ways. For example, to compare Clorox to nano-Ag using CEA, you couldn't because neither CEA exists. A traditional risk assessment with what we know currently, even using high uncertainty factors and precautionary measures, could help us do apples to apples comparisons.
- Participant S: In working with nano, do you see greater antimicrobial properties relative to other antimicrobials?
  - Answer: Everything is formulation dependent, but if you try to set them to be comparable, we don't see heightened efficacy over ions, but you see a more durable effect. A primary concern in hand sanitizers in medical community is lack of persistence. For someone who develops those for that environment, there is an interest there. AgCl also has similar release rates, but has photosensitivity issues. So heightened efficacy, no, but persistence, yes.
- Observer 2: Is it an implication that you would place more emphasis on characterization and relating older colloidal data over doing more effects studies?
  - Answer: I think these can affect effects data, but I'd hate to pick. I think you might prioritize the data mining approach over a more expensive approach. Also, we don't want to plow the same field twice when we can use data that already exist (e.g., photographic industry). I think it has to be considered sooner rather than later, but I don't want to say to ignore more effects work until historical data are mined.

- Question: Did you talk about how to characterize the historical data? There is so much emphasis on characterization.
  - Answer: Great point – the literature sometimes gives particle size, but several materials have been on market in continuum since the 1920s. Argyrol was used in millions of households in the 1920s, and it predates FDA, and is still available in its original form. That material is in a time capsule – can test now and relate back to epi studies. You have some niche areas where you have some opportunity for this using the original products.
- Participant A: Evidence for antimicrobial resistance to silver – some studies showing resistance to $AgNO_3$. Have you looked at that, and what other microbes might have stable resistance? People seem to think resistance doesn't happen.
  - Answer: Good question – I recommend bringing some of the FDA medical device professionals who have been doing approvals on these medical products for years. They distinguish between resistance in lab and clinical relevance where it could be stable and relevant to public health.
  - Participant F: We recognize those themes of resistance and tolerance are important, and we have captured these in the Word document along with the importance of appropriate controls in efficacy, resistance, and toxicities.
- Participant N: I like this data mining approach, and there is a lot to learn from this. Colloidal silver includes nanosized particles. Those studies that are still being used for regulatory purposes for silver were done in the 1930s and are called epi but are not really epi. We don't know what they were exposed to even. Since these are still on the market, why don't we now do epi studies with the current tools?
  - Answer: Could not agree more – we are on shaky ground using a constellation of various animal studies to make assumptions about human health. We don't have some history, but we have this embedded experiment with drinkers of colloidal silver, and many of them are squarely within the 1- to 100-nm range and people drink them every day. I think this is a walking epi study. I think there is little scientific support that it will do all these wonderful things.
- Participant N: Yes, it is hard to reach the people being exposed, and it is particularly challenging given why people ingest silver. In my review of the historical data, there is no epi data and no characterization, but you need to go to different literature in the medical case study literature. Mining all of the literature could result in much relevant data.

3.2.10. Biological Effects

Group Members: Participants A, H, and R

3.2.10.1 *Group Summary*

Short Description of Priority Theme:

The biological effects of nano-Ag are mostly unknown. These effects are concerned with all living biological systems, including ecological and human. The following are the main research questions to address:

- What are the sensitive endpoints (e.g., subcellular, cellular, tissue, organism, or population level) for nano-Ag exposure?
- What are the relevant susceptibility factors (e.g., changes in genetic makeup)?
- What are the short-term and long-term responses observed at current occupational and consumer exposure levels?

Why is this research theme of high importance?

Data on biological effects are necessary to compile metrics to determine hazard identification as a first step. Then, hazard identification can be combined with exposure data, where available, to facilitate risk assessment. The biological effects data are important to be able to help set health benchmarks, such as NOEL (no observed effect level), NOAEL (no observed adverse effect level), WHEL (worker health exposure level) and MRL (maximum residue level), etc. Without this knowledge, an adequate risk assessment cannot be performed.

Where does this research theme fit within the CEA process?

Assays incorporating sensitive endpoints, susceptibility factors and data covering the full range of levels from subcellular to population are needed to be performed to obtain information on biological effects. Compiling this information helps to understand the magnitude of the concern for nano-Ag exposure. Biological effects data are necessary for completion of the CEA framework and set the groundwork for guidelines, regulations, and policies.

How would answering the research questions under this theme directly support or relate to a future CEA of nanomaterials?

The biological effects data are a key step in completing future CEA frameworks of nanomaterials. The information on biological responses is used to determine impact, the end of the theoretical spectrum of the CEA framework.

For each of the research questions under this theme, indicate whether it is relevant to (1) a specific application of nano-Ag, (2) all applications of nano-Ag, or (3) nanomaterials in general (not only nano-Ag).

Each research question outlined above is relevant to 1, 2, and 3.

What challenges might arise in answering the research questions under this theme (e.g., from a technical, policy, or social perspective)?

It is important to know biological responses relative to nano-Ag, not conventional Ag. It is also important to gather information on effects at the subcellular level up to the population level. Assays

need to be developed and included in the regulatory regimen, which observes epigenetic and generational effects.

It is necessary to gather information on sources and releases and exposure levels that can be compiled with biological effects to determine risk. In addition, the whole ecosystem needs to be taken into consideration because the most sensitive species might be affected but might not be the point where the most biological effect occurs. A species farther downstream (i.e., keystone species) might be the most appropriate due to its heightened response and the spread of this effect throughout the ecosystem.

How are the research questions under this theme related to other top priority themes or questions?

Biological effects research questions go hand in hand with mechanisms. It also relates ultimately with communication and education to be able to set guidelines for worker and consumer exposure as well as setting recommendations for consumer use.

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

By answering the questions really well, a better prediction of unintended consequences can be made. For example, studies with sensitive endpoints at various biological levels can lead to a more comprehensive picture of biological effects. Effects at the subcellular level might vary greatly from effects on the tissue or organ level.

In addition, investigations into susceptibility factors, such as changes in genetic makeup among individuals in the population, could provide a greater knowledge base to prepare guidelines for the general population and define which individuals might be more sensitive.

3.2.10.2 *Group Presentation Slides*

Short description:

- The following are the main research questions to address:
  - What are the sensitive endpoints (e.g., subcellular, cellular, tissue, organism, or population level) for nano-Ag exposure?
  - What are the relevant susceptibility factors (e.g., changes in genetic makeup)?
  - What are the short-term and long-term responses observed at current occupational and consumer exposure levels?

Why is this research theme of high importance?

- Data on biological effects are necessary to compile metrics to determine hazard identification as a first step. Then, hazard identification can be combined with exposure data, where available, to facilitate risk assessment.
- The biological effects data are important to be able to help set health benchmarks, such as NOEL (no observed effect level), NOAEL (no observed adverse effect level), WHEL (worker health exposure level) and MRL (maximum residue level), etc.
- Without this knowledge, an adequate risk assessment cannot be performed.

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

- By answering the questions really well, a better prediction of unintended consequences can be made. For example, studies with sensitive endpoints at various biological levels can lead to a more comprehensive picture of biological effects. Effects at the subcellular level might vary greatly from effects on the tissue or organ level.
- In addition, investigations into susceptibility factors, such as changes in genetic makeup among individuals in the population, could provide a greater knowledge base to prepare guidelines for the general population and define which individuals might be more sensitive.

3.2.10.3 *Presentation Notes*

- No separation of effects on human and ecological populations (this was done consciously). Focus was on "biological effects."

3.2.10.4 *Questions and Answers*

- Observer 2: It always concerns me when I see a list of examples that includes a bunch of endpoints. Which of those are the more important things to focus on or why?
  - Participant E: I think the answer you are looking for should be in test methods, and no one signed up for that.
  - Participant F: I don't think it could be one in particular. Our research shows that it is synergies of properties and endpoints. I don't think scientists can answer that question.
- Observer 2: To pick a strategy, we need to focus. Where would you focus?
  - Participant S: The previous point was interesting . The myth of the most sensitive species can be adapted to the myth of the most sensitive endpoint. Maybe it is not the most sensitive endpoint that we are looking for, but what is most important to explore – like effects on children, which we are not well equipped to do.
  - Sensitive future impacts that we have no way of seeing might be the most important
  - Participant R: in response to the question. If you have no idea what your test substance will do, you start with well characterized systems. You can start with in vitro to get a better idea to start with. Starting with in vivo would result in such a complex response, you wouldn't even know what you were looking at. Can't really use single endpoint assays to understand the total characterization – need multiplex. Avoid snapshot time or dose. Where we started was the logical place to start.
- Participant E: We are not even suggesting to do basic screening assays, just a mix of in vitro and in vivo. As far as I could tell from the case study, the assays we use for screening aren't even there – *E. coli* assays, cellular. What you have now is a potpourri across a huge list of materials. Start with any nanomaterial of whatever size, and do some basic studies for a couple time periods and concentrations and see what you get, because we haven't done that.
- Participant Q: that is the goal of the OECD effort. Use the same particles in different labs throughout the world looking at eco, mammalian, and in vitro systems. It is very slow – over a year ongoing, and we still have a lot to do. At FDA, we are trying to incorporate our work into that body by using the same particles. In total, we are doing many assays. Some are being repeated in different countries. It will be a long time coming – it does not move fast.

- Observer 5: We've been testing nano-Ag and nano-TiO_2, and we can see these cells with nanoparticles going to the cells, and sometimes going in using dark-field microscopy. You can use those images for risk assessment, but it is not widely used or accepted for some reason. There is some capability here.

3.2.11. Ecological Effects Required for Risk Assessment

Group Members: Participants D, M, and O

3.2.11.1 *Group Summary*

Short Description of Priority Theme:

Examining the current state of knowledge related to responses of ecological receptors to nano-Ag in the environment. The research questions under this theme are:

- Are there sufficient data to conduct an Ecological Risk Assessment (ERA) on nano-Ag?
- What information would improve the robustness and acceptance of the ERA related to freshwater, marine, and terrestrial systems?

Why is this research theme of high importance?

Based on the information presented in the nano-Ag disinfectant spray case study that focused on the CEA framework, we conclude that sufficient information already exists on Ag^+ that would allow us to perform an ERA on nano-Ag, though there would be large uncertainties. The greatest uncertainty regarding nano-Ag is related to the "Trojan Horse" mechanism that has been postulated as a unique aspect of this nanomaterial. However, there are other parameters for which the information could be improved to minimize the uncertainties, increase our confidence level, and gain broader acceptance of the risk characterization. Some of these parameters include developing:

- Analytical methods needed to verify the occurrence and concentrations of nano-Ag in environmental media
- Toxicity test methods needed to assess the effects of nano-Ag on ecological receptors
- Exposure scenarios
- A better understanding of the uniqueness of nano-Ag with respect to bulk and Ag^+
- Communication, engagement, and education programs related to nanomaterials.

These other sources of information are critical to address ecological effects. In particular, the next steps are to construct relevant conceptual models that depict routes of exposure, select the assessment species, the assessment endpoints, and translate these to data quality objectives and sampling and analysis plans.

Where does this research theme fit within the CEA process?

The ERA framework should drive the CEA framework. Currently, the CEA appears to assemble large quantities of data that might not be used for an ERA. The nano-Ag case study using the CEA framework focuses on collating data on the effects on generic representatives of sub-individual and individual organisms, and many other parameters. A formal ERA requires expansion to include exposure and effects assessments.

How would answering the research questions under this theme directly support or relate to a future CEA of nanomaterials?

If done first, the steps of problem formulation of an ERA would streamline the CEA framework by identifying parameters that will be needed for a regulatory determination based on ERA. This would avoid the unnecessary generation and gathering of extraneous data.

For each of the research questions under this theme, indicate whether it is relevant to (1) a specific application of nano-Ag, (2) all applications of nano-Ag, or (3) nanomaterials in general (not only nano-Ag).

- RQ1: Are there sufficient data to conduct an Ecological Risk Assessment (ERA) on nano-Ag?
  - Yes, this is relevant to all metal-based nanoparticles.
- RQ2: What information would improve the robustness and acceptance of the ERA related to freshwater, marine, and terrestrial systems?
  - Yes, this is relevant to all nanomaterials.

What challenges might arise in answering the research questions under this theme (e.g., from a technical, policy, or social perspective)?

The stakeholder engagement is the most frequently skipped part of problem formulation. Eliciting the values of affected stakeholders is not only the most difficult, but the most crucial for getting the questions of the ERA articulated. Without getting the right values, ERAs tend to miss what the public cares about. The role of the science and technical practitioners is to ensure that the stakeholders' needs and concerns are addressed. Often this requires a dialog between scientists and the other stakeholders regarding what is technically feasible and economically practical.

Another major challenge is encountered in translating organism-level toxicity information to population, community, or systems levels. This is because there are emerging properties of these higher levels of organization that cannot be inferred from lower level information.

How are the research questions under this theme related to other top priority themes or questions?

The research questions for ecological effects are related to

- Analytical methods
- Exposure and susceptibility (including fate and transport, kinetics, etc.)
- Biological effects (including humans)
- Nano uniqueness
- Communication, engagement, and education.

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

If an integrated holistic approach is used in performing the problem formulation stage, unintended ecological consequences would be minimized. This systems-based approach explicitly considers alternative scenarios, the results of which can be used in active adaptive management.

3.2.11.2 *Group Presentation Slides*

Short description:

- Examining the current state of knowledge related to responses of ecological receptors to nano-Ag in the environment. The research questions under this theme are
  - Are there sufficient data to conduct an Ecological Risk Assessment (ERA) on nano-Ag?
  - What information would improve the robustness and acceptance of the ERA related to freshwater, marine, and terrestrial systems?

Why is this research theme of high importance?

- Sufficient information already exists on Ag^+ that would allow us to perform an ERA on nano-Ag, although there would be large uncertainties.
- The greatest uncertainty regarding nano-Ag is related to the "Trojan Horse" mechanism.
- Other parameters could be improved to minimize the uncertainties, increase our confidence level, and gain broader acceptance of the risk characterization (on next slide).

Why is this research theme of high importance?

Some of these parameters include the development of

- Analytical methods for verification of the occurrence and concentrations of nano- Ag in environmental media
- Toxicity test methods needed to assess the effects of nano-Ag on ecological receptors
- Exposure scenarios
- Understanding the uniqueness of nano-Ag with respect to bulk and Ag^+
- Communication, engagement, and education related to nanomaterials.

This other information is critical to construct relevant conceptual models that depict routes of exposure, select the assessment species, and the assessment endpoints and to translate these to data quality objectives and sampling and analysis plans.

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

If an integrated holistic approach is used in performing the problem formulation stage, unintended ecological consequences would be minimized

This systems-based approach explicitly considers alternative scenarios, the results of which can be used in active adaptive management.

3.2.11.3 *Presentation Notes*

- Yes, there is sufficient data to conduct an ecological assessment for nano-Ag.
- If you use silver ion data, you can conduct a nano-Ag risk assessment.
- Several members were wondering why a risk assessment was not conducted as a continuation of the CEA document.

- By and large for aquatic, terrestrial, and human health, the existing information suggests risks are low.
- Exceptions are water quality impairment in certain places.
- Will have more dispersed exposure scenario in different places.
- Because of the Trojan Horse phenomenon, it would lend some uncertainty.
- There is enough information to begin to move forward.
- Uncertainty means "we don't know what we are talking about," but there are a lot of things that we know about silver – sulfide, for example, probably trumps many of the consequences.
- Need an integrated, holistic approach to help break down artificial barriers (human, eco, etc.), and make it a systems approach. If one starts with existing eco risk framework, it works through iteratively, the development of a conceptual model. The model helps in engagement and dialog with other stakeholders about what people care about and what we can do as scientists.
- It gets at Mike's question of what endpoint would be most useful.
- Tells us about precision, numbers of needed samples, relevant endpoints, relevant species, and from there the risk assessment is reduced to easy math.
- This procedure needs to be looked at the front end of the CEA. There are so many boxes that need to be filled, it can be paralyzing. Rather than using it as a way to paralyze the process, move forward rapidly and conduct a risk assessment identifying what we know and don't know.
- Begin with what is reasonable, based on what you know. Don't use lack of knowledge as an excuse not to move forward.

3.2.11.4 *Questions and Answers*

- Observer 2: What we want to do here is engage a broader array of perspectives that we typically don't get in a regulatory setting. How is what you are proposing different from what we are doing here? Also, we are struggling with how to define these questions. Typically in a regulatory approach, we ask clients what questions they want us to answer. This, on the other hand, is not paralysis by analysis at all, we just want to raise some to the top and make sure some don't drop off the table a priori. We want the whole array of questions we should consider and which should be high priority.
  - Participant M: This is useful in one context, but the flip side is that people run out and gather data. A lot of parameters in the CEA boxes are requesting data to fill them without having done the scenario analysis to decide what information one needs before running out to get the data. We ask the questions of what we need to make the decision and what we need to get that data. This can be a living, iterative process.

3.2.12. Communication, Engagement, and Education

Group Members: Participants E, J, K, L, and N

3.2.12.1 *Group Summary*

Short Description of Priority Theme:

- A research strategy should be developed to engage or involve consumers, workers, researchers and trainees in discussions to gather information about the uses, benefits, and risks of nano-Ag sprays. Such a strategy should be informed by the principles of community-based participatory research.
- A research strategy should develop methods (with metrics where appropriate) that improve the collaboration, information exchange, and communication among multiple disciplines.
- A research strategy should be developed to effectively communicate risk/benefit information for nano-Ag to the general public for the purposes of improving the quality of the information exchange

Why is this research theme of high importance?

Comprehensive Environmental Assessment cannot be considered comprehensive if relevant information about real-world industrial and consumer uses from relevant populations is not adequately considered. This information is gathered by active engagement with consumers, workers, and others about their interactions with silver nanomaterials and the products in which they are incorporated.

Building on recommendations from the National Academies in the area of *Science and Decisions: Advancing Risk Assessment (NRC 2009)*, which calls for formal provisions for external and internal stakeholder involvement at all stages of risk assessment, this research theme should be considered to be an integral part of CEA.

Where does this research theme fit within the CEA process?

Currently it is not explicit within the CEA Framework. However, it could be incorporated into Figure 1.1 as a cross-cutting input to each stage of the CEA analogous to the cross-cutting role of analytical methods development and application. There is precedent for this in the NRC recommendations to EPA in the document, *Science and Decisions*.

How would answering the research questions under this theme directly support or relate to a future CEA of nanomaterials?

It would improve the quality of the information inputs, such as the intended and unintended consumer products usage and real-world workplace contexts, as well as the transparency, credibility, and efficiency of the CEA process.

For each of the research questions under this theme, indicate whether it is relevant to (1) a specific application of nano-Ag, (2) all applications of nano-Ag, or (3) nanomaterials in general (not only nano-Ag).

Each research question is relevant to nanomaterials in general.

What challenges might arise in answering the research questions under this theme (e.g., from a technical, policy, or social perspective)?

- Changing the CEA process to include greater stakeholder participation.
- Traditionally, stakeholders have been separated from the decision making of the technical community, resulting in an undervaluation of stakeholder knowledge that is highly relevant to the CEA process.
- CEA is a complex process requiring specialized knowledge. Engaging non-technical communities is time-consuming, expensive, and complex.
- Mindsets of the technical community and non-technical communities are vastly different. Establishing common ground for discussions is a challenge and there are very few formally trained practitioners who can facilitate these processes.
- There are few to no incentives and encouragement to the technical community to engage with stakeholders.
- It is difficult to motivate stakeholders to be involved.
- There is often lack of trust between stakeholders and technical communities that impedes meaningful engagement.
- The Technical community is encouraged only to publish adverse results. As a consequence, unbalanced data are the only kinds available for CEA.

How are the research questions under this theme related to other top priority themes or questions?

Exposure and Susceptibility: Inadequate stakeholder engagement could result in incomplete understanding of exposures and susceptibility.

Sources and Release: Better understanding of stakeholder use of nano-Ag products could provide useful information about potential sources and releases into the environment.

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

More complete information about actual uses of and interactions with nano-Ag spray products would reduce the potential for unintended health impacts by

- Providing information for interventions by public health agencies
- Supporting the development of better consumer information, including product labeling by product manufacturers
- Enabling the development of better worker training tools and exposure controls
- Creating more informed environmental and public health policies to prevent harmful consumer exposures and environmental releases.

3.2.12.2 *Group Presentation Slides*

Short description:

- A research strategy should be developed to engage or involve consumers, workers, researchers and trainees in discussions to gather information about the uses, benefits, and risks of nano-Ag

sprays. Such a strategy should be informed by the principles of community-based participatory research.

- A research strategy should develop methods (with metrics where appropriate) that improve the collaboration, information exchange, and communication among multiple disciplines.
- A research strategy should be developed to effectively communicate risk/benefit information for nano-Ag to the general public for the purposes of improving the quality of the information exchange.

Why is this research theme of high importance?

- Comprehensive Environmental Assessment cannot be considered comprehensive if relevant information about real-world industrial and consumer uses from relevant populations is not adequately considered. This information is gathered by active engagement with consumers, workers, and others about their interactions with silver nanomaterials and the products in which they are incorporated.
- Building on recommendations from the National Academies in the area of *Science and Decisions: Advancing Risk Assessment (NRC 2009)*, which calls for formal provisions for external and internal stakeholder involvement at all stages of risk assessment, this research theme should be considered to be an integral part of CEA.

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

- More complete information about actual uses of and interactions with nano-Ag spray products would reduce the potential for unintended health impacts by
  - Providing information for interventions by public health agencies
  - Supporting the development of better consumer information, including product labeling by product manufacturers
  - Enabling the development of better worker training tools and exposure controls
  - Creating more informed environmental and public health policies to prevent potentially harmful exposures to humans and environmental organisms.

3.2.12.3 *Presentation Notes*

- Education has dropped out of it.
- We thought we were making a radical statement.
- We've tried to say that more communication and engagement needs to be associated with the CEA process. Need to ask researchers how this can happen.
- This tried to address the silos and that for something as complex as nano, need to cross-cut disciplines.
- If you are only talking about risks and not benefits, you are not getting to the level where people are making a conscious decision.
- At all levels of the CEA, there should be more engagement with stakeholders.

- Resistance of the technical community to have a dialog with the community in their area was immense.
- It is hard to do – time consuming and complex, and the mindsets are vastly different.
- Establishing common ground is difficult, and there is a lack of experienced facilitators.
- It might help in focusing research design.
- Difficult to motivate stakeholders to be involved.
- Often a lack of trust between stakeholders and technical communities.
- Technical community only encouraged to publish adverse results.
- If we could implement almost any part of this, it would help a lot.

3.2.12.4 *Questions and Answers*

- Participant N: We didn't purposefully drop education. It was sort of lumped into engagement because education is inherent in engagement.
  - Participant L: Quality of information exchange – want better input from external and internal stakeholders. All part of the process of understanding why you want information, why you think it is important, and how you will use it. A two-way communication.
- Participant F: Academic community – publish or perish. What is easiest to get published is hazard. All of us have the negative results that should be published as well, and we get grants based on publication record. In this particular thing, it is just important to publish the stuff that gives us no toxic effects and no exposure, etc.
- Participant L: I have seen negative results get into the literature as controls or comparative studies. They are not the part of the paper that is emphasized. This is a communication issue – a lot of the negative results are in the literature, just not emphasized.

3.2.13. Fate and Transport of Nano-Ag

Group Members: Participants I, P, U, and W

3.2.13.1 *Group Summary*

Short Description of Priority Theme:

Knowing the fate and transport is essential to understanding how nano-Ag gets to the point of release to its receptors, the different paths it takes, and the rates at which it moves through those different paths. By that, we mean it ultimately leads to prediction of exposure concentrations at the organism (both media concentration and target organisms), which is essential for ecological and human risk assessment.

Why is this research theme of high importance?

It would be desirable to have tools that are predictive of the kinetics of nanomaterial interactions in a wide variety of environments. Given the fact that every experimental or theoretical environmentally relevant scenario cannot be performed, we need to use models to breach these knowledge gaps. In particular, we need to be able to distinguish properties, and other aspects, that are specific to nanomaterials, and how these might be distinctive from classical models of contaminants.

Where does this research theme fit within the CEA process?

Identifying pathways of relevance/importance to human exposure and ecological receptors. Fate and transport fits under fate and transport in the CEA. External factors represent sensitivity factors for each of the models. By performing sensitivity analysis on the external factors, we can determine controlling mechanisms governing transformations, etc.

Oxidation, subsequent dissolution, and binding are an important part of fate and transport. Dispersion characteristics drive fate and transport. This builds on the fundamental chemistry and expands to relevant properties in the appropriate environmental compartments.

There is a feedback between the models and experiments.

How would answering the research questions under this theme directly support or relate to a future CEA of nanomaterials?

If we are to predict nano-Ag behavior, we need to know its properties, and interaction in the environment; otherwise, we will not have accurate or relevant exposures, assessments, or effects of different control strategies.

For each of the research questions under this theme, indicate whether it is relevant to (1) a specific application of nano-Ag, (2) all applications of nano-Ag, or (3) nanomaterials in general (not only nano-Ag).

All – having a model that predicts nano-Ag fate is applicable to nanomaterials in general; even if the constants are different, the concepts are useful. Nano-Ag and its particular properties can be very useful for modeling other nanomaterials, given the inherent complexity of dispersion and dissolution processes. This model could be useful for other nanomaterials that are known to exhibit more simplistic behaviors.

What challenges might arise in answering the research questions under this theme (e.g., from a technical, policy, or social perspective)?

Technical: Centered around difficulties of making measurements. Hard to find, very dilute in the environment, making validation of models very difficult. Also, to arrive experimentally at half-lives that are relevant for input into the models.

Policy: Because half-lives are tied in with so many aspects of the CEA, its implications are obvious. Fate and transport will represent the endpoints on which regulations will be crafted.

Social: Fate and transport are complementary to the source information, but important for communicating risk to the public. This information, however, is not communicated separately to the public, but lumped into the overall assessment.

How are the research questions under this theme related to other top priority themes or questions?

These questions are related to many of these themes. Kinetics needs to be run for the models, and vice versa. Surface characteristics help to provide basic tenets for physical modeling. They are related to exposure in identifying which routes are relevant, as well as material species, etc. Also, they are related to analytical methods as they provide an anchor for the models. Models can dictate the kind of measurements that need to be made.

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

They can be used as a predictive tool for policymakers, risk assessors, control strategists, material developers, etc. If we did not do this, we could easily miss an important pathway or route to exposure. The questions can help determine the relevant toxicological models and species that can be employed, such as benthic vs. aquatic. The questions can also help assess the efficacy of different control strategies.

3.2.13.2 *Group Presentation Slides*

Short description:

- Essential to understanding how nano-Ag gets
  - from the point of release to its receptors
  - the different paths it takes
  - the rates at which it moves through those different paths.
- Leads to prediction of exposure concentrations at the organism (both media concentration and target organisms), which is key for ecological and human risk assessment.

Why is this research theme of high importance?

- Desire to have tools that are predictive of the kinetics of nanomaterial interactions in a wide variety of environments.
- Given the fact that every experimental or theoretical environmentally relevant scenario cannot be performed, need to use models to breach these knowledge gaps.
- In particular, need to be able to distinguish properties, etc., that are specific to nanomaterials, and how these might be distinct from models for classical contaminants.

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

- Can be used as a predictive tool for policymakers, risk assessors, control strategists, material developers, etc. If we did not do this, could easily miss out on an important pathway or route to exposure.
- Can help determine the relevant toxicological models and species that can be employed, such as benthic vs. aquatic.
- Can help assess efficacy of different control strategies.

3.2.13.3 *Presentation Notes*

- Different pathways – soils, aerosols
- Fate and transport leads to exposure concentrations and species to which organisms are exposed
- Highly integrative
- Ability to develop particular tools – cannot experiment and develop experimental scenarios that are applicable to everything in the environment, so we need to generalize

- Use models with lots of constants and then we plug those into other models, which is how we get around having to chase a nanomaterial through compartments
- Helps comprehend data we have for other disciplines
- For people that deploy the technology, we can develop a predictive model for them to utilize, and they can add in some site-specific data and tailored kinetic data
- Good geochemical speciation models and surface complexation models exist, and these can be applied to tox models

3.2.13.4 *Questions and Answers*

- Where are we with fate and transport modeling?
  - Participant I: We are well off for conventional materials, but we need to think about how to apply them to nano. I am pessimistic about applying existing models to nano, but if we are smart enough and if we collect all the possible mechanistic data and calculate a rate constant for removal of parent material, I think we could do it, but it would require the additional step of developing a rate constant.
- Participant S: The prevailing models that are being looked at for stability of particles in water generally show us that ionic composition and pH will do things to the particles to make them settle out, etc., but we don't know the lifetime of the process and if they will become resuspended.
  - Participant P: Basic models deal with shifting of solutes between solid and solute phase, but we have two behaviors to track – particulate and dissolution behavior. Once they diverge, they interact in different ways. The colloid chemistry is probably the first approach, but we need more sophisticated and accurate colloid theory. Also, mathematics on colloid theory is terrible, and you need to know how to translate into the field, and then how to deal with the ionic factor. It is a substantial challenge.
  - Participant U: I was thinking about gas phase, and the models that exist now for organic materials can be adapted to nano if we know rate constants.

4. Other Workshop Documents

4.1. Workshop Agenda

All events in EPA conference room C111B/C unless otherwise specified.

11:30 AM	Shuttle from Hilton to EPA
3:30 – 4:00 PM	**Registration / Check In –** outside of EPA conference room C111B/C
4:00 – 4:45 PM	Introductions
4:00 – 4:15 PM 4:15 – 4:30 PM 4:30 – 4:45 PM	Purpose of workshop Review agenda and establish ground rules Brief participant introductions
4:45 –5:00 PM	Presentation of Pre-Workshop Ranking Results
5:00 –5:30 PM	Introduction of Nominal Group Technique (NGT)
5:30 PM	**End of Day 1**
6:30 PM	**Optional Group Dinner at Tyler's Tap Room** – meet in hotel lobby

7:30 AM 7:45 AM	Shuttles from Hilton to EPA
8:00 – 8:30 AM	**Participant and Observer Check In**
8:30 – 8:35 AM	Welcome Back
8:35 – 10:00 AM	NGT Round Robin Discussions – Round 1
10:00 – 10:30 AM	Break
10:30 – 12:00 PM	NGT Round Robin Discussions – Rounds 2 and 3
12:00 – 1:00 PM	Lunch – EPA Lakeside Café (on your own)
1:00 – 3:30 PM	NGT – Consolidation and Multi-Vote
3:30 – 4:00 PM	Break
4:00 – 4:30 PM	Discuss Vote Results
4:30 – 5:00 PM	Breakout Group Assignments
5:00 – 5:30 PM	Breakout Groups Meet (Topic 1) – Rooms C111B, C111C, and C113
5:30 PM	**End of Day 2**
6:30 PM	**Optional Group Dinner at Pop's Trattoria** – meet in hotel lobby

Day 3 – Thursday, January 6, 2011	
7:30 AM 7:45 AM	Shuttles from Hilton to EPA
8:00 – 8:30 AM	**Participant Check In**
8:30 – 12:00 PM	Breakout Groups Meet (Topic 1) – Rooms C111B, C111C, and C113 Groups take 30 minute break at their discretion.
12:00 – 1:00 PM	Lunch – EPA Lakeside Café (on your own)
1:00 – 1:30 PM	Breakout Group Assignments (Topic 2) – Room C111C
1:30 – 5:30 PM	Breakout Groups Meet (Topic 2) – Rooms C111B, C111C, and C113 Groups take 30 minute break at their discretion.
5:30 PM	**End of Day 3**
6:30 PM	**Optional Group Dinner at Nantucket Grill** – meet in hotel lobby
7:30 AM 7:45 AM	Shuttles from Hilton to EPA
8:00 – 8:30 AM	**Participant and Observer Check In**
8:30 – 8:35 AM	Welcome Back
8:35 – 10:30 AM	Breakout Group Presentations (Topic 1)
10:30 – 10:45 AM	Break
10:45 – 12:45 PM	Presentation of Breakout Group Results (Topic 2)
12:45 – 1:00 PM	Conclusion and Closing Remarks
1:00 PM	**Workshop Adjourns**

4.2. Workshop Participants and Observers

4.2.1. Participants

Mary Boudreau

Mary Boudreau is a Research Toxicologist in the Division of Biochemical Toxicology at the FDA National Center for Toxicological Research (NCTR). The Division conducts fundamental and applied research designed specifically to define the biological mechanisms of action underlying the toxicity of products regulated by, or of interest to, the FDA. Dr. Boudreau received a Ph.D. in Veterinary Medical Sciences with a major in toxicology from Louisiana State University and completed a post-doctoral program at the Pennington Biomedical Research Center. She was recruited by the NCTR in 2000 and has served as the principal investigator on three National Toxicology Program (NTP) studies, including the cosmetic ingredients aloe vera and retinyl palmitate, and the dietary supplement aloe vera. She has served as co-investigator on photococarcinogenicity studies of tattoo pigments and nanoscale titanium dioxide and zinc oxide. At present, she is the principal investigator on the NTP study of nano-Ag that is designed to evaluate the effects of particle size on the bioavailability, distribution, and toxicity in rats. She is manager of the FDA/NTP/NCTR Phototoxicology Laboratory Facility, a member of the NCTR Nanotechnology Working Group, reviewer for the Office of Women's Health, and has 24 years of research experience in applied biochemistry and nutritional toxicology.

Mark Chappell

Dr. Mark Chappell is a Research Physical Scientist and leader of the Soil and Sediment Geochemistry Team in the Environmental Laboratory, U.S. Army Engineer Research and Development Center (ERDC) in Vicksburg, MS. Dr. Chappell earned a Ph.D. in Soil Science from Iowa State University in 2004. He served as an Oak Ridge Institute Science and Education Postdoctoral Fellow with the U.S. Environmental Protection Agency before joining ERDC in 2007. One of Dr. Chappell's main research interests involves understanding the impact of humic materials on the dispersion stability of natural colloids. With respect to nanomaterials, he serves as a Co-Technical Lead for ERDC's research program into the ecological risk associated with these materials. Within that effort, Dr. Chappell heads up the investigations on the environmental fate of nanomaterials, leading studies into their dispersion stability and dissolution potential in natural aqueous systems.

Hongda Chen

Dr. Hongda Chen is the National Program Leader for Bioprocess Engineering and Nanotechnology in National Institute of Food and Agriculture (NIFA) of U.S. Department of Agriculture (USDA). He has represented USDA on the National Science and Technology Council (NSTC) subcommittee on Nanoscale Science, Engineering, and Technology (NSET) since 2001. Currently, he is a co-chair of the National Nanotechnology Initiative (NNI) Strategic Planning Task Force. He provides national leadership to develop, coordinate, and manage research, education, and extension programs in the areas of value-added novel products for food and nonfood applications. He has spoken frequently on nanotechnology for agriculture and foods at professional conferences, symposia, and strategic planning meetings both in the U.S. and internationally. He received his Ph.D. in engineering from University of California–Davis and served as professor of food engineering at the University of Vermont for more than 10 years before joining USDA/CSREES in December 2000.

Mary Jane Cunningham

Dr. Mary Jane Cunningham is the President and Founder of Nanomics Biosciences, Inc., a Delaware incorporated nanobiotechnology company in Cary, North Carolina. Nanomics Biosciences offers global screening services in genomics and proteomics for manufacturers and developers of a variety of substances, including nanomaterials. Dr. Cunningham received her B.A. degree with double majors in biology and chemistry from Case Western Reserve University and her Ph.D. in Physiological Chemistry from The Ohio State University. She completed postdoctoral fellowships in genetic toxicology at Haskell Laboratory of E.I. du Pont de Nemours & Co., Inc. and in prostate cancer and molecular biology at Stanford University. Dr. Cunningham worked in several biotech start ups in Northern California and was one of the first investigators to apply gene expression microarrays and proteomics to study the adverse effects of chemical compounds. She is an inventor on several gene expression patents and has presented and published on the use of OMICs technologies in toxicology. For the last seven years, Dr. Cunningham's research focus has been in applying OMICs technologies to predict the efficacy and safety of nanomaterials.

James Delattre

Dr. James Delattre is Vice President of NanoHorizons Inc., a spin-out of Penn State University and manufacturer of nanoscale materials based in Bellefonte, Pennsylvania. He received his bachelors with honors from Penn State University, where he studied inorganic chemistry. After working in electronic materials research at Novellus Systems, Inc. in Santa Clara, California, he earned his Ph.D. in Chemistry from the University of California–Berkeley with a focus on processes for reducing greenhouse gas emissions from semiconductor manufacturing and the development of new synthetic techniques for the solid state. Following postdoctoral work investigating the plasma treatment of polymers at the Consiglio Nazionale delle Ricerche at the University of Bari, Italy, Dr. Delattre joined the research team at NanoHorizons as Product Development Manager in 2005. He has presented at many international conferences and is the author of numerous peer-reviewed scientific and trade magazine articles. As a representative of NanoHorizons, he is active in the Silver Nanotechnology Working Group, a program of the Silver Institute, and the U.S. Silver Task Force.

David Ensor

Dr. David Ensor is a Distinguished Fellow at RTI International. He has a Ph.D. in engineering and an M.S. in chemical engineering from the University of Washington and a B.S. in chemical engineering from Washington State University. He has over 40 years experience in the field of aerosol science. His current research is in the area of nanofiber applications and nanomaterial characterization. Dr. Ensor is a member of the Homeland Security Subcommittee of U. S. EPA Science Advisory Board. He is Convener of the International Organization of Standards (ISO) technical committee (TC) 209 “Cleanrooms and associated controlled environments” working group (WG) 7 Separative devices and WG10 Nanotechnology. As a U.S. Delegate and Expert to ISO/TC 229 “Nanotechnologies” since the initial meeting in 2005, Dr. Ensor is active on WG1 Terminology, WG2 Metrology, WG3 Health, safety and environment, and WG4 Material specifications. He was President of the American Association for Aerosol Research (1988-1990) and is a Founding Editor of *Aerosol Science and Technology*. Dr. Ensor is an Adjunct Professor of Environmental Engineering at the University of North Carolina at Chapel Hill. He has over 85 peer reviewed publications, 7 patents, and over 200 presentations.

Michael Hansen

Dr. Michael Hansen, a Senior Staff Scientist with Consumers Union (CU), publisher of Consumer Reports, currently works primarily on food safety issues. He has been largely responsible for developing CU positions on safety, testing and labeling of genetically engineered food and "mad cow" disease. Since 2003, he has worked on a multi-state effort to ban the use of food crops to produce pharmaceutical drugs and industrial chemicals. He also represents Consumers International, a federation of more than 250 organizations in 110 countries, at Codex Alimentarius and other international fora on issues. Dr. Hansen speaks on CU's concerns on mad cow disease, GMOs, pest management, and antibiotics in animal feed, at meetings and conferences throughout the world. Dr. Hansen served on the USDA Advisory Committee on Agricultural Biotechnology from 1998–2002, and on the California Department of Food and Agriculture Food Biotechnology Advisory Committee, from 2001–2002. Dr. Hansen received his undergraduate degree from Northwestern University and his doctorate in ecology and evolutionary biology from the University of Michigan.

Carol Henry

Dr. Carol Henry is a Professorial Lecturer at the George Washington University School of Public Health and Health Services and a consultant to Society of Automotive Engineers (SAE) International. She advises organizations on issues in toxicology, risk assessment, public and environmental health, and sustainable green chemistry and engineering practices. She retired as Vice President, Industry Performance Programs at the American Chemistry Council in November 2007. Previously, Dr. Henry served as Director of the Health and Environmental Sciences Department of the American Petroleum Institute; Associate Deputy Assistant Secretary for Science and Risk Policy at the U.S. Department of Energy; Director of the Office of Environmental Health Hazard Assessment (OEHHA) at the California Environmental Protection Agency. She is Chair of the Federal Advisory Committee for the National Children's Study, Co-Chair of the Montgomery Country Maryland Water Quality Advisory Group, and President of the Chemical Society of Washington of the American Chemical Society. Dr. Henry received her undergraduate degree in chemistry from the University of Minnesota and doctorate in microbiology from the University of Pittsburgh. She is a diplomate of the American Board of Toxicology, certified in general toxicology.

Matthew Hull

Matthew Hull is President of NanoSafe, Inc., a provider of nanotechnology environmental health and safety services founded in 2007 and headquartered in Blacksburg, VA. In 2009, Hull co-edited the book *Nanotechnology Environmental Health and Safety: Risks, Regulation, and Management*. In 2008, Hull developed NanoSafe Inc.'s NanoSafe Tested™ program and companion Nanotech Register™, which provides independent verification of nanomaterials and nanotechnology products. Hull also holds an NSF IGERT fellowship in Civil and Environmental Engineering at Virginia Tech, where his doctoral research is focused on understanding the factors influencing partitioning of engineered nanomaterials in the environment. Previously, Hull served as Senior Research Scientist at Luna Innovations Incorporated, where in 2003 he developed the concept for the NanoSafe™ framework—an integrated approach for addressing nanotechnology environment, health, and safety issues in nanotechnology facilities. That framework has gone on to spin-off programs focused on web-enabled nanotechnology EHS management systems, nanotechnology waste recovery and recycling processes, and life-cycle ecotoxicological studies of nanomanufacturing. Hull has an M.S. in Biology from Virginia Tech and a B.S. in Environmental Science from Ferrum College in Virginia.

Ian Illuminato

Ian Illuminato is the Health and Environment Campaigner at Friends of the Earth U.S. and serves on the Executive Committee of Friends of the Earth International. At the international level he helps direct the work of more than one thousand employees and five thousand volunteers throughout the world as they foster solidarity and human/environmental justice in some of the planet's most vulnerable places. Ian's mandate at Friends of the Earth U.S. is to encourage the safe and precautionary management of nanotechnology. He has worked for Greenpeace Italy, Greenpeace International, and the United Nations Environmental Program in Italy and has extensive experience monitoring the impact of technological change on the environment. At Greenpeace he helped lead an international movement against genetically engineered crops in Europe and the Middle East. He persuaded Europe's largest rice company to stop importing American rice to keep its stock GM-free. He also works closely with the Campaign for Safe Cosmetics to remove toxins from beauty products. He has authored reports including, "Nano and biocidal silver: extreme germ killers present a growing threat to public health" and "Nanotechnology, climate and energy: over-heated promises and hot air?" His writing has appeared in publications including the *Journal of Nanoparticle Research* and the *European Journal of Oncology*. He has also appeared in numerous media outlets including the *New York Times*, *Scientific American*, *Business Week,* and *Reuters*. Ian has a Bachelor's of Arts degree in Human Ecology from the College of the Atlantic in Bar Harbor, Maine.

Larry Kapustka

Larry Kapustka (LK Consultancy) has been a leader in the field of environmental risk assessment since the late 1980s. He has held positions in academia, government and the private sector. Currently, he chairs the ASTM-I E47 Committee on Biological Effects and Environmental Fate, which is examining the efficacy of existing toxicity test methods to evaluate nanoscale products. He also serves as the Assistant Chair of the Society of Environmental Toxicology and Chemistry (SETAC) Nanotechnology Advisory Group. He has extensive publications in the fields of ecology, risk assessment, toxicology, and environmental management including a framework for addressing emerging issues in nanotechnology.

Bojeong Kim

Dr. Bojeong Kim is a postdoctoral associate in the Department of Geosciences at Virginia Tech. Her recent work has focused on the fate, transport, and transformation of engineered nanoparticles in the environment. Her latest research article "Discovery and Characterization of Silver Sulfide Nanoparticles in Final Sewage Sludge Products" published in *Environmental Science and Technology* (2010) has received much media coverage, including articles in *Chemical & Engineering News*, *Environmental Health Perspectives*, and a perspective paper in *Science*. She was also named as a 2010 Trendsetter by *Public Works Magazine* for her exceptional research on the environmental impact of silver nanoparticles. She holds a B.S. in chemistry from Sungkyunkwan University, an M.S. in chemistry from Seoul National University and a Ph.D. in environmental toxicology from Cornell University, where she studied long-term environmental perturbations in terrestrial ecosystems by anthropogenic activities.

Kristen Kulinowski

Dr. Kristen Kulinowski is a Faculty Fellow in the Department of Chemistry at Rice University and Director for External Affairs for the Center for Biological and Environmental Nanotechnology (CBEN). She currently serves as the Director of the International Council on Nanotechnology (ICON), an international, multi-stakeholder organization whose mission is to develop and communicate information regarding potential environmental and health risks of nanotechnology thereby fostering risk reduction while maximizing societal benefit. She has experience as a chemical researcher, educator, curriculum developer, administrator, outreach coordinator, and policy fellow. Since 2004, Dr. Kulinowski has been actively engaged in developing and promoting the International Council on Nanotechnology (ICON) which provides a neutral forum in which experts from academia, governments, industry and nonprofit organizations can explore questions of nanotechnology's impact on environment, health, and safety (EHS). She directed an effort that resulted in the web publication of the first publicly available database of citations to peer-reviewed papers on nano EHS. Other activities of ICON include a survey of best practices for nanomaterial handling in the workplace and a public portal of information on nanotechnology EHS. Dr. Kulinowski earned a B.S. in chemistry at Canisius College and her M.S. and Ph.D. in chemistry at the University of Rochester.

Debbie Lander

Dr. Debbie Lander received a B.S. in chemistry from McGill University and obtained a Ph.D. in physical chemistry from Rice University. She has served as a plant chemist for Exxon Chemical Company, and later worked in contamination control technologies at W.L. Gore. She used her field experience of analytical chemistry and process engineering to work as Chief Scientist at the chemical weapons neutralization facility in Maryland, maximizing safety of workers and preventing any chemical release into the environment. This led her to begin estimating exposures of chemical weapons to workers and the environment in order to enable the safe destruction of the facility and restoration of the environment. Afterwards, she joined DuPont as a risk assessor and became involved in exposure assessments for REACH. Over the last three years at DuPont, she has focused on exposure assessments for industrial chemicals. This requires an understanding of the use scenarios of workers, professionals, and consumers, in order to define potential routes of exposure to humans and the environment. She uses available data and modeling tools to estimate exposures and characterize risk by comparing exposure estimates to the developed health or environmental benchmarks.

Paul Lioy

Dr. Paul Lioy is a Professor and Vice Chair of the Department of Environmental and Occupational Medicine at UMDNJ-Robert Wood Johnson Medical School (RWJMS), Piscataway, N.J. He is Deputy Director for Government Relations at the Environmental and Occupational Health Sciences Institute (EOHSI) of Rutgers University and RWJMS, and Director of the Exposure Science Division. He is a member of EPA's Science Advisory Board was a member of the National Academy of Sciences Board of Toxicology and Environmental Studies, and was the Chair of the National Research Council's first committee on Exposure Assessment. Now Dr. Lioy is Vice Chair of the NRC Committee on Exposure Science in the 21st Century. He was a founder of International Society for Exposure Analysis (Science) and President from 1993-94. Dr. Lioy has published 255 scientific papers, and is identified by the *Information Science Institute* as a highly cited scientist in *Environment/Ecology*, and published the book *Dust: the Inside Story of its role in the September 11th Aftermath*. He is an Associate editor for *Environmental Health Perspectives*, and the *Journal of Exposure Science and Environmental Epidemiology*.

Brian O'Connor

Dr. Brian O'Connor obtained his Ph.D. in organic chemistry from McGill University, Canada in 1987 and started his career at FPInnovations (a Forest Products Research Institute) in 1988. He is currently Program Manager in charge of the Environment Research Program which covers a variety of issues of concern to the pulp and paper industry such as environmental assessment of new products, environmental impact in receiving waters, effluent treatment, and energy and resource recovery from solid residues. One new product that is being examined is nanocrystalline cellulose (NCC), a renewable nanomaterial that is formed from kraft cellulose pulp. An environmental assessment of NCC is currently being conducted in order to ensure its safe use in products for sale in Canada and the United States.

Maria Powell

Dr. Maria Powell is a community-based participatory researcher and community organizer with the Nanotechnology Citizen Engagement Organization and the Midwest Environmental Justice Organization (MEJO) in Madison, Wisconsin. She has a B.A. in biology from the University of California and M.S. and Ph.D. from the University of Wisconsin, Madison, both in environmental studies. From 2004–2009 she was a postdoctoral researcher with the University of Wisconsin's Nanoscale Science and Engineering Center and co-leader of the center's societal group. Her research focused on outlining factors that shape uncertainties about risks of nanotechnologies and in developing meaningful ways for citizens to engage with scientists and policymakers in decision-making on these issues. She has published numerous peer-reviewed papers on citizen engagement, nanotechnology risk assessment and policy, and environmental justice. She co-created the Madison Nano Cafés, which developed into NanoCEO. She also led a multidisciplinary team of local and state government scientists who collaboratively outlined potential environmental health risks of emerging nanotechnologies and developed strategies to proactively address them in the context of their agencies. Nanosilver became a priority issue for this team and in collaboration with NanoCEO they are currently analyzing levels and forms of silver/nanosilver released from several consumer products on the market.

Gurumurthy Ramachandran

Dr. Gurumurthy Ramachandran is a Professor of Industrial Hygiene in the Division of Environmental and Occupational Health in the School of Public Health at the University of Minnesota–Minneapolis. He conducts research in various areas relating to human exposure assessment in occupational and non-occupational settings. His recent research includes occupational exposure assessment for nanoparticles including the development of robust strategies and analyzing measurement data and the use of expert judgment in risk assessment for nanomaterials. Additional areas of expertise include retrospective exposure assessment using Bayesian methods for silica, asbestos fibers, and a variety of gases and vapors; occupational hygiene decision-making; and developing mathematical methods for exposure modeling and analyzing occupational measurements. The focus of these interests is the development of more effective and accurate methods to assess health-related human exposure. He has a Bachelor's degree in electrical engineering, a Master's degree in environmental engineering, and a Ph. D. in environmental sciences and engineering from the University of North Carolina.

Christie Sayes

Dr. Christie Sayes is a tenure-track Assistant Professor at Texas A&M University where she is principal investigator of the *Nanomaterials, Bioavailable Metals, & Toxicology Research Laboratory* in the Department of Physiology & Pharmacology and the Department of Biomedical Engineering. She completed a post-doctoral fellowship at the DuPont Global Centers Haskell Laboratory for Health and Environmental Sciences and is actively studying the health effects of various nanomaterials in *in vitro* and *in vivo* systems. She has made correlations between physicochemical properties and toxicological effects. Dr. Sayes has a Ph.D. in chemistry, specializing in nanoscience, from Rice University. She pioneered many cytotoxicity studies, including biocompatibility investigations with carbon, metal, and oxide nanomaterials to various *in vitro* systems. She is on the Executive Committees of Texas A&M Toxicology Program and Texas A&M Biotechnology Professional program. She is also a member of the Intercollegiate Faculty of Material Science and Engineering. She is a member of Society of Toxicology, Society of Environmental Toxicology & Chemistry, and American Chemical Society.

Maria Sepulveda

Dr. Marisol Sepulveda received a Doctorate in Veterinary Medicine in 1991. Between 1993 and 2000, she obtained a M.S. in wildlife ecology and a Ph.D. in veterinary sciences from the University of Florida. Her Masters was focused on evaluating the impact of mercury in great egrets from the Everglades. Her dissertation was centered around the effects of paper mill effluents on fish reproduction. She then joined the USGS Florida Integrated Science Center as a post-doctoral researcher where she studied the influence of chlorinated pesticides in growth and development of fish-eating birds and alligators. In 2004, she joined the Department of Forestry and Natural Resources at Purdue University as an Assistant Professor of Ecotoxicology and Aquatic Animal Health and was promoted to Associate Professor in 2009. Her laboratory conducts research evaluating the sublethal effects of a wide-range of environmental contaminants on the physiology of different species of aquatic animals. One major research area consists of developing molecular biomarkers of exposure and effects to pollutants. For that purpose, her laboratory utilizes “omic” approaches, including transcriptomics, proteomics, and metabolomics. In relation to nanotoxicology, her laboratory is working with nano-Ag and its effects on fish and aquatic invertebrates.

Brian Strohmeier

Dr. Brian Strohmeier has over 30 years experience in applied surface science. Brian holds a Ph.D. in analytical chemistry from the University of Pittsburgh and an M.A. in business leadership from Duquesne University. He is currently Manager of the Surface Analysis Laboratory at RJ Lee Group, Inc., an analytical services and consulting firm located in Monroeville, PA. Brian's interests involve applications of surface analytical and microscopic techniques for industrial problem solving, product/process development, and the characterization of complex materials. His expertise includes: X-ray photoelectron spectroscopy (XPS), Auger electron spectroscopy (AES), secondary ion mass spectrometry (SIMS), scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS), and various other analytical techniques. His technical experience includes the study of adhesion, corrosion, oxidation, and wetting phenomena; chemical and plasma modification of material surfaces; and the surface and microscopic characterization of asbestos and associated minerals, catalysts, ceramics, glass and fiberglass, metals, oxides, paints and coatings, polymers, semiconductors, and vacuum-deposited thin films. Dr. Strohmeier is the co-inventor of one patent and the author/co-author of 51 publications and 74 technical presentations (30 invited).

Michael Tolocka

Dr. Michael Tolocka received a Ph.D. in physical chemistry from George Washington University after earning a B.S. in chemistry from Fairleigh Dickinson University in Madison, New Jersey. He is an expert in spectroscopy, separation methods, and mass spectrometry techniques. Until 2001 he worked as a Physical Scientist for U.S. EPA comparing chemical composition of ambient aerosols in the Eastern and Southwestern United States. From 2001 to 2006 he conducted postdoctoral research at the University of Delaware as well as the University of North Carolina at Chapel Hill, where he was recently hired to develop an aerosol ion trap mass spectrometer for use in indoor air quality studies.

Dik van de Meent

Dr. Dik van de Meent is a senior scientist with the National Institute of Public Health and the Environment (RIVM) in Bilthoven, The Netherlands, where he has worked since 1982. He is leading expert in modeling of fate and ecological effects of toxic substances in the environment. He has contributed to this field by developing the multimedia fate model SimpleBox, which has become one of the pillars of the European Union System for Evaluation of Substances (EUSES). He is currently involved in research aimed at making EU exposure and risk assessment models suitable for predicting environmental fate of nanomaterials. Dr. Van de Meent obtained an Engineering degree in chemistry and a Ph.D. in environmental science, both from Delft University of Technology. He has taught Environmental Quality at Radboud University Nijmegen since 2004. He serves as an editorial board member for several scientific journals. He actively contributes to various professional organizations as a board member or meeting organizer. Dr. Van de Meent has adopted as his scientific mission to quantify the impact of toxic emissions on biodiversity and indicate how these impacts can best be controlled.

4.2.2. Observers

Observer	Affiliation
Lauren Barton	Duke
Jed Costanza	EPA/OCSPP/OPP/AD
Genya Dana	EPA National Center for Environmental Assessment
J. Michael Davis	EPA National Center for Environmental Assessment
Patricia Gillespie	EPA National Center for Environmental Assessment
Maureen Gwinn	EPA National Center for Environmental Assessment
Jaydee Hanson	International Center for Technology Assessment
Murray Height	HeiQ
Christine Hendren	EPA National Center for Environmental Assessment
Ross Highsmith	EPA National Exposure Research Laboratory
Michael Hughes	EPA
William Jordan	EPA Office of Pesticide Programs
Kirk Kitchin	EPA National Health and Environmental Effects Research Laboratory
Tom Long	EPA National Center for Environmental Assessment
Tara Lyons-Darden	NiPERA, Inc.
Jennifer McClain	EPA Office of Pesticide Programs
Dotti Miller	EPA Office of Research and Development
Eric Money	Duke
Christy Powers	Duke University
Alan Rae	NanoMech Inc.
Jo Anne Shatkin	CLF Ventures
Julian Taurozzi	National Institute of Standards & Technology
Mathieu Therezien	Duke
David Thomas	EPA
Thabet Tolaymat	EPA Office of Research and Development
John Vandenberg	EPA National Center for Environmental Assessment
Rosalind Volpe	Silver Nanotechnology Working Group
Debra Walsh	EPA National Center for Environmental Assessment
Ron White	Johns Hopkins Bloomberg School of Public Health
Robert Zucker	EPA National Health and Environmental Effects Research Laboratory

4.3. Pre-Workshop Charge to Workshop Participants

IMPORTANT: Please read this entire charge and the accompanying instructions before starting your review.

Introduction and Objectives

We request that you complete five tasks prior to the Nanomaterial Case Study Workshop:

1) Review *Nanomaterial Case Study: Nanoscale Silver in Disinfectant Spray*
2) Comment on the case study document
3) Rank potential research or information gap questions listed in the case study document
4) Add new questions or modifications of existing ones (optional)
5) Provide a biosketch

The *Nanomaterial Case Study: Nanoscale Silver in Disinfectant Spray* document is one step in the development of a research strategy for the comprehensive environmental assessment of nanomaterials such as nanoscale silver (nano-Ag). It serves as a starting point for the Nanomaterial Case Study Workshop. Prior to the workshop (by December 15th), please submit review comments and ranking of research questions (information gaps), as explained below. The preliminary ranking results will be provided at the workshop. Any new questions submitted by reviewers by December 15th will be distributed to workshop participants prior to the workshop via email and at the workshop.

The case study attempts to take a holistic view of a selected use of nano-Ag and the potential ecological and health implications of nano-Ag products across their life cycle. Although the case study report presents a great deal of information, many questions remain to be answered. Many of these questions, which can also be thought of as information or research needs, are listed throughout the case study report.

- The document is meant to stimulate thinking about potential release scenarios and implications, both direct and indirect. The case study is a starting point for your thinking, not an end in itself.
- A key aspect of the review is to identify and rank the research or information that is most needed to conduct a comprehensive environmental assessment of nano-Ag used in disinfectant sprays. Separate instructions for the ranking process are provided below and should be read *before* reviewing the document.

Instructions for Completing Pre-Workshop Tasks

Instructions are provided below for accessing the case study draft report, preparing comments on the draft report, preparing a brief biosketch, ranking research/information needs, and submitting all of your materials prior to the workshop. A checklist is provided at the end of this Charge to assist you.

Thank you for your thoughtful review and participation in this endeavor.

Accessing the Draft Case Study

You can download an Adobe PDF version of the document at the following Web URL:

http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=226723

If you prefer to have a hardcopy version, you can email a request to NanoWorkshop@icfi.com. ICF will send it to you by FedEx within three days of receipt of your email.

Reviewing the Draft Case Study

You are asked to read the entire document, not just your own areas of expertise or interest. We want reviewers to take a "big picture" view and not focus exclusively on a particular chapter or section. As you review the document, please consider this overarching question:

"**What research or information is most needed to conduct a comprehensive environmental assessment of nano-Ag used in disinfectant spray?**"

Preparing Comments on the Draft Case Study

In your review comments, please indicate:

1) Is the information presented in the document accurate, objective, and logical? Are statements properly supported by references? Note that we have by necessity had to rely on gray literature and personal communications at times. If you have better sources to cite for such information, please provide them.

2) Is information clearly and concisely presented? If not, please suggest alternative wording.

3) Is the information complete? Have any important points been omitted? Do you know of other information that bears directly or indirectly on the case study? Can you provide a source (e.g., a document, Web site, person) for additional information?

For comments on specific text in the document:

1) Please "triage" your comments for us by noting your 5 most important substantive comments.

2) Please indicate the specific document page and line number. For comments about the questions that are listed at the ends of chapters, please indicate the question number in your comment (e.g., 2.1, 5.10)

Email your review comments to NanoWorkshop@icfi.com by December 15, 2010.

Submitting Pre-Workshop Rankings

The research/information needs for conducting a comprehensive environmental assessment of nano-Ag in disinfectant spray are posed as questions at the end of chapters 2–6 in the draft case study.

You will need to identify what you perceive as the highest- and lowest-priority needs through the ranking system described below. You will record your rankings in an Excel workbook provided by ICF. **(Please read the detailed ranking instructions embedded in the first tab of the workbook.)** Within the Excel workbook, you will be asked to:

1) **Rank the top 10 needs:** Identify and rank the top 10 priorities by assigning a score of 10 to the question you believe is the most important of all identified, a score of 9 to the question you think is the second most important, a score of 8 for the third most important, and so on. In the workbook, select "Top ten, [priority number]" from the options in the dropdown list (e.g., "Top ten, 9; Top ten, 4).

2) **Identify 15 unranked high-priority needs:** Select an additional 15 questions you believe are among your 25 most important. (Your top 10 priorities selected in the previous step will automatically be included in this group.) In the workbook, select "High (unranked)" for these 15 questions.

3) **Identify the 10 lowest or "zero" priority needs:** Identify up to 10 questions that you believe are not important or are the lowest priority of all of the questions listed in the document. In the workbook, select "Low (unranked)" for these 10 questions.

Email your completed Excel workbook to NanoWorkshop@icfi.com by December 15, 2010.

Adding New Questions and Modifying Existing Questions

You also may submit new questions and revisions of existing questions. Any new research/information needs should be significantly and directly relevant to a comprehensive environmental assessment of nano-Ag. (Many interesting questions could be asked, for example, about uses of nanomaterials or about policies or regulations that could be applied to them, but these types of questions are outside the intended purview of this exercise.)

- Please add only questions that you would consider among your top-ranked issues. New questions will not be ranked in the pre-workshop rankings, but all new questions will be distributed in advance to the workshop participants, and participants will have an opportunity to discuss their highest priority issues (including new questions) during the workshop.
- You will need to type (or copy and paste) any new questions in the spaces provided on the third tab of the Excel workbook. You should identify the case study report chapter to which each question belongs (provided in a drop-down menu in the Excel worksheet).
- If you are modifying an existing question, please indicate the number of the original question, and enter the revised wording (otherwise select <none> if it is a new question). Please limit modifications to questions that are among your top 25. You should rank the original question if it is among your top 10.
- The Excel workbook can accommodate submittal of up to 10 new/revised questions, each with a maximum of 250 characters. If you have more than 10 new questions, please email your entire list to NanoWorkshop@icfi.com.

Preparing a Brief Biosketch

Please prepare a brief biosketch of up to 200 words. This information will be shared with the other workshop participants and will facilitate introductions and interactions.

Email your biosketch to NanoWorkshop@icfi.com by December 15, 2010.

Check List for Pre-Workshop Activities

By <u>December 15</u>:

___ Provide review comments on the draft case study report
___ Rank the questions (research/information needs) in the ICF Excel workbook
    ___ Top 10 highest-priority needs
    ___ Additional 15 high-priority needs
    ___ Lowest 10 priority needs

___ (Optional) Add new or modified questions

___ Provide a 200-word biosketch

Email all materials (completed Excel workbook, review comments, and biosketch) to NanoWorkshop@icfi.com.

4.4. Research Questions

Questions about Physicochemical Properties and Analytical Methods

2.1.	What information could be provided about the nano-Ag contained in spray disinfectants to enable adequate characterization of exposure routes and toxic effects?
2.2.	How can engineered nano-Ag particles be distinguished from incidental, background, or naturally occurring nano-Ag particles?
2.3.	Which physicochemical properties of conventional silver can be applied to nano-Ag?
2.4.	Does the morphology of nano-Ag determine the efficacy of use in spray disinfectants?
2.5.	How does surface coating affect: a. the physicochemical properties of nano-Ag? b. toxicity to humans or biota?
2.5.a.	How does surface coating affect the physicochemical properties of nano-Ag?
2.5.b.	How does surface coating affect toxicity to humans or biota?
2.6.	What physicochemical properties of nano-Ag can be used to: a. predict fate and transport in environmental media? b. predict toxicity to humans or biota?
2.6.a.	What physicochemical properties of nano-Ag can be used to predict fate and transport in environmental media?
2.6.b.	What physicochemical properties of nano-Ag can be used to predict toxicity to humans or biota?
2.7.	Which physicochemical properties of nano-Ag are most essential to characterize before and during toxicity experiments?
2.8.	What standardized test methods or characterization protocols are necessary to ensure that research results generated in multiple laboratories are consistent, reproducible, and reliable?
2.9.	Are there standard nano-Ag reference materials that can be used in exposure and effects testing to aid in comparison of results among investigators?
2.10.	Do adequate analytical methods exist to detect and characterize nano-Ag in environmental compartments and in biota?
2.11.	What analytical methods are available to disaggregate nano-Ag particles in preparing environmental samples for analysis?
2.12.	Do adequate analytical methods exist to detect and characterize exposure to nano-Ag via soil, water, and air?
2.13.	What new analytical methods would enhance characterization of nano-Ag particles?

2.14.	For the purpose of assessing potential risk, what metrics are most informative for quantifying dose of nano-Ag?

New Questions about Physicochemical Properties and Analytical Methods

2.15.	What are the particle sizes of silver products currently registered under FIFRA?
2.16.	Does nano-Ag react with materials (i.e. organic matter, other metals, polymers) and alter properties such as REDOX potential or leached metal ion rates?
2.17.	How can engineered nano-Ag particles be routinely, inexpensively detected, monitored, or distinguished from incidental, background, or naturally occurring nano-Ag particles?
2.18.	Can electron microscopy or other imaging methods be used to QUANTITAVELY measure nano-Ag in tissues?
2.19.	Do any methods exist that can accurately measure ABSORBED DOSE of nano-Ag and distinguish total amount of silver in tissues from conventional silver?
2.20.	What would constitute good reference positive and negative controls for nano-Ag experiments?
2.21.	For the purpose of assessing potential risk, what metrics are most informative for quantifying exposure and dose of nano-Ag?

Questions about Life-Cycle Stages

3.1.	What is a reliable estimate of worldwide and domestic nano-Ag production?
3.2.	What data regarding the physicochemical properties, concentrations, and formulations in nano-Ag spray disinfectants are appropriate for assessing their behaviors in and impacts on the environment?
3.3.	What are realistic strategies for collecting data on production quantities and product characteristics given that much of this information is proprietary?
3.4.	What properties of engineered nano-Ag particles that are incorporated in spray disinfectants are different from known properties of colloidal silver?
3.5.	Which manufacturing methods for nano-Ag and spray disinfectants containing nano-Ag are most common at the industrial scale?
3.5.a.	What are the associated feedstocks and by-products; of these feedstocks and by-products, which might be released, in what quantities, and via which pathways?
3.5.b.	Does the choice of manufacturing method for nano-Ag or spray disinfectant containing nano-Ag affect the release rate of silver ions?
3.6.	What changes occur to the physicochemical properties of nano-Ag throughout the material life cycle stages, either as a function of process and product engineering or as a function of incidental encounters with other substances and the environment?

3.6.a.	Do the changes that occur as a function of process and product engineering (e.g., the incorporation of nano-Ag into disinfectant sprays) affect the release rate of silver ions such that the rate might differ throughout the life cycle stages?
3.7.	What are the potential exposure vectors by which nano-Ag or nano-Ag by-products could be released to the environment at the various life-cycle stages?
3.7.a.	What information is most relevant (e.g., product handling throughout different life cycle phases, product use patterns, and nanoparticle release rates from products) for determining which of these potential exposure vectors represent the most significant pathway(s) for environmental release?
3.7.b.	What are the prevailing release pathways expected to be for nano-Ag and disinfectant sprays containing nano-Ag into the environment?
3.7.c.	What are the frequencies and durations of releases of nano-Ag during various life-cycle stages?

New Questions about Life-Cycle Stages

3.8.	Is there any experimental evidence that indicates that nano-Ag disposed down the drain would not rapidly react with sulfides, thiols, chlorides or other ions entrained in the sewer system?
3.9.	Do explosion risks exist for dried nano-Ag powders or nano-Ag powders modified with certain types of surface coatings?
3.10.	How persistent are the antibacterial activity or otherwise toxic characteristics of nano-Ag?
3.11.	What other examples of widespread use of toxic metals in consumer products can we look to for information on nano-Ag in disinfectant sprays? In general, how have those applications faired?
3.12.	Can we start measuring silver/nanosilver right now using available, existing methods to see where it is concentrated and begin to at least get general baseline data and track changes over time?
3.13.	What are the mechanisms during the application of disinfectant spray that affect availability of nanoscale silver?

Questions about Fate and Transport in Environmental Media

4.1.	Do the properties of nano-Ag that differ from those of well-characterized colloidal silver, if any, cause them to behave differently in aquatic, terrestrial, and atmospheric environmental compartments? a. If they do differ, how do they differ? b. Can information about how colloidal silver behaves in these environments be used to understand how nano-Ag behaves?

4.1.a.	Do the properties of nano-Ag that differ from those of well-characterized colloidal silver, if any, cause them to behave differently in aquatic, terrestrial, and atmospheric environmental compartments? If they do differ, how do they differ?
4.1.b.	Do the properties of nano-Ag that differ from those of well-characterized colloidal silver, if any, cause them to behave differently in aquatic, terrestrial, and atmospheric environmental compartments? Can information about how colloidal silver behaves in these environments be used to understand how nano-Ag behaves?
4.2.	Does particle size of nano-Ag affect the rate of release of silver ions in environmental compartments?
4.3.	Does the aggregation state, aggregate size, or aggregate density of nano-Ag affect the rate of release of silver ions in environmental compartments?
4.4.	Which physicochemical properties of nano-Ag and nano-Ag coatings can best be used to predict its fate and transport in different environmental media?
4.5.	Is nano-Ag as environmentally persistent as conventional silver?
4.6.	Does nano-Ag form the same strong complexes with anions as conventional silver, and if so, is it also effectively immobilized in aquatic environments?
4.7.	How does nano-Ag partition among soil, water, sediment, and air, and what are the key parameters determining this partitioning behavior?
4.8.	Which environmental factors significantly affect the behavior of nano-Ag in aquatic and terrestrial ecosystems, and by what mechanisms do they impart these effects?
4.9.	What are the characteristics of nano-Ag surface coatings that affect the transport behavior of nano-Ag within and between environmental compartments, and how is the transport affected?
4.10.	How effectively is nano-Ag removed from sewage and industrial process water by wastewater treatment technology, and can information on the removal of conventional silver be applied to nano-Ag removal?
4.11.	To what extent does nano-Ag bind to wastewater sludge and settle out or remain with treated water and enter the downstream aquatic environment?
4.12.	How could existing models applicable to conventional silver be used to adequately predict the transport and fate of nano-Ag through environmental compartments, or how could they be modified to do so?
4.13.	What role, if any, does temperature play in the behavior of nanoparticles?

New Questions about Fate and Transport in Environmental Media

4.14.	Leaching and run-off are two terms mentioned frequently as means for introducing nano-Ag to the natural environment. How applicable are these fate and transport processes to nano-Ag disinfectant sprays?

4.15.	Surface properties factor prominently into nano-Ag toxicity, fate and transport; yet surface attributes are poorly characterized in most published nano-Ag studies. How can we improve understanding of nano-Ag surface properties, and what tools can enhance our understanding of nanoscale surface features?
4.16.	How does the path of nanoscale silver affect its persistence and fate in the environment? For example, if the material is oxidized or agglomerated with inert, sorptive materials, its impact may be greatly reduced.

Questions about Exposure, Uptake, and Dose

5.1.	Are available methods adequate to characterize nano-Ag concentrations and associated exposure via relevant matrices such as: a. air? b. water? c. food?
5.1.a.	Are available methods adequate to characterize nano-Ag concentrations and associated exposure via relevant matrices such as air?
5.1.b.	Are available methods adequate to characterize nano-Ag concentrations and associated exposure via relevant matrices such as water?
5.1.c.	Are available methods adequate to characterize nano-Ag concentrations and associated exposure via relevant matrices such as food?
5.2.	To what extent is information on conventional silver applicable to nano-Ag, particularly regarding: a. uptake? b. biopersistence? c. bioaccumulation? d. biomagnification?
5.2.a.	To what extent is information on conventional silver applicable to nano-Ag, particularly regarding uptake?
5.2.b.	To what extent is information on conventional silver applicable to nano-Ag, particularly regarding biopersistence?
5.2.c.	To what extent is information on conventional silver applicable to nano-Ag, particularly regarding bioaccumulation?
5.2.d.	To what extent is information on conventional silver applicable to nano-Ag, particularly regarding biomagnification?
5.3.	What effect, if any, do surface treatments of nano-Ag particles have on: a. uptake? b. biopersistence? c. bioaccumulation?

	d. biomagnification?
5.3.a.	What effect, if any, do surface treatments of nano-Ag particles have on uptake?
5.3.b.	What effect, if any, do surface treatments of nano-Ag particles have on biopersistence?
5.3.c.	What effect, if any, do surface treatments of nano-Ag particles have on bioaccumulation?
5.3.d.	What effect, if any, do surface treatments of nano-Ag particles have on biomagnification?
5.4.	Which sources, pathways, and routes offer the greatest exposure potential to nano-Ag for humans and biota?
5.5.	Do particular species of biota and particular human populations have greater potential for exposure to nano-Ag?
5.6.	By region and environmental segment (e.g., air, water, soil), what are the background concentrations and characteristics of nano-Ag in air, water, and soil due to natural (non-anthropogenic) processes?
5.7.	Ecologically, is nano-Ag a point-source or regional exposure problem? If a regional distribution issue, what are the exposure concentrations and concentration gradients in key media (e.g., air, water, soil)?
5.8.	What is the potential for uncoated nano-Ag particles to interact with or form complexes with constituents in water, and what impact do these interactions have on particle bioavailability and release of silver ions?
5.9.	What is the impact of environmental characteristics such as water chemistry (e.g., pH, ionic strength), the presence of suspended solids, and the concentration of sulfides and other dissolved ligands on: a. the potential for uptake of nano-Ag from the environment? b. tissue distribution and dose of nano-Ag and silver ions?
5.9.a.	What is the impact of environmental characteristics such as water chemistry (e.g., pH, ionic strength), the presence of suspended solids, and the concentration of sulfides and other dissolved ligands on the potential for uptake of nano-Ag from the environment?
5.9.b.	What is the impact of environmental characteristics such as water chemistry (e.g., pH, ionic strength), the presence of suspended solids, and the concentration of sulfides and other dissolved ligands on tissue distribution and dose of nano-Ag and silver ions?
5.10.	To what extent does nano-Ag facilitate the uptake of other contaminants in the environment?
5.11.	What is the impact of organism characteristics such as physiology (e.g., cell membrane structure for single-celled organisms; respiratory physiology for multicellular organisms), behavior (e.g., filter feeding, habitat), and lifestage on:

	a. the potential for uptake of nano-Ag from the environment? b. tissue distribution and dose of nano-Ag and silver ions?
5.11.a.	What is the impact of organism characteristics such as physiology (e.g., cell membrane structure for single-celled organisms; respiratory physiology for multicellular organisms), behavior (e.g., filter feeding, habitat), and lifestage on the potential for uptake of nano-Ag from the environment?
5.11.b.	What is the impact of organism characteristics such as physiology (e.g., cell membrane structure for single-celled organisms; respiratory physiology for multicellular organisms), behavior (e.g., filter feeding, habitat), and lifestage on tissue distribution and dose of nano-Ag and silver ions?
5.12.	What is the relative bioavailability of nano-Ag and silver ions in aquatic environments, and how might the presence of nano-Ag alter the bioavailability of silver ions in sediments, water, and biota?

New Questions about Exposure, Uptake, and Dose

5.13.	Can estimates of the historical number of exposures to nano-Ag in wound care, medicinal, algaecidal, and disinfection be used to estimate a frequency for adverse effects?
5.14.	Many effects of emerging substances are not known until many years after their introduction and use in commerce. What are the chronic and subchronic effects of nano-Ag, and how can we accelerate our understanding of them?
5.15.	What benefits do nano-Ag disinfectant sprays offer over conventional sprays? Do these benefits warrant the risks already identified for nano-Ag disinfectant sprays?
5.16.	What effect, if any, do surface treatments of nano-Ag particles have on human exposures and uptake?
5.17.	How should dose and exposure be characterized for human exposures and how do the following parameters affect it: (1) physiological characteristics, (2) behavior, (3) lifestage, (4) susceptibility factors?
5.18.	What is the distribution of exposure intensities and frequencies of such exposures among homemakers, children, and maintenance personal, and are these of concern for acute and or chronic health effects?
5.19.	Is the form of the nano-sliver released in from the source the most important factor in affecting human exposure or does the final form after agglomeration deposition on surfaces have a greater influence on human exposure?
5.20.	Are available methods adequate to characterize nano-Ag concentrations and associated exposure via relevant matrices such as: a. air? b. water?

	c. food? d. surface dust?
5.21.	What information exists on the temporal changes in the release of ionic silver by nano-Ag in relation to particle physicochemical and environmental characteristics?
5.22.	Which sources, pathways, and routes offer the greatest exposure potential to nano-Ag for humans?
5.23.	Which sources, pathways, and routes offer the greatest exposure potential to nano-Ag for biota?

Questions about Ecological and Human Health Effects

6.1.	To what extent do particle properties (e.g., size, shape, chemical composition, surface treatments) determine biological responses to nano-Ag?
6.2.	Are there physicochemical properties of nano-Ag that could change significantly between the initiation and termination of toxicity studies, thereby affecting biological responses?
6.3.	Are the effects observed for exposure to nano-Ag due to silver ion release or the presence of nanoparticles? Can this be distinguished?
6.4.	Do nano-Ag particle size and phase partitioning (i.e., nano-Ag particle, nano-Ag clustering, dissolved silver ions from nano-Ag) affect organ distribution and biological effects?
6.5.	Is the available ecological effects evidence adequate to support ecological risk assessment for nano-Ag? If no, what research is needed to make an assessment possible?
6.6.	At a minimum, what assays could be considered in a harmonized test guideline for determination of the ecological effects of nano-Ag?
6.7.	How do abiotic factors in the environment affect nano-Ag effects in biota? These include but are not limited to: a. UV light; b. Water quality; and c. Other chemicals.
6.7.a.	How do abiotic factors in the environment affect nano-Ag effects in biota? These include but are not limited to UV light.
6.7.b.	How do abiotic factors in the environment affect nano-Ag effects in biota? These include but are not limited to water quality.
6.7.c.	How do abiotic factors in the environment affect nano-Ag effects in biota? These include but are not limited to other chemicals.

6.8.	What are the most sensitive ecological endpoints to nano-Ag exposure? Are there sufficient data/analytical techniques to determine how sensitive specific endpoints and organisms are to nano-Ag exposure, including: a. Benthic invertebrates; b. Marine invertebrates; and c. Freshwater invertebrates?
6.8.a.	What are the most sensitive ecological endpoints to nano-Ag exposure? Are there sufficient data/analytical techniques to determine how sensitive specific endpoints and organisms are to nano-Ag exposure, including benthic invertebrates?
6.8.b.	What are the most sensitive ecological endpoints to nano-Ag exposure? Are there sufficient data/analytical techniques to determine how sensitive specific endpoints and organisms are to nano-Ag exposure, including marine invertebrates?
6.8.c.	What are the most sensitive ecological endpoints to nano-Ag exposure? Are there sufficient data/analytical techniques to determine how sensitive specific endpoints and organisms are to nano-Ag exposure, including freshwater invertebrates?
6.9.	Are there secondary human health effects resulting from the ecological impacts of nano-Ag exposure? For example, exposure of terrestrial biota to sewage sludge contaminated with nano-Ag?
6.10.	At a minimum, what assays could be considered in a harmonized test guideline for determination of the human health effects of nano-Ag?
6.11.	Is there sufficient information available to determine appropriate standard reference materials for use in analysis of nano-Ag ecological and human health effects?
6.12.	What is the primary mechanism of action for nano-Ag in different species?
6.13.	What are the fundamental biological responses to and associated mechanisms of nano-Ag exposure at the cell, organ, and whole-animal levels?
6.14.	What are the biological responses observed at current nano-Ag occupational exposure levels?
6.15.	Do current publications describing the health effects of nano-Ag particles and laboratory-generated nano-Ag particles accurately depict the toxicity of commercially available nano-Ag materials?

New Questions about Ecological and Human Health Effects

6.16.	Are the current tests for regulatory acceptance relevant to nano-Ag?
6.17.	What relevance do acute, subchronic and chronic toxicity tests have in the prediction of adverse effects for nano-Ag?
6.18.	Are the results from regulatory tests for colloidal Ag sufficient to apply to nano-Ag?
6.19.	What route of exposure in in vivo preclinical testing is the most relevant?

6.20.	In several regulatory tests, a metabolic system is added to help with full metabolism of the test substance. Is this relevant to nano-Ag itself? Is this relevant to surface-modified or surface-coated nano-Ag? Is it relevant to functionalized nano-Ag because of the added functionalized groups?
6.21.	Can differences in toxicity observed between nano-Ag and silver nitrate in in vitro testing be extrapolated to human health toxicity?
6.22.	What rapid screening tests are available to identify relevant eco-endpoints for nano-Ag?
6.23.	What rapid screening tests are available to identify relevant human health endpoints for nano-Ag?
6.24.	What rational steps can be taken to assure that risks to sensitive populations, particularly children are minimized?
6.25.	In the absence of rigorously defined threshold limit values (TLVs) or water quality criteria–and with the understanding that development of such values may still be years away–can a rational and conservative approach be devised to establish and implement interim compliance standards for working with nano-Ag?
6.26.	Is there evidence of adaptive tolerance developing in microorganisms to Ag and to nano-Ag that would render the products useless, especially as the products gain widespread use?
6.27.	Are there sufficient data to develop concentration- or dose-response relationships instead of the current emphasis on point estimates or narratives of relative effects?
6.28.	Are there any parallels between health effects of conventional silver and those in emerging studies on nanosilver?
6.29.	What have the long-term effects (including sub-clinical) been to people who chronically ingested or applied conventional and colloidal silver–which includes nanoparticulate? Are there any studies on this?
6.30.	Given what is already known about conventional silver and nanosilver, what might the long term human and ecological effects of the increasing levels of silver (in a variety of forms) be?
6.31.	Can we predict the long-term effects to ecosystems of the disturbance to microbial communities caused by increasing levels of nanosilver in the environment?
6.32.	Can we predict the potential effects on human and ecological systems over the long-term from microbial resistance that may develop as a result of all this silver/nanosilver use?
6.33.	Can we predict whether widespread resistance to silver ions may develop and if so, are silver and/or nanosilver likely to be useful antimicrobials in the future?

6.34.	What are the epidemiological hypotheses that have to be investigated among the potential populations at risk due to nano-silver exposures in residential setting and maintenance work locations?
6.35.	The majority of toxicity studies with conventional silver were conducted over a decade ago. Are more studies needed that utilize state-of-the-art technology for comparing its mode of toxicity to that of nano-Ag? In other words, can we accurately say that nano-Ag and conventional silver have different modes of toxicity if most of the studies available for conventional silver were not conducted using current methods?

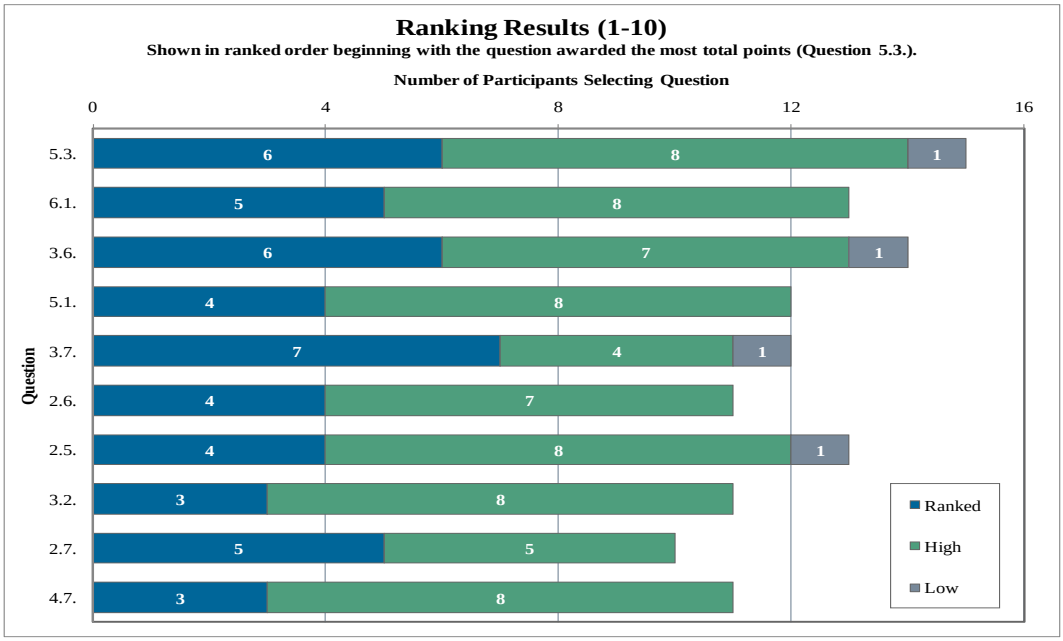
New Questions Corresponding to Multiple Topics

0.1.	Safety factors have historically been used to group of bulk silver, colloidal silver and silver compounds with vastly differing physiochemical properties and toxicity for risk assessment purposes. Is there any evidence that nano-Ag is not adequately covered by these safety factors?
0.2.	Could historical colloidal silver products with 100% of particles in the 1-100 nm range be categorized as nano-Ag for risk assessment purposes? 99%? 90%?
0.3.	Colloidal silver algaecides for swimming pools with reported 7.5 nm particles have been registered under FIFRA and commercially available in continuum since 1954. Has incidents data, or the lack thereof, been considered for environment fate and human health risk assessment?
0.4.	Has the database and risk assessment methodology used by FDA during approval of nano-Ag medical devices been integrated with EPA's database and risk assessment processes?
0.5.	Are there sufficient commonalities in nanomaterials reactivity, toxicology, and environmental fate to warrant grouping nanomaterials for risk assessment purposes?
0.6.	Have adverse incidents, or the lack thereof, recorded in EPA's OPP Incident Database System (IDS) for FIFRA registered nano-Ag products be considered for risk assessment purposes?

4.5. Pre-Workshop Ranking Results

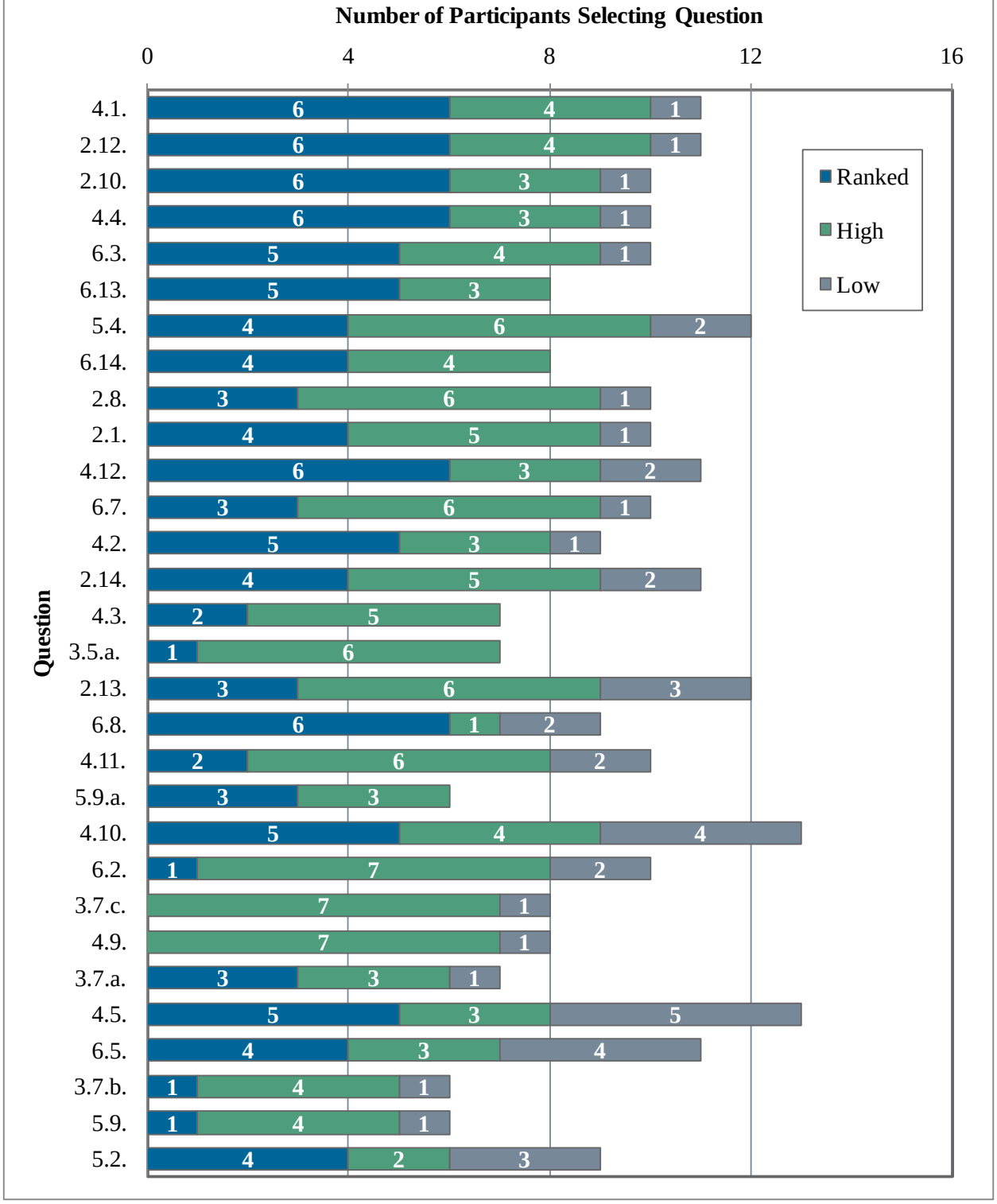
The following steps describe the methodology used to analyze the pre-workshop question rankings based on the rankings received from the participants.

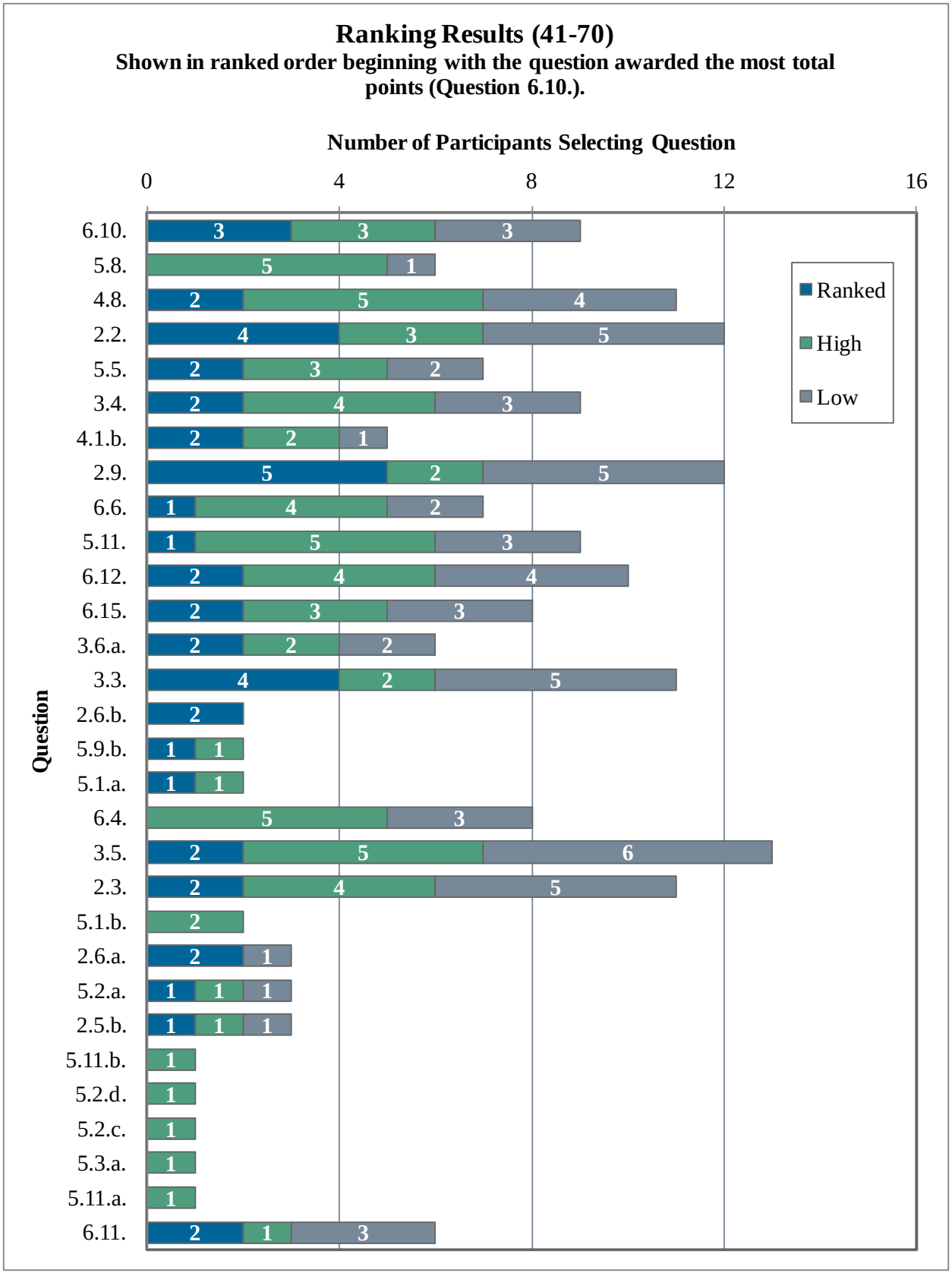
1. Added 6 placeholder questions so there are a total of 100 questions.
2. For the top 10 ranked questions, converted the score of 10 to 100, score of 9 to 99, score of 8 to 98, etc.
3. For the unranked high questions, assigned a random value between 76 and 90. Not all participants selected unranked high questions, so for those participants, skipped this step.
4. For the unranked low questions, assigned a random value between 1 and 10. Again, this range of random numbers may be smaller or larger than 10, depending on how many low questions the participant submitted. This range always began at 1. Not all participants selected unranked low questions.
5. For the ones that were not ranked or selected as high or low (left blank in the ranking spreadsheet), assigned a random value between 11 and 75. The placeholder questions were included in this group. This range of random numbers varied from participant to participant based on how many ranked, low, and high questions were submitted by that participant.
6. Calculated total points, mean score, and standard deviation for each question.
7. Ran a Monte Carlo simulation 500 times, storing the total points, mean score, and standard deviation for each run. Only the randomly assigned numbers changed from run to run – the ranked questions always kept the same order and points from 100 down to 91.
8. Averaged the results of all Monte Carlo simulations to get the final results shown in the charts and tables below.

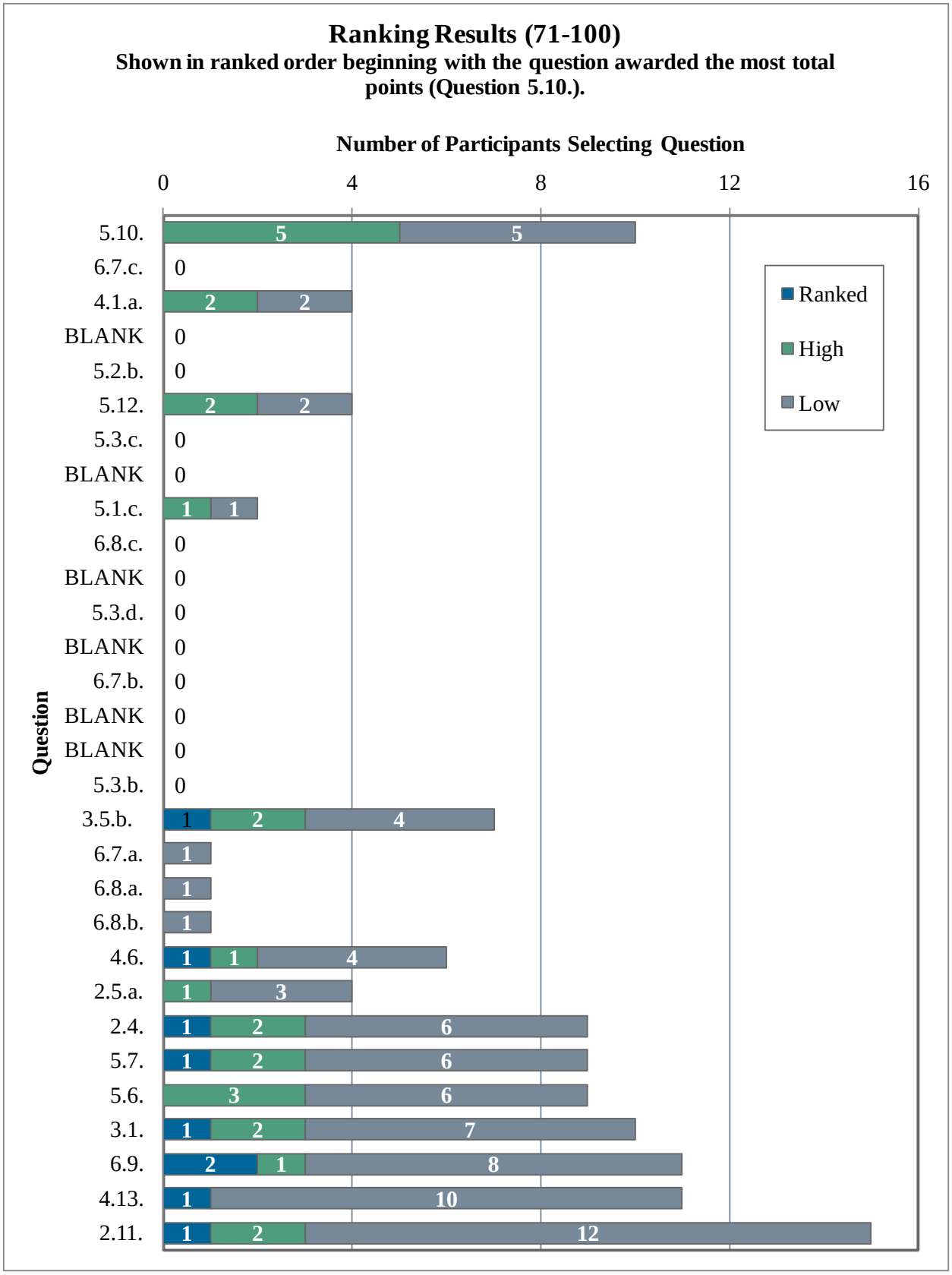


Ranking Results (11-40)

Shown in ranked order beginning with the question awarded the most total points (Question 4.1.).




Ranking Results (41-70)
Shown in ranked order beginning with the question awarded the most total points (Question 6.10.).
Number of Participants Selecting Question
0
4
8
12
16
Question
6.10. 3 3 3
5.8. 5 1
4.8. 2 5 4
2.2. 4 3 5
5.5. 2 3 2
3.4. 2 4 3
4.1.b. 2 2 1
2.9. 5 2 5
6.6. 1 4 2
5.11. 1 5 3
6.12. 2 4 4
6.15. 2 3 3
3.6.a. 2 2 2
3.3. 4 2 5
2.6.b. 2
5.9.b. 1 1
5.1.a. 1 1
6.4. 5 3
3.5. 2 5 6
2.3. 2 4 5
5.1.b. 2
2.6.a. 2 1
5.2.a. 1 1 1
2.5.b. 1 1 1
5.11.b. 1
5.2.d. 1
5.2.c. 1
5.3.a. 1
5.11.a. 1
6.11. 2 1 3
Ranked
High
Low


Ranking Results (71-100)
Shown in ranked order beginning with the question awarded the most total points (Question 5.10.).
Number of Participants Selecting Question
0
4
8
12
16
Question
5.10.
6.7.c.
4.1.a.
BLANK
5.2.b.
5.12.
5.3.c.
BLANK
5.1.c.
6.8.c.
BLANK
5.3.d.
BLANK
6.7.b.
BLANK
BLANK
5.3.b.
3.5.b.
6.7.a.
6.8.a.
6.8.b.
4.6.
2.5.a.
2.4.
5.7.
5.6.
3.1.
6.9.
4.13.
2.11.
Ranked
High
Low

Rank	Question		Number of Participants Who Selected the Question			Total Points	Strength of Feeling	Mean	Std. Dev.
			Ranked in Top 10	High	Low				
1	5.3.	What effect, if any, do surface treatments of nano-Ag particles have on: a. uptake? b. biopersistence? c. bioaccumulation? d. biomagnification?	6	8	1	1486	71%	70.76	28.21
2	6.1.	To what extent do particle properties (e.g., size, shape, chemical composition, surface treatments) determine biological responses to nano-Ag?	5	8	0	1482	71%	70.55	25.84
3	3.6.	What changes occur to the physicochemical properties of nano-Ag throughout the material life cycle stages, either as a function of process and product engineering or as a function of incidental encounters with other substances and the environment?	6	7	1	1464	70%	69.69	28.69
4	5.1.	Are available methods adequate to characterize nano-Ag concentrations and associated exposure via relevant matrices such as: a. air? b. water? c. food?	4	8	0	1427	68%	67.95	26.03
5	3.7.	What are the potential exposure vectors by which nano-Ag or nano-Ag by-products could be released to the environment at the various life-cycle stages?	7	4	1	1404	67%	66.87	30.70
6	2.6.	What physicochemical properties of nano-Ag can be used to: a. predict fate and transport in environmental media? b. predict toxicity to humans or biota?	4	7	0	1396	66%	66.48	27.51
7	2.5.	How does surface coating affect: a. the physicochemical properties of nano-Ag? b. toxicity to humans or biota?	4	8	1	1390	66%	66.17	28.78

Rank	Question		Number of Participants Who Selected the Question			Total Points	Strength of Feeling	Mean	Std. Dev.
			Ranked in Top 10	High	Low				
8	3.2.	What data regarding the physicochemical properties, concentrations, and formulations in nano-Ag spray disinfectants are appropriate for assessing their behaviors in and impacts on the environment?	3	8	0	1389	66%	66.14	26.74
9	2.7.	Which physicochemical properties of nano-Ag are most essential to characterize before and during toxicity experiments?	5	5	0	1383	66%	65.86	28.46
10	4.7.	How does nano-Ag partition among soil, water, sediment, and air, and what are the key parameters determining this partitioning behavior?	3	8	0	1383	66%	65.86	26.07
11	4.1.	Do the properties of nano-Ag that differ from those of well-characterized colloidal silver, if any, cause them to behave differently in aquatic, terrestrial, and atmospheric environmental compartments? a. If they do differ, how do they differ? b. Can information about how colloidal silver behaves in these environments be used to understand how nano-Ag behaves?	6	4	1	1346	64%	64.09	30.79
12	2.12.	Do adequate analytical methods exist to detect and characterize exposure to nano-Ag via soil, water, and air?	6	4	1	1327	63%	63.18	30.47
13	2.10.	Do adequate analytical methods exist to detect and characterize nano-Ag in environmental compartments and in biota?	6	3	1	1317	63%	62.72	31.61
14	4.4.	Which physicochemical properties of nano-Ag and nano-Ag coatings can best be used to predict its fate and transport in different environmental media?	6	3	1	1301	62%	61.97	30.72
15	6.3.	Are the effects observed for exposure to nano-Ag due to silver ion release or the presence of nanoparticles? Can this be distinguished?	5	4	1	1301	62%	61.93	30.98

Rank	Question		Number of Participants Who Selected the Question			Total Points	Strength of Feeling	Mean	Std. Dev.
			Ranked in Top 10	High	Low				
16	6.13.	What are the fundamental biological responses to and associated mechanisms of nano-Ag exposure at the cell, organ, and whole-animal levels?	5	3	0	1281	61%	61.02	28.95
17	5.4.	Which sources, pathways, and routes offer the greatest exposure potential to nano-Ag for humans and biota?	4	6	2	1276	61%	60.77	31.38
18	6.14.	What are the biological responses observed at current nano-Ag occupational exposure levels?	4	4	0	1276	61%	60.76	28.35
19	2.8.	What standardized test methods or characterization protocols are necessary to ensure that research results generated in multiple laboratories are consistent, reproducible, and reliable?	3	6	1	1269	60%	60.44	29.22
20	2.1.	What information could be provided about the nano-Ag contained in spray disinfectants to enable adequate characterization of exposure routes and toxic effects?	4	5	1	1267	60%	60.35	30.16
21	4.12.	How could existing models applicable to conventional silver be used to adequately predict the transport and fate of nano-Ag through environmental compartments, or how could they be modified to do so?	6	3	2	1262	60%	60.11	33.21
22	6.7.	How do abiotic factors in the environment affect nano-Ag effects in biota? These include but are not limited to: a. UV light b. Water quality c. Other chemicals	3	6	1	1256	60%	59.82	28.85
23	4.2.	Does particle size of nano-Ag affect the rate of release of silver ions in environmental compartments?	5	3	1	1240	59%	59.06	30.17
24	2.14.	For the purpose of assessing potential risk, what metrics are most informative for quantifying dose of nano-Ag?	4	5	2	1231	59%	58.60	31.91

Rank	Question		Number of Participants Who Selected the Question			Total Points	Strength of Feeling	Mean	Std. Dev.
			Ranked in Top 10	High	Low				
25	4.3.	Does the aggregation state, aggregate size, or aggregate density of nano-Ag affect the rate of release of silver ions in environmental compartments?	2	5	0	1217	58%	57.94	26.36
26	3.5.a.	What are the associated feedstocks and by-products; of these feedstocks and by-products, which might be released, in what quantities, and via which pathways?	1	6	0	1186	56%	56.49	25.84
27	2.13.	What new analytical methods would enhance characterization of nano-Ag particles?	3	6	3	1182	56%	56.28	32.47
28	6.8.	What are the most sensitive ecological endpoints to nano-Ag exposure? Are there sufficient data/analytical techniques to determine how sensitive specific endpoints and organisms are to nano-Ag exposure, including: a. Benthic invertebrates b. Marine invertebrates c. Freshwater invertebrates	6	1	2	1181	56%	56.23	32.34
29	4.11.	To what extent does nano-Ag bind to wastewater sludge and settle out or remain with treated water and enter the downstream aquatic environment?	2	6	2	1175	56%	55.94	30.24
30	5.9.a.	What is the impact of environmental characteristics such as water chemistry (e.g., pH, ionic strength), the presence of suspended solids, and the concentration of sulfides and other dissolved ligands on: the potential for uptake of nano-Ag from the environment?	3	3	0	1172	56%	55.80	26.83
31	4.10.	How effectively is nano-Ag removed from sewage and industrial process water by wastewater treatment technology, and can information on the removal of conventional silver be applied to nano-Ag removal?	5	4	4	1170	56%	55.72	35.22

Rank	Question		Number of Participants Who Selected the Question			Total Points	Strength of Feeling	Mean	Std. Dev.
			Ranked in Top 10	High	Low				
32	6.2.	Are there physicochemical properties of nano-Ag that could change significantly between the initiation and termination of toxicity studies, thereby affecting biological responses?	1	7	2	1166	56%	55.51	30.11
33	3.7.c.	What are the frequencies and durations of releases of nano-Ag during various life-cycle stages?	0	7	1	1145	55%	54.52	27.06
34	4.9.	What are the characteristics of nano-Ag surface coatings that affect the transport behavior of nano-Ag within and between environmental compartments, and how is the transport affected?	0	7	1	1144	54%	54.49	26.90
35	3.7.a.	What information is most relevant (e.g., product handling throughout different life cycle phases, product use patterns, and nanoparticle release rates from products) for determining which of these potential exposure vectors represent the most significant pathway(s) for environmental release?	3	3	1	1138	54%	54.20	29.24
36	4.5.	Is nano-Ag as environmentally persistent as conventional silver?	5	3	5	1090	52%	51.90	36.50
37	6.5.	Is the available ecological effects evidence adequate to support ecological risk assessment for nano-Ag? If no, what research is needed to make an assessment possible?	4	3	4	1083	52%	51.58	34.58
38	3.7.b.	What are the prevailing release pathways expected to be for nano-Ag and disinfectant sprays containing nano-Ag into the environment?	1	4	1	1073	51%	51.12	27.02

Rank	Question		Number of Participants Who Selected the Question			Total Points	Strength of Feeling	Mean	Std. Dev.
			Ranked in Top 10	High	Low				
39	5.9.	What is the impact of environmental characteristics such as water chemistry (e.g., pH, ionic strength), the presence of suspended solids, and the concentration of sulfides and other dissolved ligands on: a. the potential for uptake of nano-Ag from the environment? b. tissue distribution and dose of nano-Ag and silver ions?	1	4	1	1073	51%	51.09	26.93
40	5.2.	To what extent is information on conventional silver applicable to nano-Ag, particularly regarding: a. uptake? b. biopersistence? c. bioaccumulation? d. biomagnification?	4	2	3	1073	51%	51.08	32.25
41	6.10.	At a minimum, what assays could be considered in a harmonized test guideline for determination of the human health effects of nano-Ag?	3	3	3	1072	51%	51.06	32.18
42	5.8.	What is the potential for uncoated nano-Ag particles to interact with or form complexes with constituents in water, and what impact do these interactions have on particle bioavailability and release of silver ions?	0	5	1	1065	51%	50.72	26.09
43	4.8.	Which environmental factors significantly affect the behavior of nano-Ag in aquatic and terrestrial ecosystems, and by what mechanisms do they impart these effects?	2	5	4	1063	51%	50.63	33.08
44	2.2.	How can engineered nano-Ag particles be distinguished from incidental, background, or naturally occurring nano-Ag particles?	4	3	5	1055	50%	50.25	36.17
45	5.5.	Do particular species of biota and particular human populations have greater potential for exposure to nano-Ag?	2	3	2	1053	50%	50.16	29.53

Rank	Question		Number of Participants Who Selected the Question			Total Points	Strength of Feeling	Mean	Std. Dev.
			Ranked in Top 10	High	Low				
46	3.4.	What properties of engineered nano-Ag particles that are incorporated in spray disinfectants are different from known properties of colloidal silver?	2	4	3	1052	50%	50.11	30.89
47	4.1.b.	Do the properties of nano-Ag that differ from those of well-characterized colloidal silver, if any, cause them to behave differently in aquatic, terrestrial, and atmospheric environmental compartments? Can information about how colloidal silver behaves in these environments be used to understand how nano-Ag behaves?	2	2	1	1049	50%	49.97	26.92
48	2.9.	Are there standard nano-Ag reference materials that can be used in exposure and effects testing to aid in comparison of results among investigators?	5	2	5	1047	50%	49.86	35.97
49	6.6.	At a minimum, what assays could be considered in a harmonized test guideline for determination of the ecological effects of nano-Ag?	1	4	2	1038	49%	49.43	28.58
50	5.11.	What is the impact of organism characteristics such as physiology (e.g., cell membrane structure for single-celled organisms; respiratory physiology for multicellular organisms), behavior (e.g., filter feeding, habitat), and lifestage on: a. the potential for uptake of nano-Ag from the environment? b. tissue distribution and dose of nano-Ag and silver ions?	1	5	3	1036	49%	49.35	30.18
51	6.12.	What is the primary mechanism of action for nano-Ag in different species?	2	4	4	1021	49%	48.60	32.10
52	6.15.	Do current publications describing the health effects of nano-Ag particles and laboratory-generated nano-Ag particles accurately depict the toxicity of commercially available nano-Ag materials?	2	3	3	1019	49%	48.53	30.68

Rank	Question		Number of Participants Who Selected the Question			Total Points	Strength of Feeling	Mean	Std. Dev.
			Ranked in Top 10	High	Low				
53	3.6.a.	Do the changes that occur as a function of process and product engineering (e.g., the incorporation of nano-Ag into disinfectant sprays) affect the release rate of silver ions such that the rate might differ throughout the life cycle stages?	2	2	2	1017	48%	48.44	28.79
54	3.3.	What are realistic strategies for collecting data on production quantities and product characteristics given that much of this information is proprietary?	4	2	5	1010	48%	48.08	35.51
55	2.6.b.	What physicochemical properties of nano-Ag can be used to: predict toxicity to humans or biota?	2	0	0	1003	48%	47.75	24.62
56	5.9.b.	What is the impact of environmental characteristics such as water chemistry (e.g., pH, ionic strength), the presence of suspended solids, and the concentration of sulfides and other dissolved ligands on: tissue distribution and dose of nano-Ag and silver ions?	1	1	0	997	47%	47.50	23.07
57	5.1.a.	Are available methods adequate to characterize nano-Ag concentrations and associated exposure via relevant matrices such as: air?	1	1	0	992	47%	47.22	22.58
58	6.4.	Do nano-Ag particle size and phase partitioning (i.e., nano-Ag particle, nano-Ag clustering, dissolved silver ions from nano-Ag) affect organ distribution and biological effects?	0	5	3	988	47%	47.06	28.88
59	3.5.	Which manufacturing methods for nano-Ag and spray disinfectants containing nano-Ag are most common at the industrial scale?	2	5	6	985	47%	46.93	35.36
60	2.3.	Which physicochemical properties of conventional silver can be applied to nano-Ag?	2	4	5	983	47%	46.81	33.76

Rank	Question		Number of Participants Who Selected the Question			Total Points	Strength of Feeling	Mean	Std. Dev.
			Ranked in Top 10	High	Low				
61	5.1.b.	Are available methods adequate to characterize nano-Ag concentrations and associated exposure via relevant matrices such as: water?	0	2	0	976	46%	46.48	22.18
62	2.6.a.	What physicochemical properties of nano-Ag can be used to: predict fate and transport in environmental media?	2	0	1	972	46%	46.28	26.41
63	5.2.a.	To what extent is information on conventional silver applicable to nano-Ag, particularly regarding: uptake?	1	1	1	950	45%	45.26	24.81
64	2.5.b.	How does surface coating affect: toxicity to humans or biota?	1	1	1	947	45%	45.10	24.23
65	5.11.b.	What is the impact of organism characteristics such as physiology (e.g., cell membrane structure for single-celled organisms; respiratory physiology for multicellular organisms), behavior (e.g., filter feeding, habitat), and lifestage on: tissue distribution and dose of nano-Ag and silver ions?	0	1	0	944	45%	44.94	20.96
66	5.2.d.	To what extent is information on conventional silver applicable to nano-Ag, particularly regarding: biomagnification?	0	1	0	943	45%	44.88	20.76
67	5.2.c.	To what extent is information on conventional silver applicable to nano-Ag, particularly regarding: bioaccumulation?	0	1	0	940	45%	44.77	20.90
68	5.3.a.	What effect, if any, do surface treatments of nano-Ag particles have on: uptake?	0	1	0	939	45%	44.71	20.71
69	5.11.a.	What is the impact of organism characteristics such as physiology (e.g., cell membrane structure for single-celled organisms; respiratory physiology for multicellular organisms), behavior (e.g., filter feeding, habitat), and lifestage on: the potential for uptake of nano-Ag from the environment?	0	1	0	937	45%	44.60	20.68

Rank	Question		Number of Participants Who Selected the Question			Total Points	Strength of Feeling	Mean	Std. Dev.
			Ranked in Top 10	High	Low				
70	6.11.	Is there sufficient information available to determine appropriate standard reference materials for use in analysis of nano-Ag ecological and human health effects?	2	1	3	933	44%	44.45	29.25
71	5.10.	To what extent does nano-Ag facilitate the uptake of other contaminants in the environment?	0	5	5	914	44%	43.50	31.01
72	6.7.c.	How do abiotic factors in the environment affect nano-Ag effects in biota? These include but are not limited to: Other chemicals	0	0	0	910	43%	43.31	19.04
73	4.1.a.	Do the properties of nano-Ag that differ from those of well-characterized colloidal silver, if any, cause them to behave differently in aquatic, terrestrial, and atmospheric environmental compartments? If they do differ, how do they differ?	0	2	2	909	43%	43.28	24.76
74	BLANK	Used for statistical analysis purposes only	0	0	0	909	43%	43.26	19.40
75	5.2.b.	To what extent is information on conventional silver applicable to nano-Ag, particularly regarding: biopersistence?	0	0	0	908	43%	43.23	19.19
76	5.12.	What is the relative bioavailability of nano-Ag and silver ions in aquatic environments, and how might the presence of nano-Ag alter the bioavailability of silver ions in sediments, water, and biota?	0	2	2	906	43%	43.15	24.68
77	5.3.c.	What effect, if any, do surface treatments of nano-Ag particles have on: bioaccumulation?	0	0	0	906	43%	43.13	19.23
78	BLANK	Used for statistical analysis purposes only	0	0	0	906	43%	43.12	19.26
79	5.1.c.	Are available methods adequate to characterize nano-Ag concentrations and associated exposure via relevant matrices such as: food?	0	1	1	905	43%	43.10	22.14

Rank	Question		Number of Participants Who Selected the Question			Total Points	Strength of Feeling	Mean	Std. Dev.
			Ranked in Top 10	High	Low				
80	6.8.c.	What are the most sensitive ecological endpoints to nano-Ag exposure? Are there sufficient data/analytical techniques to determine how sensitive specific endpoints and organisms are to nano-Ag exposure, including: Freshwater invertebrates	0	0	0	903	43%	42.99	19.27
81	BLANK	Used for statistical analysis purposes only	0	0	0	902	43%	42.97	19.30
82	5.3.d.	What effect, if any, do surface treatments of nano-Ag particles have on: biomagnification?	0	0	0	902	43%	42.96	19.22
83	BLANK	Used for statistical analysis purposes only	0	0	0	902	43%	42.93	19.11
84	6.7.b.	How do abiotic factors in the environment affect nano-Ag effects in biota? These include but are not limited to: Water quality	0	0	0	901	43%	42.89	19.30
85	BLANK	Used for statistical analysis purposes only	0	0	0	900	43%	42.84	19.20
86	BLANK	Used for statistical analysis purposes only	0	0	0	897	43%	42.72	19.20
87	5.3.b.	What effect, if any, do surface treatments of nano-Ag particles have on: biopersistence?	0	0	0	895	43%	42.61	19.15
88	3.5.b.	Does the choice of manufacturing method for nano-Ag or spray disinfectant containing nano-Ag affect the release rate of silver ions?	1	2	4	881	42%	41.93	28.98
89	6.7.a.	How do abiotic factors in the environment affect nano-Ag effects in biota? These include but are not limited to: UV light	0	0	1	866	41%	41.23	20.40
90	6.8.a.	What are the most sensitive ecological endpoints to nano-Ag exposure? Are there sufficient data/analytical techniques to determine how sensitive specific endpoints and organisms are to nano-Ag exposure, including: Benthic invertebrates	0	0	1	866	41%	41.21	20.45
91	6.8.b.	What are the most sensitive ecological endpoints to nano-Ag exposure? Are there sufficient data/analytical techniques to determine how sensitive specific endpoints and organisms are to nano-Ag exposure, including: Marine invertebrates	0	0	1	857	41%	40.82	20.57

Rank	Question		Number of Participants Who Selected the Question			Total Points	Strength of Feeling	Mean	Std. Dev.
			Ranked in Top 10	High	Low				
92	4.6.	Does nano-Ag form the same strong complexes with anions as conventional silver, and if so, is it also effectively immobilized in aquatic environments?	1	1	4	841	40%	40.07	27.40
93	2.5.a.	How does surface coating affect: the physicochemical properties of nano-Ag?	0	1	3	827	39%	39.37	24.21
94	2.4.	Does the morphology of nano-Ag determine the efficacy of use in spray disinfectants?	1	2	6	814	39%	38.78	30.69
95	5.7.	Ecologically, is nano-Ag a point-source or regional exposure problem? If a regional distribution issue, what are the exposure concentrations and concentration gradients in key media (e.g., air, water, soil)?	1	2	6	804	38%	38.27	30.49
96	5.6.	By region and environmental segment (e.g., air, water, soil), what are the background concentrations and characteristics of nano-Ag in air, water, and soil due to natural (non-anthropogenic) processes?	0	3	6	797	38%	37.94	29.26
97	3.1.	What is a reliable estimate of worldwide and domestic nano-Ag production?	1	2	7	779	37%	37.08	31.23
98	6.9.	Are there secondary human health effects resulting from the ecological impacts of nano-Ag exposure? For example, exposure of terrestrial biota to sewage sludge contaminated with nano-Ag?	2	1	8	740	35%	35.24	31.67
99	4.13.	What role, if any, does temperature play in the behavior of nanoparticles?	1	0	10	580	28%	27.60	27.79
100	2.11.	What analytical methods are available to disaggregate nano-Ag particles in preparing environmental samples for analysis?	1	2	12	577	27%	27.47	31.61

4.6. Template and Instructions for Breakout Group Reports

4.6.1. Group Summary

Nanomaterial Case Study Workshop—Report from Breakout Group [insert name of group]

Breakout group members: [insert group member names]

Short Description:
[Prepare a short paragraph, individual sentences, or bullet statements referring to specific questions subsumed under this priority area. Synthesis of questions is encouraged.]

1. Why is this research theme of high importance?

2. Where does this research theme fit within the CEA process?

3. How would answering the research questions under this theme directly support or relate to a future CEA of nanomaterials?

4. For each of the research questions under this theme, indicate whether it is relevant to (1) a specific application of nano-Ag, (2) all applications of nano-Ag, or (3) nanomaterials in general (not only nano-Ag).

5. What challenges might arise in answering the research questions under this theme (e.g., from a technical, policy, or social perspective)?

6. How are the research questions under this theme related to other top priority themes or questions?

7. How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

4.6.2. Group Presentation Slides

Nanomaterial Case Study Workshop
Developing a Comprehensive Environmental Assessment Research Strategy for Nanoscale Silver

Breakout Group [insert number of group] **Summary**

Title of priority theme:

Group members: [insert group member names]

Short description:

- [Insert one to two sentences or bullet statements describing questions under this theme; synthesis of questions is encouraged.]

1

Nanomaterial Case Study Workshop
Developing a Comprehensive Environmental Assessment Research Strategy for Nanoscale Silver

Why is this research theme of high importance?

- [Insert one to two sentences or bullet statements describing why this research theme is of high importance.]

How might answering the research questions under this theme reduce the chances of unintended ecological, human health, or other consequences?

- [Insert one to two sentences or bullet statements describing why this research theme is of high importance.]

5. References

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