

PEER REVIEW SUMMARY REPORT

External Peer Review of EPA's Draft Document *Nanomaterial Case Study: Nanoscale Silver in Disinfectant Spray*

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Contract No. EP-C-07-025
Task Order 149

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January 31, 2012

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I. INTRODUCTION

The Environmental Protection Agency (EPA) *Nanotechnology White Paper*¹, under the heading of Risk Assessment (section 6.2.7), recommended developing case studies of engineered nanomaterials and conducting workshops to identify data gaps and research needs related to assessment efforts. Subsequently, the EPA's National Center for Environmental Assessment (NCEA) prepared the draft *Nanomaterial Case Study: Nanoscale Silver in Disinfectant Spray*² and held a *Public Information Exchange on EPA Nanomaterial Case Studies* along with a separate *Nanomaterial Case Study Workshop: Developing a Comprehensive Environmental Assessment Research Strategy for Nanoscale Silver* on January 4 through 7, 2011. The goal in preparing the draft document and holding the workshop was to identify and prioritize research needed to support a comprehensive environmental assessment (CEA) of nanoscale silver (nano-Ag). The *Nanomaterial Case Study: Nanoscale Silver in Disinfectant Spray* document is the second in a series of such documents³ and serves as another step in refining a strategic approach for nanomaterials risk assessment research, consistent with objectives described in the EPA *Nanomaterial Research Strategy*⁴.

The case studies were constructed with the CEA framework, which is a holistic approach that incorporates a life-cycle perspective in the risk assessment paradigm⁵. However, the case studies were not intended to be actual or even preliminary assessments, nor were they meant to provide an immediate basis for risk management, regulatory, or policy decisions. Instead, the intent was to organize information on nanomaterials in a manner that would facilitate thinking about information gaps that would need to be filled to support future assessment efforts. Each chapter is briefly described below.

- Chapter 1 is an introductory chapter that describes the CEA approach used for the case studies and presents other background information.
- Chapter 2 provides basic information on silver and nano-Ag as well as analytical methods for detecting and characterizing nano-Ag.
- Chapter 3 describes life cycle stages of nano-Ag as potentially used in disinfectant spray products, including feedstocks, manufacturing, distribution, use, and disposal.
- Chapter 4 reviews the state of knowledge regarding the environmental transport, transformation, and fate of nano-Ag.
- Chapter 5 presents information on exposure and dose characterization of nano-Ag in humans and other biota.

¹ U.S. Environmental Protection Agency (2007) *Nanotechnology White Paper*. Science Policy Council, Washington, DC. EPA 100/B-07/001, February. Available at: http://www.epa.gov/nanoscience/files/epa_nano_wp_2007.pdf

² U.S. Environmental Protection Agency (2010) *Nanomaterial Case Study: Nanoscale Silver in Disinfectant Spray* (External Review Draft). ORD, NCEA, Washington, DC. EPA/600/R-10/081, August. Available at: <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=226723>

³ U.S. Environmental Protection Agency (2010) *Nanomaterial Case Studies: Nanoscale Titanium Dioxide in Water Treatment and Topical Sunscreen* (Final). ORD, NCEA, Washington, DC. EPA/600/R-09/057F, November. Available at: <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=230972>

⁴ U.S. Environmental Protection Agency (2009) *Nanomaterial Research Strategy*. Washington, DC. EPA 620/K-09/011, June. Available at: http://www.epa.gov/nanoscience/files/nanotech_research_strategy_final.pdf

⁵ U.S. Environmental Protection Agency (2011) *Comprehensive Environmental Assessment: A Meta-Assessment Approach to Increase Effectiveness of Risk Management and Research Planning*. ORD, NCEA, RTP, NC. Available at: <http://www.epa.gov/nanoscience/files/CEAPrecis.pdf>

- Chapter 6 discusses research on ecological and health effects of nano-Ag.
- Chapter 7 provides a summary of the information and information gaps identified in the case study and prioritized in the accompanying workshop, as well as a discussion of the role of the case study in research planning and future assessment efforts.

The external review draft of EPA's *Nanomaterial Case Study: Nanoscale Silver in Disinfectant Spray* has been previously revised based on comments received from workshop participants and the public. This report reflects the outcomes of NCEA's request for a letter review of this revised draft by individuals with expertise in one or more topic areas related to life cycle and risk assessment of nanomaterials.

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II. CHARGE TO REVIEWERS

The external review draft of EPA's *Nanomaterial Case Study: Nanoscale Silver in Disinfectant Spray* has been revised based on comments received from workshop participants and the public. NCEA is requesting a letter review of this draft by individuals with expertise in one or more topic areas related to life cycle and risk assessment of nanomaterials. Charge questions to guide the review are listed below. Reviewers should provide detailed responses to each charge question. However, if a question requires a response outside of the reviewer's expertise or general knowledge, then the reviewer may so indicate. Following the review, NCEA staff will revise the case studies to consider comments from the peer reviewers.

Charge Questions:

1. Chapter 1 provides introductory material regarding the CEA approach used in these case studies along with other background information and a discussion of terminology. Is this information accurately and clearly presented? Please comment on the utility of the chapter in providing background and support for the remainder of the document. In particular, are the figures summarizing the CEA framework and process clear? How might this chapter be improved?
2. Chapter 2 presents basic information on conventional silver, including data on usage and historic environmental levels. Information on the physical-chemical properties of nanoscale silver and analytic methods makes up the rest of the chapter. Is this information clear and accurate? How might this chapter be improved?
3. Chapter 3 summarizes information on the lifecycle stages of nano-Ag disinfectant spray products, including potential releases to the environment of nano-Ag and by-products. To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-Ag as it might be used in disinfectant spray products? To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?
4. Information on the transport, transformation, and fate of nano-Ag in air, water, sediment, and soil is discussed in Chapter 4. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of understanding regarding the known and anticipated behavior of nano-Ag in the environment. To what extent is this information presented in a manner that would inform consideration of likely exposure routes relevant to biota and human health? For each of the environmental media discussed, to what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?
5. Chapter 5 provides information on exposure, dose, and translocation of nano-Ag in humans and other biota. Please comment on the extent to which this chapter accurately and sufficiently characterizes this information and forms a basis for considering the health and ecological impacts of nano-Ag. To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

6. Chapter 6 characterizes factors that influence ecological and health impacts of nano-Ag and discusses the currently available scientific evidence regarding these impacts. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of the science. To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

7. Chapter 7 summarizes the information and research questions presented in the nano-Ag case study, as well as discusses the role of case studies in the refinement of research strategies and potential future assessment efforts. We would appreciate comment from the peer reviewers on the integration of evidence in this chapter and its usefulness in supporting future development of research strategies and assessments. How might this chapter be improved?

8. For the document as a whole, are there ways to improve the structure, scope or presentation of information to better support the identification and prioritization of research needs by diverse stakeholders?

9. The case study follows the CEA framework, which combines a product life-cycle perspective with the risk assessment paradigm to support subsequent steps in the CEA process. Please comment on aspects of the CEA framework and process that can be improved in future applications of CEA. We would appreciate input on the overall structure and scope of the framework and process and the extent to which they support the development and refinement of research directions for future CEAs of nano-Ag in particular and nanomaterials in general.

III. QUALITY NARRATIVE STATEMENT

This section details the quality assurance procedures that were followed to conduct this external peer review. Versar has a well-established approach to conducting peer reviews, from reviewer selection through completion of the final report. Within this approach are several quality assurance protocols to ensure that: qualified individuals are selected to participate, they are free from conflict of interest, and a thorough review is completed.

Reviewer Identification and Selection

Versar's approach for selecting the technical expert reviewers consisted of the following five key steps: (1) development of selection criteria, (2) identification of experts, (3) conflict of interest (COI) screening, (4) selection of peer reviewers, and (5) confirmation of peer reviewer participation.



The experts that participated in this review were identified by literature searches of scientific journals, professional societies, and scientific meetings, as well as searches of Versar's internal peer review database of more than 3,000 scientists. As a result of this search, Versar identified a total of 40 potential scientific experts with expertise in the general area of life cycle and risk assessment of nanomaterials. Interested candidates provided a current *curriculum vitae* which was reviewed by two Versar staff members to ensure that each candidate had the appropriate scientific credentials and evidence of expertise through a listing of their publications and professional affiliations. The specific areas of expertise included: (1) analytical methods for characterizing nanomaterials, (2) life cycle stages for nanomaterials, (3) transport, transformation, and fate of nanomaterials (4) exposure, dose, and translocation of nanomaterials, (5) ecological effects of nanomaterials, and (6) health effects of nanomaterials.

Versar also conducted COI screening to make certain that the experts would provide unbiased, objective scientific input. This screening involved sending the potential candidates a series of COI screening questions that helped us to determine if they were involved with any other work and/or organizations that might create a real or perceived conflict of interest for the current task. Additionally, each expert signed forms certifying that, to the best of their knowledge, they did not have any conflict of interest related to the task. Upon completion of the COI screening, Versar selected seven experts, based on their credentials, to conduct the review. Versar requested consent from the EPA Task Order Project Officer (TOPO) and EPA acknowledged that the proposed candidates were well qualified to participate in the review. Versar contacted the seven reviewers to notify them of their selection.

Conducting the Review

Versar distributed to the reviewers EPA's draft document *Nanomaterial Case Study: Nanoscale Silver in Disinfectant Spray*. Versar also distributed a draft workshop report *Nanomaterial Case Study Workshop*, which was provided for background information only. Along with the document and draft workshop report, Versar included a work assignment authorization letter, which included instructions for the preparation of the written comments to ensure that the experts provided comments according to a prescribed format. Versar also specified the amount of time the external reviewers had to complete their reviews and submit written comments. During the review period, Versar monitored the progress of the reviewers in order to make sure there was timely delivery of the written comments.

Review of Expert Comments

At the completion of the review period, Versar evaluated the experts' comments for completeness and scientific quality, organized them into a final report, and submitted them to EPA. The seven reviewers all submitted thorough reviews of the draft document. The experts provided (1) general comments which included their overall impressions of the document, addressing the accuracy of information presented, clarity of presentation, and soundness of the conclusions, (2) responses to nine charge questions, and (3) specific observations which included any corrections or changes to the document.

IV. GENERAL IMPRESSIONS OF THE DOCUMENT IN ITS ENTIRETY

Paul M. Bertsch

The document purports to be a “case study” of nanoscale silver in disinfectant spray. However, the document contains a good deal of information and material both on nano silver and nanomaterials, generally that extend well beyond what might be anticipated in a “case study.” While much of the discussion surrounding what is known about nano Ag and its environmental fate, transport, bioavailability, and toxicity might be considered pertinent to disinfectant sprays containing nano Ag, the presentation is broad and comprehensive relative to Ag and nano-Ag in the environment quite generally and elements related to the actual focus of the case study seem to become lost. Chapter 7 does not help bring the case study back into focus, as much of what was covered previously in the document relative to a broad discussion of Ag and nano-Ag is repeated and there are some lists of research gaps interspersed with larger research themes, again making it difficult to wade through the material to identify those areas critical to moving a risk analysis forward.

The failure to identify what major stakeholder or stakeholders were the primary target audience for this document made it difficult to evaluate the appropriateness of the depth and breadth of much of the information presented.

Identifying key knowledge gaps and defining risk trade-offs leading to prioritized research and adaptive risk management plans is certainly an important goal; however the document is very long, covering a lot of material with significant overlap and redundancy throughout (sometimes inconsistently presented) and a concise prioritized list aimed at advancing research and adaptive risk management plans never really emerges, although section 7.3.1 is the most useful in this regard.

Overall the document is well written and the material is presented clearly. The redundancy in the document might be justified if the intention is to have most of the chapters read as stand-alone contributions; however this is not a stated goal. One major problem with the repetition and redundancy is that the information covered multiple times is not always internally consistent. For the most part, the information presented appears to be accurate and conclusions sound, albeit most conclusions posed are rather general in nature. There are a number of specific areas needing attention (with respect to clarity or accuracy) are indicated below.

Jaclyn Cañas

Overall, the case study document is very well written, organized, and very informative. To my knowledge, although limited with regards to nano-Ag, the information presented is heavily cited with the most current nano-Ag literature. I can, however, confirm that the way data/information was interpreted based on the literature and with regards to general fate, toxicity, and nanotoxicology principles was definitely accurate. Sufficient evidence was provided to fully support any conclusions outlined in the case study. The CEA approach was effective at gathering all the relevant nano-Ag information to assess the state of the science and direct future research planning. The document is not only useful with regards to nano-Ag research, but there are several overarching themes or questions related to nanomaterials in general that are raised

throughout. Therefore, this document will be of use to nano-Ag researchers as well as to those working with other nanomaterials.

With regards to presentation of the document, it is well organized and presented in a very logical manner. The introductory chapter does an excellent at setting the stage for the remainder of the document. The extensive use of headings and subheadings is very useful to the reader. The use of summaries at the end of each chapter is also very useful. Overall, the document is easy to read and is presented in a clear and logical manner.

Robert I. MacCuspie

See comments below.

Peter R. McClure

The accuracy of information presented and the clarity of presentation in Chapters 1, 3, 5, 6 and 7 of this document are excellent, and the conclusions reached are sound. The document should be an excellent resource for planning for future research on ecological and human health impacts of silver nanoparticles in disinfection sprays. Unfortunately, much of Chapters 2 and 4 are poorly written. While I essentially agree with the conclusions for these chapters, I think they should be rewritten to reflect the high quality of organization, presentation, and evaluation demonstrated in the other chapters. Specific suggestions for improvement of the document are presented below.

Bernd Nowack

The document is pretending to make a “case study;” however, it is rather using one application of nano-Ag to organize to some extent the information extracted from the scientific literature and to guide the questions. However, this is not a real case study. From a case study, I would expect that only the information that is pertinent to the case study is summarized. My expectation was that the document contains some general chapters that are then followed by the “case study,” where the information that is relevant for the case is listed based on the life cycle aspects of the application. At the moment, just the information about nano-Ag is listed and the case study information (that is in some chapters covered quite nicely) gets completely lost. In principle, this document is a normal review of nano-Ag with some additional focus on sprays. But it's not really a case study. This could be solved by writing an additional chapter that collects all of the case-specific information in a single place.

The document is extremely long and goes, especially in Chapters 5 and 6, into very small details. Chapter 6 is almost not readable due to the huge amount of information that is presented – what is missing are tables and figures that help to organize the information and provide a critical discussion. The document is a summary of the work performed so far rather than a critical review. This is what makes the document so difficult to read. The different pieces of information are never really connected to each other. This is especially true for exposure and effect concentrations that are never compared to each other – however, this is the most crucial information that is needed for any risk assessment.

It is also surprising that from the wealth of information on use and human health effects of the historic use of nanosilver, e.g., Collargol or Argyrol, almost nothing is cited and used. The basic book about the human health effects of silver should be acknowledged as it forms the basis for all human health standards up to today (Hill, W.R.; Pillsbury, D.M. *Argyria, the Pharmacology of Silver*; The Williams & Wilkins Co.: Baltimore, MD, 1939).

Stig I. Olsen

Overall, the document is a very comprehensive overview of the current knowledge on nano-Ag. I have learnt a lot reading the document, and although I am not an expert in many of the fields based on my knowledge from literature and conferences, I believe that the accuracy of the presented information is high. The clarity of presentation suffers a bit from the comprehensiveness. The wealth of information being served would require more small summaries, e.g., after each subchapter as well as more condensed presentation in the form of tables and figures. I find that the conclusions reflect the information presented in a sound way.

James F. Ranville

As stated frequently, the purpose of the document is to begin the CEA process for nano-Ag in disinfectant sprays. The role of this document is specifically to fulfill the first step in the CEA framework "Compile Information in CEA Framework" (Figure 1-2), and in this respect it achieves its purpose. The document is effective as a non-critical literature review, being a compilation of most of the relevant papers on studies of nano-Ag and "conventional" silver, although I believe there is a body of literature on the environmental health and safety of dissolved ionic Ag that was not included. Given that the document is primarily a literature review, I cannot comment on the accuracy of the information it contains, but assume that results of the studies cited have been accurately presented.

The document also serves as a brief tutorial, both on the relevant processes for nano-Ag, including behavior in the environment (e.g. aggregation, dissolution, etc.), organism exposure, and organism health effects, and on analytical methodology. This aspect of the document is also useful to a reader having minimal background in nanomaterials, but is rather basic for those already practicing in this field.

Perhaps it is an unavoidable consequence of the format of the document, but it suffers from some redundancy, which makes it quite tedious to read the document in its entirety. Many of the concepts and implications seem to be discussed in nearly every chapter. This is especially true of the summary chapter (Chapter 7), which instead of being a succinct, to the point summary, is "watered down" with restatements of things presented in the preceding chapters. Much of Chapter 7 seems to actually be "cut and pasted" from previous sections. I suggest that Chapter 7 should be looked at to see if it could be streamlined. The addition of a 2-3 page executive summary would be very beneficial.

Finally, the document is not designed to draw any conclusions, but is intended to direct efforts at identifying current information gaps, which future research will hopefully close. Therefore, I cannot comment on the soundness of the conclusions as requested.

V. RESPONSE TO CHARGE QUESTIONS

Charge Question 1

Chapter 1 provides introductory material regarding the CEA approach used in these case studies along with other background information and a discussion of terminology. Is this information accurately and clearly presented? Please comment on the utility of the chapter in providing background and support for the remainder of the document. In particular, are the figures summarizing the CEA framework and process clear? How might this chapter be improved?

Paul M. Bertsch

The chapter attempts to introduce the document and provide background information relative to the motivation for generating the document. In terms of an overall introduction, the chapter is adequate and appears accurate and reasonably clear. I would suggest moving the discussion of naturally occurring, incidental, and engineered nanoparticles (nanomaterials?) from the "Terminology section" to page 1-1, first paragraph, since this is touched upon in this section and this distinction represents more of a higher level definition/classification of nanoscale materials.

The discussion relative to how the nano-Ag in disinfectant spray was chosen for a case study is not particularly clear or compelling. It would be useful if a more specific conceptual model for nano-Ag in disinfectant sprays be developed to illustrate a life-cycle inspired risk analysis for these products. Much of the subsequent discussion (later chapters) seems to amplify the relative relevance/importance and existing data for nano-Ag in such products as fabrics, personal care products, and food storage devices, etc.

The CEA framework is fairly well presented. The captions for figures 1-1 and 1-2 need to be expanded. I do not understand the abiotic resources component in the exposure-dose facet of the CEA. It seems to me that these would be part of the product life cycle or transport/transformation/fate facets. While there is a statement concerning the relative simplicity of the CEA not capturing the real-life complexity (Page 1-2, lines 30-31), this discussion could be expanded a bit to introduce the notion of coupled processes and feedbacks between elements listed in environmental conditions, environmental media, and between each of the facets of the CEA framework. I believe this is what the authors were attempting to articulate with the comment "...numerous linkages and transfers..."

Section 1.3 could be beefed up. It seems that the chapters are organized to introduce the production and use of Ag and nano-Ag, and provide background on important characteristics of nano-Ag (Chapter 2) and then describe the facets of the CEA framework, i.e., life cycle (Chapter 3), transport/transformations, and fate (Chapter 4), exposure-dose (Chapter 5), and then impacts (Chapter 6). Outlining this in section 1.3 might help. I would also suggest having the terminology section (1.4) prior to section 1.3.

Jaclyn Cañas

The information presented in Chapter 1 is very clear and appropriately presented. The chapter is extremely helpful in laying the foundation for and providing the context in which the remainder

of the document is presented. I found both the figures and the text regarding the figures to be extremely clear. The text was helpful and easy to read and follow the steps/compartments in the figures. I also believe the inclusion of the Purpose of the Document to be useful in setting the stage and expectations for the remainder of the document. As an experienced nanotoxicology researcher of carbon nanotubes, I appreciated the Terminology section as it clearly outlined the definitions of terms that often have different meanings depending on their use. Overall, I think Chapter 1 was well written and served as a good introduction to the document. I do not have any suggestions with regards to how the chapter might be improved.

Specific observations:

Page xv, line 14. Why is this reference used when U.S. EPA 2010a is used on pg 1-1 line 15 and both lines refer to the same case study document?

Page 1-2, line 18. Same comment as the previous comment. It is not clear why sometimes the 2010a reference is used and other times the 2009d (which is the external review) reference is used. It should be consistent or made clear why the different stages of the document are referenced.

Page 1-2, line 19. CEA is first written in line 2 on the same page and therefore the acronym should be in line 2 not 19.

Page 1-2, line 26. Extra space before the period.

Robert I. MacCuspie

Chapter 1 provides a concise discussion of the relevant terminology involved. The information is presented accurately and clearly with good citations. Chapter 1 clearly states what this document intends and does not intend to accomplish.

Specific suggestions for improvement:

Figure 1-1. Summarizing the CEA framework is probably the best that can be achieved given the vast complexity of this approach. Does the color gradient from left to right signify anything beyond visual appeal?

Figure 1-2. It was quite clear CEA is meant to be an iterative process.

Page 1-9, lines 1-10. ASTM & ISO also have similar definitions to the BSI definitions of aggregate and agglomerate. Lines 10-27 make an excellent point.

Peter R. McClure

The background information presented in this chapter is clearly presented. The information on the CEA framework and process is important for the reader to understand the purpose of the document. The terminology information presented in Section 1.4 (on silver nanoparticles; conventional silver; agglomeration/aggregation/cluster and colloid; and naturally occurring, incidental, and engineered nanoparticles) is important to frame the analyses presented in

subsequent chapters. I have no general suggestions for improvement, but see a few specific comments below.

Specific observations:

Page 1-2, L8-10. The framework indicates that impacts other than human health and ecological impacts can be accommodated. The last sentence in this paragraph states that "... the focus in this case study is limited to environmental impacts." Does "environmental impacts" refer only to health and ecological impacts or some subset of "other" impacts in addition to health and ecological impacts?

Page 1-4, L14-18. This text clearly and importantly restricts the focus of the present case study to identifying and prioritizing information gaps.

Bernd Nowack

This chapter provides a good overview on what the review is about. However, what needs to be improved is the explanation for why the spray application was chosen. It is only very briefly covered that it was based on an expert process. What other products were under discussion? How was the evaluation carried out?

In Chapter 1.4, "Terminology: Colloidal Silver," more background discussion is needed. The only definition for colloids that should be used is that commonly given in textbooks on aquatic chemistry, so particles between 1 and 1000 nm (e.g., Stumm and Morgan, *Aquatic Chemistry*). The "colloidal silver" mentioned in historic publications and patents is often actually nanosilver and not particulate silver between 100 and 1000 nm, as the definition of colloids might suggest.

Stig I. Olsen

I was not knowledgeable about the CEA framework before. I find it very comprehensive, but nonetheless well described both in the text and in the two figures.

Page 1-5, line 20-21. The list of other efforts (references) is far from exhaustive, so there should be an "e.g." in front of the references.

Bottom of page 1-5 and top of page 1-6. Does raise some doubts as to what is the real purpose of the document. On the one hand it represents the "Compile Information in CEA Framework" step, but apparently only as an example since "it attempts to provide a framework for considering current and future information systematically." But isn't that framework already defined in the CEA? I don't understand why it has not gone through the whole process of compiling information and I don't think the chapter explains the reasoning sufficiently. On page 1-6, line 21 it says that the case study is meant to assist in identifying and prioritizing research – but if the study is not exhaustive how can it be used for that?

James F. Ranville

I found this chapter to be very useful, perhaps the most so of all the chapters. The figures were very clear and helpful. I don't think this chapter needs any improvement in general. It could be

helpful to list other assessments (non-nano) that have successfully used a CEA approach to provide some more background on CEA.

I'm not sure I like the term "cluster", but agree that both "agglomeration" and "aggregation" are used interchangeably and are not well defined. I might argue for "weak-aggregates" and "strong-aggregates" as descriptors that try to convey the same distinction as is attempted with the agglomerate/aggregate terminology. No mention of the term "flocculation" was made, which generally refers to processes involving polymers whereby particles are "bridged" by a single molecule. This could be an important process for constituents such as humic substances in water and high-MW proteins within cells. In the case of the former, the "bridging" effect could overcome the effects of increased surface charge, and in fact lead to a decrease in NP stability in the presence of humic substances.

Charge Question 2

Chapter 2 presents basic information on conventional silver, including data on usage and historic environmental levels. Information on the physical-chemical properties of nanoscale silver and analytic methods makes up the rest of the chapter. Is this information clear and accurate? How might this chapter be improved?

Paul M. Bertsch

Overall, the information is clearly presented and for the most part accurate. Some specific items that should be addressed for clarity and accuracy:

Page 2-3, lines 13-19. The Hornberger et al. (2000) study is discussed in detail with the suggestion that Ag was the primary cause of reproductive failure. The authors themselves indicate that Cu co-varied with Ag in the field site under question and they acknowledge that other co-contaminants not accounted for could have been involved in the observed effects. This is probably not a good example for Ag effects given the confounding variables.

Page 2-4, lines 22-32. I realize there is another section on Nanoscale silver (2.2), but neither discussion includes estimates of the quantities of nano Ag produced or how this compares to the information here (not mentioned until the life cycle 3.1). There are several papers that attempt to estimate nano Ag production, beyond the number of products discussed as part of the Woodrow Wilson Center's inventory (e.g., see Hendren et al., 2011 ES&T). Also, related to the point above about the method and rationale for choosing spray disinfectants for this case study.....how will the use of Ag in these products compare to others in terms of overall mass? Even back-of-the-envelope estimates would be useful to gain a perspective of the relative importance of this scenario.

Page 2-10, lines 1-2. In recent years, synthesis methods have been developed to produce nanoparticles, ~~and~~ including silver nanoparticles ~~in particular~~, of various shape and size distributions.

Page 2-10, line 21. ~~dissociate~~ dissolve

Page 2-10, line 26. Suggest silver ions (Ag^+), ~~sometimes referred to as Ag+ ions,~~

Page 2-10, line 27. From the particle and **influence** the particle's behavior in the environment --- **this is not clear**

Section 2.3.2. While morphology is an important parameter of nanoparticles, the discussion of crystal structure is important but not especially relevant to the particle morphology. Many particles having different morphologies can have similar crystal structures. Nanoparticles, such as TiO_2 , which can have different crystal structures (anatase vs. rutile), can have similar spherical morphologies but much different toxicities. This should be the primary point of this discussion. Furthermore, Figure 2-2 does not offer any relevant information to the discussion and I suggest deleting.

Page 2-14, lines 21-25. Charge is not the only way particles are stabilized as implied in this discussion. Amphiphilic polymers are also used as a stabilizer, as discussed on p 2-15. Table 2-2

would be more useful if the different coatings were put into some sort of perspective in terms of commercial production. For instance, PVP stabilized nano-Ag seems to be the most readily available product commercially available in large quantities. Many of the others in the table are rather exotic and either available in small quantities with special order or are not commercially available at all.

Page 2-15, lines 10-14. This is an important point that should be expanded here, i.e., the importance of the coating. It is especially important since not all components of coatings are revealed by the manufacturers or certain mixtures of materials remain on the particle surfaces following synthesis and not recognized or revealed by the manufacturers.

Section 2.3.6. The discussion on solubility needs to be beefed up. The solubility of nano Ag is a critical part of its efficacy as a biocide. While there exists emerging evidence that there are particle specific effects, many observed effects are a result of particle dissolution and release of Ag⁺. It is important that the reader realize that oxidation of Ag (0) at the surface is critical reaction prior to dissolution, since Ag(0) is very insoluble.

Page 2-18, line 18. Ensemble methods are certainly important, but the authors need to define what they mean by “preferred”, as there are differences between hydrodynamic radii and crystalline radii that are typically obtained by ensemble vs. individual particle methods.

Page 2-18, line 29. salt precipitation?

Page 2-18; 2-19. Isolating and measuring nanoparticles from/in environmental media is not only challenging, it represents a grand challenge that needs to be urgently addressed if environmental nanotechnology and risk assessment is to advance. This needs to be stressed in this section.

Table 2-3. It would be good to have representative references in this table.

Page 2-20, lines 3-8. TEM is not an ensemble method, but rather a single particle method. Is the intent to indicate multiple or orthogonal methods? You may want to include examples where micro-XRF imaging coupled to XANES and EXAFS as well as TEM have been used.

Jaclyn Cañas

I am not knowledgeable regarding the uses and environmental levels of conventional silver and thus, cannot comment on the accuracy of the information presented. However, what was presented was definitely presented in a clear and concise manner. While I am not an expert in the area of nano-Ag, I have experience with other nanomaterials, how physical-chemical properties dictate fate and transport of contaminants, and analytical chemistry. The information regarding nano-Ag physical-chemical properties and analytical methods was clearly and accurately presented. The information provided a good introduction to topics that will be discussed in the remaining chapters with regards to physical-chemical properties and analytical methods. The text accurately reflected the current issues and challenges in working with nanomaterials, especially with regards to lack of appropriate or feasible analytical methods and characterization. Therefore, I do not have any suggestions with regards to how the chapter might be improved.

Robert I. MacCuspie

Chapter 2 overall presents the information in a clear and accurate fashion. Specific points are outlined below. The authors need to make sure the point of multiple, orthogonal techniques employed to provide size distribution plots for initial stock characterization is driven home more emphatically. Many of their citations reach this conclusion as well, and it is essential for research papers to be effective and intercomparable. Further, reporting all details is critical, including those beyond the “Minimum Characterization” lists, timing of measurements, processing history such as sonication parameters (see for example Taurozzi et al., *Nanotoxicology*, 2011), dilution orders of addition, and age of stocks before use in experiments.

Specific suggestions for improvement:

Section 2.1. It would be nice to know the forms of silver most commonly found in soils, waters, etc., and the relative distribution of the most abundant forms (i.e., does Ag₂S make up 40% of all silver compounds or 98%?) across environmental compartments, if this information is known. If not, it should be identified as a critical knowledge gap.

Page 2-2, line 1. Silver nitrate solution is also used in modern medicine, for example to cauterize and/or sterilize wounds, from nosebleeds to infant bellybuttons.

Page 2-3, Table 2-1. Suggest to also include mid 1990's study on Texas estuaries, Wen, L.; Santschi, P. H.; Gill, G. A.; Paternostro, C. L.; Lehman, R. D. Colloidal and particulate silver in river and estuarine waters of Texas *Environ. Sci. Technol.* 1997, 31, 723– 731. While outside the US, this study of silver mines shows nanometric silver in mine tailings, Gomez-Caballero, J. A.; Villasenor-Cabral, M. G.; Santiago-Jacinto, P.; Ponce-Abad, F. Hypogene Ba-rich todorokite and associated nanometric native silver in The San Miguel Tenango mining area, zacatlan, puebla, Mexico *Can. Mineral* 2010, 48, 1237–1253.

Page 2-6, lines 4-5. It would appear 3.2 ppb concentrations of silver, regulated through discharge permits, is orders of magnitude lower than the 0.1 ppm (100 ppb) drinking water concentration ceiling. Please discuss why is there such a significant difference, and if one regulation makes the other unnecessary.

Section 2.3.1, Size. The document needs to discuss how the measurement methods used affect the reported size distribution, for example see MacCuspie, Rogers, et al., *Journal of Environmental Monitoring*, 2011, 13, 1212. The discussion probably belongs here, but may also be mentioned in a 2.4 Analytical Methods section. Additionally, this section should discuss the importance of reporting the handling/processing history of the materials, along with the time elapsed at each step. Silver nanoparticles are inherently unstable, releasing silver ions into solution, thereby potentially altering the size and size distribution over time. Also, recommend pointing to later discussions on metrologies of measuring size, and point to Appendix A.

Page 2-13, lines 3-4. Auffan's estimates seem off. In the reference, this claim is made in the second paragraph of the introduction, with no explanation or subsequent reference for the methodology of determining these values. A more analytical way to solve this would be as follows:

- Determine the number of silver atoms in the sphere of diameter dNP.
- Determine the number of silver atoms in a sphere of diameter (dNP - 2dAg), assuming only one layer of silver atoms is on the surface (for silver metal particles dAg = 0.288 nm, which may be much less than the diameter to subtract for metal oxides, which Auffan tended to focus on in the reference).
- The difference of these two values is the number of silver atoms on the surface, and the fraction on the surface can be determined by dividing by the number of silver atoms in the sphere of diameter dNP.

See Leff, et al., J. Phys. Chem., Vol. 99, No. 18, 1995, Page7036 for further details of how this was implemented (they used gold nanoparticles, but the same method can be extended to silver). By this method, a 10nm silver nanoparticle would have 16.3% of its atoms on the surface, while a 30nm silver nanoparticle would have only 5.65% of its atoms on the surface.

Sections 2.3.4, 2.3.5. Suggest all surface coating issues should be in one spot, and point to that section elsewhere as needed. Surface chemistry makes most sense to place length discussion.

Section 2.3.5. No reactivity is discussed in this section, thus suggest changing section title to Surface Chemistry and Coatings.

Sections 2.3.5, 2.3.6. Please discuss how formation of silver oxide or silver sulfide on surface can affect solubility, for example, Liu & Hurt's three papers in 2010-2011 in Environmental Science & Technology and ACS Nano.

Section 2.4.1, page 2-18, lines 3-13. TEM and SEM are important tools. The limitation of SEM, unable to see particles smaller than 10 nm, in most instrumental implementations, could pose a significant gap in the size analysis.

Section 2.4.1. Also, atomic force microscopy (AFM) should be considered here as well. In Appendix A, it is identified as a frequently or commonly used size technique.

Page 2-18, lines 18-19. "Ensemble methods are preferred" should be removed, as relying upon DLS alone could underrepresent or entirely miss small nanoparticles in polydisperse samples. The light hitting the photodetector in a DLS instrument is due to Rayleigh scattering, which is proportional to radius to the sixth power, r^6 . Thus, one particle (silver nanoparticle or dust) or cluster that is 10 times larger than the primary particle size will provide half of the intensity on the photodetector. DLS provides an advantage at identifying early signs of clustering. It is unrealistic to expect a TEM/SEM/AFM microscopist to count well over one million particles to identify these clusters or large particles. However, microscopies will identify small nanoparticles in polydisperse samples that DLS may never resolve. Line 23 should be amplified. The best approach is multiple and orthogonal measurement techniques (such as pairing DLS and TEM).

Section 2.4.1. TEM/SEM/AFM sample prep - Timing is critical! Glover et al., ACS Nano, 2011, 5(11), 8950 identify that high humidity and a few weeks between prep and analysis leads to artifacts of small nanoparticles.

Chapter 2 may not be the best place for this discussion, but after such attention to definitions of agglomerates and clusters, a discussion about measuring clusters and reporting them in the size distribution is needed. Additionally, suggest discussing methods of making controlled agglomerates, such as Zook et al., *Nanotoxicology* 2011, 5(4), 517.

It is worth discussing in the metrology sections the Kennedy et al., *Environmental Science & Technology* 2010 article "Fractioning Nanosilver-Importance for Determining Toxicity to Aquatic Test Organisms" (referenced in later chapters), which demonstrates an easily transferrable method for measuring dissolved silver fractions and particle fractions.

Peter R. McClure

The chapter is difficult to read and should be rewritten. It does not reflect the high quality of organization, writing, and evaluation displayed in Chapters 1, 3, 5, and 6.

The chapter could be improved by preparing more accurately descriptive titles for the various subsections. I suggest the following:

Chapter 2. Introduction to Silver and Nanoscale Silver

- 2.1. Conventional Silver: Uses, Occurrence in the Environment, and U.S. Standards
 - 2.1.1. Uses of Silver and Silver Compounds
 - 2.1.2. Occurrence of Silver in the Environment
 - 2.1.3. U.S. Standards for Environmental Silver (*Use the text starting on p 2-4, L28 and extending through p 2-6, L18*)
- 2.2. Historical and Emerging Uses of Nanoscale Silver
- 2.3. Physical and Chemical Properties of Nanoscale Silver
 - 2.3.1. Size
 - 2.3.2. Morphology
 - 2.3.3. Surface Area
 - 2.3.4. Chemical Composition
 - 2.3.5. Surface Chemistry and Reactivity
 - 2.3.6. Solubility
 - 2.3.7. Conductive, Magnetic, and Optical Properties
- 2.4. Analytical Methods to Characterize Nanoscale Silver
 - 2.4.1. Methods for Laboratory Research
 - 2.4.2. Methods to Assess Environmental Occurrence
 - 2.4.3. Methods to Assess Workplace Occurrence
 - 2.4.4. Methods for Assessing Dose and Dose Metrics
- 2.5. Summary of Physicochemical Properties and Analytical Methods

See other specific comments below for improving the writing in Chapter 2.

Specific observations:

Chapter 2. Difficult to read and often confusing. Needs rewriting.

Page 2-1, L10-13. References for these statements about background levels should be added.

Page 2-9, L8-9. Replace “as well as disperse into smaller particles or ions” with “as well as disperse into smaller particles or dissociate into ionic forms of silver.”

Page 2-10, L18-33. This information about influences of nanosilver size is repeated in Section 4.1.1. Leave here and discard Section 4.1.

Page 2-15, L12-14. Consider replacing “Their findings imply that the surface coating on the particles in a nano-Ag spray disinfectant, and not necessarily the nano-Ag per se, might be the key to product effectiveness” with “Their findings imply that surface coating characteristics can impact bactericidal effectiveness of nano-Ag sprays.”

Page 2-15 to 2-16, two paragraphs on solubility. These paragraphs on solubility should be rewritten to: (1) note that silver nanoparticles are essentially insoluble in water (since they are composed of Ag₀); (2) describe evidence that Ag₀ nanoparticles can remain suspended longer in aqueous solutions (depending on particle and solution characteristics) than larger sized Ag₀ particles; and (3) describe evidence that dissociation of Ag ions into aqueous solutions may be greater or faster from Ag₀ nanoparticles (depending on particle and solution characteristics) than from larger-sized Ag₀ particles. The current descriptions of observations by Cumberland and Lead (2009) and Griffett et al. (2009) are more confusing than enlightening. For example, did Cumberland and Lead (2009) show that dissociation from silver nanoparticles in aqueous systems was greater when human substances and sodium and calcium were added at environmentally relevant concentrations (1% “dissolved” versus what percentage in absence?)? Did they use methods that could adequately discern “dissolved” Ag (i.e., Ag⁺) from suspended Ag₀ nanoparticles? See next row for comments on the Griffett et al. (2009) citation.

Page 2-16, L3-6. Wording and citation of this finding from Griffett et al. (2009) is confusing and misleading here. They added 1000 µg/L of silver nanoparticles to 2L of 0.02-µm filtered water, noticed that most of the material sedimented within 15 minutes, characterized the suspended material as showing a range of sizes from single nanoparticles to aggregates of several hundred nm size, determined that Ag concentrations in water samples taken at 2, 24 and 48 hours after addition of the silver were constant at about 50 µg/L, and determined that Ag concentrations in samples filtered through 0.02 µm filters were about 10 times lower than Ag concentrations in unfiltered samples. Griffett et al. (2009) called the filtrate Ag “soluble silver,” but their methods could not determine what fraction of the silver in the filtrates was suspended Ag₀ nanoparticles with diameters < 20 nm versus dissociated silver ions. I could not access the Cumberland and Lead (2009) paper through HERO, so I could not determine what methods these authors used to determine “dissolved” Ag.

Page 2-18, L3-4. Replace “atomic adsorption” with “atomic absorption.”

Page 2-23, L28. Replace “e.g. in solution or in air” with “e.g., in water, air, sediments, or soil.”

Bernd Nowack

In Chapter 2.2, more information on the historic use of nanosilver is needed: much of the “colloidal silver” used in the last 100 years is actually nano-silver. The paper by Nowack et al. in ES&T (Environ. Sci. Technol. 2011, 45, 1177–1183) has shown that before 1995 all EPA-registered silver-products are actually based on nano-silver and only after this date “conventional” Ag-products were registered. So one could also say that nano-Ag is the conventional form of silver!

It should also be made clear that nanosilver was produced by scientists and companies around 1900, so it's not a new compound but has just recently gotten a lot of attention because of the “nano-hype”. A thorough review of nano-silver needs to consider more than 100 years of production and use. It is important to note that shortly after 1900, scientists knew that they were producing particulate silver with a size in the nano-range (and were able to determine the size even without TEM!). These early nano-silvers were therefore deliberately made and were not an unintentional and unrecognized formation of nanoparticles. Many patents, e.g., from the 1960s, make it clear that the companies knew that for certain functionality the silver needed to be in the nano-range (at that time called micro-micro). On page 2-8, it seems that the authors consider historic uses of colloidal silver (which actually is nano-silver) as “conventional silver”. This of course is wrong. What matters is if there were particles present in the nano-range and then it needs to be considered as nano-Ag. It is not correct to name the same compound before 1990 “conventional Ag” and after that date “nano-silver” because then this term was invented. “Conventional silver” should be restricted to uses of dissolved silver in all forms and metals salts and metals in particle sizes larger than 100 nm.

Chapter 2.3.6 covers dissolution of nano-Ag. This chapter needs to be rewritten. Metallic Ag first needs to be oxidized to Ag⁺, which can then be released into solution and it is therefore different to AgCl or other silver salts that can release Ag without any oxidation step. The oxidation of metallic Ag is covered later in the review (Chapter 4), but it needs to be made clear here that “dissolution” of nano-Ag involves two coupled processes.

Specific observations:

Figures 2.2 and 2.3 are not necessary – they contain too much detail that is not important for the document.

Chapter 2.3.6. AgCl is not nearly insoluble. Compared to environmental Ag concentrations the solubility limit is far away.

Stig I. Olsen

The chapter does not represent any of my expertise, but I do find that the chapter provides a good overview not just on conventional silver, but when it comes to physiochemical properties very much so on nano-Ag. Since the chapter provides information on both conventional silver and nano-Ag, I think it would be worthwhile to present the data on the use (and amounts) more clearly giving a better overview, e.g., how much additional Ag could be expected in the environment through the use of nano-Ag.

Specific observations:

Page 2-2, 5-17. Would be useful to have a table on the distribution of silver usage among different applications.

Page 2-8, 11-14. Considering the large use of silver in many other industrial application, I don't find it substantiated that back-ground concentrations will increase in the environment. But background for human exposure probably will.

James F. Ranville

I found this to also be a useful chapter, its contents were clearly presented, and does not need any improvement. I cannot assess its accuracy, but I assume that the data from the studies cited have been accurately presented. The great deal of concern over dissolved ionic silver during the time of heavy use in the photography industry generated many studies on its environmental fate and effects. I feel like there is more data out there on "conventional" silver that was not included. Given that many of the effects of nano-Ag may be a result of the formation of ionic silver, it is paramount to glean as much information as possible from the historical database. Were any authors from these studies or researchers from Kodak involved in the compilation of these data? There are a number of researchers I know that worked extensively on Ag and could be useful sources of data.

In discussing the chemical forms of Ag, there was no mention of silver oxides or hydroxide phases. Given that the common state of bulk silver metal is to have a "tarnish" composed of Ag₂O, I'm surprised there was no discussion of this phase, which could potentially affect nano-Ag behavior. Was Ag₂O eliminated as a result of the investigation of the literature or was it overlooked?

The PEN report is mentioned several times as a listing of nano-Ag containing consumer products. It is stated that no verification is made as to whether the products listed actually contain nano-Ag or some other form of Ag. However, another important point is not made. Once a product appears on the list it is never tracked to see if it remains on the market, so the list continues to grow and is never adjusted downward to reflect the loss of products from the market. As such the current consumer products lists may, or may not, be greatly inflated.

Although Table 2-3 is not intended to be an exhaustive list of methods, it is missing a few key techniques under the category of size distribution. Both nanotracking analysis (NTA) and disc centrifugation (DSC) are important techniques that should be included. Furthermore, we have recently published two papers on single particle ICP-MS, and at least a half dozen other investigators are preparing documents. We feel this approach may prove to be the best approach for detecting and characterizing nano-Ag. Although still relatively new, it would be useful to include this method in Table 2-3.

Pace, H.E., Rogers, N.J., Jarolimek, C., Coleman, V.A., Higgins, C.P., and Ranville, J.F. 2011. Determining transport efficiency for the purpose of counting and sizing nanoparticles via single particle inductively coupled plasma mass spectrometry. Anal. Chem., 83, 9361-9369.

Mitrano, D., Leshner, E., Bednar, A., Higgins, C.P., and Ranville, J.F. 2012. Detecting nanoparticulate silver using single particle inductively coupled plasma mass spectrometry. Environ. Toxicol. Chem. 31, 115-121.

Specific observations:

Page 2-15, Table 2.2. I would argue that hydrocarbons are not surfactants and as such should not be listed in this category

Charge Question 3

Chapter 3 summarizes information on the lifecycle stages of nano-Ag disinfectant spray products, including potential releases to the environment of nano-Ag and by-products. To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-Ag as it might be used in disinfectant spray products? To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

Paul M. Bertsch

The chapter does a decent job of summarizing the life-cycle stages of nano-Ag. The big challenge is that there is so little information. As indicated below, there needs to be greater discussion concerning by-products and multiple surface stabilizers that are not revealed by the manufacturer. In terms of disinfectant spray products, there is even less information for fabrics, food storage devices, personal care products, etc. The organization of the chapter is reasonable. There are parts of the discussion that seem irrelevant to the disinfectant spray story. However, in terms of informing planning for future research.....I do not think that the major gaps and issues are adequately defined and prioritized.

Page 3-2, lines 5-11. As indicated above, there are several papers that attempt to estimate nano Ag production in the U.S., beyond the number of products discussed as part of the Woodrow Wilson Center's inventory (e.g., see Hendren et al., 2011 ES&T).

Page 3-2, lines 12-18. This section seems out of place. As stated before, the process of oxidative dissolution of Ag (0) is important in terms of Ag⁺ release and the efficacy of many nano-Ag products as biocides and is not introduced until 4-3, and even then not clearly. The discussion on AgNO₃ and halides does not seem to fit here or at least the relevance is not clear.

Page 3-6, line 3. One problem with evaluating the particles is related to the removal of surface active agents as stated. However, this is a very common problem and our experience indicates that many manufacturers are not sure what the composition of the surface active agents are and they simply report what they believe to be the primary agent. This seems even more complicated for nano-Ag sprays, as described on page 3-8 and 3-9/10. The possible presence of multiple surface stabilizers and other by-products has many implications for evaluating materials in a systematic way and should be discussed.

Page 3-10, lines 1-20. It is not clear how this discussion relates to the nano-Ag spray solutions.

Page 3-11, lines 1-8. What about Ag speciation and Ag⁺ release?

Jaclyn Cañas

Chapter 3 does an excellent job at describing what is known and what is unknown regarding the life-cycle stages of nano-Ag. It is very clear what information is lacking, especially with regards to manufacturing and synthesis of nano-Ag in general as well in the manufacturing of nano-Ag for disinfectant sprays.

The material presented in Chapter 3 is very well organized and presented in a logical manner starting with the extraction or mining of conventional silver to synthesis of nano-Ag to potential uses of disinfectant sprays with nano-Ag to disposal (both proper and improper) of disinfectant sprays with nano-Ag. From Section 3.2.1 to the end of the chapter, the information provided is definitely informative to support planning of future research. Section 3.4 is especially informative and raises numerous needed areas of research to identify the true risk associated with using disinfectant sprays with nano-Ag. The whole chapter, from a toxicologist's perspective, is informative and raised numerous research questions to assess exposure and fate of nano-Ag used in disinfectant sprays.

The chapter could be improved by the inclusion of tables and figures. The inclusion of a summary table of the synthesis techniques with references would be useful. In addition, a figure that illustrates the life-cycle of nano-Ag from mining of conventional silver to disposal (both proper and improper) would be useful for the reader to follow along. Perhaps a table of potential uses of disinfectant sprays with nano-Ag would also be useful. Adding tables and/or figures will really add to the chapter.

Specific observations:

Page 3-1, line 14. There appear to be at least 2 different font sizes.

Robert I. MacCuspie

Chapter 3 is a clear and complete description of the life cycle analysis of nano-Ag disinfecting sprays. The information is presented concisely, and with as thorough of a literature citation as possible.

Specific suggestions for improvement:

Section 3.2.1, lines 4-5. Some nutritional supplements claim electrolysis generates their nano-Ag.

Section 3.2.1, line 17. After Faraday, Carey Lea reported in 1889 on the reduction of silver nitrate in the presence of trisodium citrate. Carey Lea M. (1889) On allotropic forms of silver. *Am J Sci* 37:476–491.

Section 3.2.1, lines 18-19. Revise to: Subsequently, many nanoscientists now view the Carey Lea method as the Turkevich gold method extended to silver.

Page 3-3, lines 5-9. This is true, as recently reported by Takesue et al., *Journal of the American Chemical Society*, 2011, 133, 14164.

Peter R. McClure

I found this chapter informative and easy to read. See comments below for suggestions for improvement.

Specific observations:

Chapter 3. Well written and informative.

Page 3-3, L7-10. Please clarify here the chemical state of the silver spherical particles produced – elemental, not ionic, silver Ag₀.

Page 3-3, Paragraph L10-24. The opening topical sentence should be amended to note whether or not data on air concentrations in, or emissions from, nano-Ag spray manufacturing facilities are available. I suspect data are not available, otherwise I would expect it to be cited and described in this section.

Page 3-2, L17-18. It would be informative to describe some of the results of the Park et al. (2009) study. What was the range of concentrations and the characteristics of the particles (i.e., size distribution, degree of clustering) detected in workplace air samples?

Page 3-2, L20-21. Replace “Available data on exposures are described in Section 5.3.2.” with “Available data on occupational exposure are described in Section 5.3.2.”

Page 3-9, L12-14. Replace “including the humans, pets....” with “including humans, pets....”

Page 3-11 to 3-12, Summary paragraph. A statement should be added stating whether or not data on air concentrations in, or emissions from, nano-Ag spray manufacturing facilities are available.

Page 3-12, L5-6. “Results of bench-scale syntheses of nano-Ag suggest that wet chemical processing is more efficient than other production processes; wet chemical processing is likely to result in lower inhalation exposures during the manufacturing stage than solid- or vapor-phase processes.” What is the evidence for the latter contention in this summary sentence? It is not mentioned, but should be, in Section 3.2.

Bernd Nowack

On a qualitative level this chapter lists what is already known about the lifecycle of nano-Ag and what information we have about sprays. However, what is missing is a quantitative evaluation. I expected from this chapter to get numbers of release to the environment during the different life cycle stages. The whole chapter remains very vague and again, it is not really a case study, but listing of qualitative data. From a case study, I expect to see quantitative data. For example, a material flow diagram showing the flows of Ag from production, formulation, use and disposal to the environment. Even if a lot of data is missing, we can still make some estimations and best guesses.

Chapter 3.1, production amount of nano-Ag. In the beginning of 2011, a paper was published that gave an estimate of the US nano-Ag production (Hendren et al. *Environ. Sci. Technol.*, 2011, 45 (7), pp 2562–2569). Why is this information not used? It's a much better value than the estimate by Mueller and Nowack (2008) that is cited in this chapter. The value by Hendren is not an estimate, but a range based on different pieces of information about the companies producing nano-Ag.

In Chapter 3.2.1, why are flame and plasma-processes not mentioned in this part? See for example Sotiriou (Environ. Sci. Technol. 2010, 44, 5649–5654) that used a flame-synthesized nano-Ag or NanoGrade, a company that produces nano-Ag based on flame processes. Demou et al. (Ann. Occup. Hyg., Vol. 52, No. 8, pp. 695–706, 2008) measured particle exposure in a nano-Ag producing facility that uses flame-synthesis. At least some of the nano-Ag on the market is therefore produced by flame-methods and need to be covered in this review.

Stig I. Olsen

Overall, the chapter includes all relevant life cycle stages in a fairly comprehensive way. The Manufacturing of Nano-Ag seems to be a bit exaggerated in comparison to the other chapters, most likely due to much more available information. Reading through the chapter, it seems that the focus on potential releases is not equal throughout the chapter – the chapters on use, distribution, and disposal have a high focus on this whereas the two preceding chapters do not. I find that the chapter on feedstock is more focused on total quantities produced than on quantities that may enter the environment, but this is a general remark to the chapter. It would be good to summarize what are the main processes for release of which compounds and what are the potential quantities (in line with my comment above, I think it would make sense to provide an overview of the potential releases. I realize it is written several times in different chapters and that there are limited data, but nonetheless this is information, even if estimated, will support planning of future research).

I am wondering about the use stage – all the potential releases mentioned are of course relevant, but will it not be all that is sprayed on that will eventually be released somehow? Or will the surfaces accumulate nano-Ag? I don't think this question has been addressed.

Specific observations:

Page 3-1, line 25. The study by Johnson et al. is not a life-cycle analysis but a mass flow analysis (MFA).

James F. Ranville

This chapter is well organized, and presents a good overview, in the abstract sense, of the issues that are likely to be important in the life cycle of nano-Ag in disinfectant sprays. Of course the issue is that no data was presented, and likely does not exist, specifically on spray disinfectants. It is fairly clear from the discussion that almost any research into how nano-Ag behaves after it is sprayed on surfaces would be valuable.

The chapter does not need to be improved for the most part. One suggestion is to try and obtain more information on the other components of the sprays. Do these sprays contain alcohols, surfactants, volatile solvents, etc.? These other, non-metal, components could be problems in themselves.

Specific observations:

Page 3-5, line 30. I would suggest changing collide to coalesce

Charge Question 4

Information on the transport, transformation, and fate of nano-Ag in air, water, sediment, and soil is discussed in Chapter 4. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of understanding regarding the known and anticipated behavior of nano-Ag in the environment. To what extent is this information presented in a manner that would inform consideration of likely exposure routes relevant to biota and human health? For each of the environmental media discussed, to what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

Paul M. Bertsch

The chapter is a comprehensive list of the major factors that are known to or thought to influence the behavior, transport, bioavailability and toxicity of manufactured nanomaterials. While it is correctly stated that there are relatively few studies on many of the processes and pathways discussed, there are a number of key publications on nano-Ag that have emerged in the past few years that are not included (indicated below). While some areas needing additional research are at least eluded to in the discussion, there needs to be a distilled prioritized list of research needs at the end of the chapter that links back to the life-cycle inspired CEA and knowledge gaps so that one does not need to wade through the voluminous material (this is true for all subsequent chapters as well). This chapter could be organized more efficiently. The environmental factors that influence nano Ag behavior are largely the same for both terrestrial and aquatic systems so repeating these in each subsection is redundant and is also confusing.

Page 4-3, lines 5-29. As mentioned previously, it is the oxidation of Ag (0) to Ag₂O that is the critical intermediate step in dissolution.

Page 4-5, lines 19-25. There are a number of papers published in 2011 that examine the transformation of Ag NPs as influenced by sulfidation as well as one that provides evidence for Ag₂S nanoparticles in biosolids from waste water treatment plants. These should definitely be included in this discussion.

Page 4-5, line 27. Abiotic factors.....what about biotic factors? Biota in soil and sediments drive many geochemical reactions that are discussed subsequently and can also have a direct influence via exudates, biofilms, etc.

Page 4-6, lines 15-18. The sulfides are very insoluble ($K_{sp} \sim 10^{-51}$) which leads to their immobilization potential. The discussion here should focus on soluble complexes.

Page 4-7, lines 1-4. As mentioned several times previously, the dissolution of Ag (0) is largely dependent on the formation of Ag₂O and the pH dependence of Ag₂O solubility (that is why dissolved oxygen is an important player).

Page 4-7, lines 14-32. It should be noted that the CCC is highly dependent on the characteristics of the surface coating, thus presenting specific concentrations (Page 4-8, lines 1-2) is only useful in this context.

Page 4-8, lines 3-18. See comments above about Ag (0) oxidation. A discussion of sulfidation of Ag (0) NPs should be included since the transformation to Ag₂S has recently been demonstrated to be a dominant transformation product and this is, of course, redox sensitive reaction.

Page 4-11, lines 28-34. There are several newer references that point to the importance of biosolids and biosolid applications to agricultural lands as an important vector for nanomaterials to be introduced to the environment.

Page 4-12, lines 10-24. There is a good deal of literature on colloid facilitated transport of contaminants that involves naturally occurring or incidental nano sized mineral phases being transported through porous media that would be relevant to this discussion.

Page 4-13, lines 6-28. There are a few more recent references on plant uptake of nano Ag and Au that are be relevant to this discussion. There also have been many studies published recently examining the uptake and effects of Au, Cu, and Ag NPs from soil to earthworms and a few on *C. elegans* (soil nematode) that should be included in 4.3.

Section 4.4.2.1, line 17. Water chemistry vs. nature of the water? Lines 17-21, Table 4-1. This is very confusing as the verbiage mentions Ag complexes, but the data is on solubility products. Complexation and solubility need to be discussed separately and association constants for Ag complexes should also be presented. Many of the solid phases listed in Table 4-1 are not environmentally relevant.

Sections 4.4.2.2/4.4.2.3. This information is largely redundant with the earlier discussion. Suggest reorganizing the chapter to cover important environmental factors that are common to nano Ag in both aquatic and terrestrial systems and then discuss specific life-cycle inspired pathways for the terrestrial and aquatic systems separately.

Page 4-18, lines 1-5. Again, this discussion seems to be mixing up solubility and complexation. AgCl is very insoluble, but in the presence of high Cl⁻ concentrations, soluble negatively charged Ag chloro complexes are formed.

Page 4-18, lines 14-18. It should be clear what media was/were used to derive K_d's.

Section 4.4.3. This section covers data and studies focused on the biosolids produced in the WWTP, which is more relevant to the terrestrial section. Also, this is the first mention of Ag₂S formation, which as described earlier is emerging as a critical transformation process (sulfidation).

Page 4-20, lines 9-15. This section needs to be re-written.

Page 4-20, lines 24-31 and Page 4-21, lines 1-12. There is a rich literature dealing with colloid facilitated transport that considers transport through porous media as a three phase system.

Pages 4-21 and 4-22. It is noteworthy that the Gottshalk study estimated biosolid Ag concentrations from nano Ag use to be ~5-6 mg/kg *currently*. There mixing scenario for biosolids in soil was extremely conservative. The EPA 503 document on risk of biosolids uses a

1:1 biosolid to soil ratio to estimate metal loading from long term biosolid application to agricultural land. Thus, the concentrations using this approach would be orders-of-magnitude higher than their estimates.

Jaclyn Cañas

Chapter 4 most definitely, accurately, and sufficiently (perhaps with exception of fate of nano-Ag in soil – see below) characterizes what is known regarding the fate of nano-Ag in the environment. The Chapter also does a good job at including expectations of anticipated behavior of nano-Ag based on conventional Ag, other nanoparticles, or the properties of nano-Ag in general. This chapter is a great resource to spark and guide future research planning. As presented, this chapter definitely has enough good information, related to both general fate principles as well as to nano-Ag specifically, to seriously inform considerations of likely exposure routes and drive future research directions in fate and toxicity of nano-Ag.

Air: The text related to the fate of nano-Ag in air is well organized and presented in a logical manner. The necessary and relevant information is present to support planning of future research. Even though there is limited fate and transport data for nano-Ag in air, the section provides sufficient information to begin to develop research questions and identifies research gaps related to nano-Ag fate in air. The only suggestion to improve this section would be to use bullets for the paragraphs that identify how nano-Ag might be released into the air, as is done for the other two environmental media sections.

Terrestrial: The text related to the fate of nano-Ag in soil was brief but informative enough to at least start to think about future research. The text presented seems very basic soil fate material that is taught in environmental chemistry courses with a few sentences specific to nanoparticles (rarely to nano-Ag). Researchers would definitely need to consult other sources to fully develop a research question related to fate of nano-Ag in soil. Perhaps the brevity of this section is due to the lack of research in this area.

Aquatic: The text related to the fate of nano-Ag in water was well organized and logically presented. Additionally, the aquatic text was very thorough and sufficiently informative to fully support planning of future research. Inclusion of conventional Ag fate in water is excellent and useful to begin to plan future research with nano-Ag instead.

Specific observations:

Page 4-6, lines 8-14. The last half of the paragraph seems a little disjointed from the first part of the paragraph. Perhaps a transition sentence to tie the two sections of the paragraph together might help it to flow better.

Page 4-8, line 26. Also appears twice in 3 words. Only one is needed.

Page 4-11, line 27. Groundwater is presented as two separate words when it is one word.

Page 4-12, lines 1-5. The two sentences presented here seem contradictory. If nanoparticles are unlikely to resuspend, then why even include it as a possible route of release into the terrestrial environment?

Page 4-12, lines 23 and 24. Groundwater is presented as two separate words when it is one word.

Page 4-12, line 16. Groundwater is presented as two separate words when it is one word.

Page 4-18, line 27. Groundwater is presented as two separate words when it is one word.

Page 4-19, line 21. Groundwater is presented as two separate words when it is one word.

Page 4-21, line 18. Groundwater is presented as two separate words when it is one word.

Page 4-23, line 25. Groundwater is presented as two separate words when it is one word.

Robert I. MacCuspie

Chapter 4 is less effectively organized. The outline is so much more subdivided compared to previous chapters; it gives some sections a stunted and incomplete feeling, and leads to much repetition. However, this does make the table of contents easier to navigate.

Specific suggestions for improvement:

General Comment. Suggest changing "throughout this document" to "throughout this chapter."

Page 4-1, line 2, Footnote 7. This important point is presented too late in the document; this should be introduced at the first usage of nano-Ag.

Page 4-1, line 10, Footnote 8. It is the opinion of this reviewer that trying to extend trends to all "nanoparticles" is too broad, much the same way extending trends to all "chemicals" is too broad. Classes of chemicals such as "cationic surfactants" have general trends, while still requiring careful analysis of specific chemicals (quaternary vs. tertiary cations, lengths and/or branching of alkyl chains, etc.); classes of nanoparticles such as "silver nanoparticles" may be more appropriate for general trends, while still requiring careful analysis of specific nano-Ag particles (surface coating, size, shape, etc.)

Page 4-2, line 12, Footnote 9. Excellent point, worth mentioning again. Please ensure this terminology is in fact used consistently throughout the entire document (not just Ch.4), to avoid propagating the confusion.

Section 4.1.1.1, Persistence page 4-3. Here, persistence only talks about dissolution into Ag+. Title should be dissolution. Persistence should include both dissolution (and reformation from ions, see comment below), and colloidal stability. It seems to this reviewer that persistence in 4.1.1.1 only refers to particles remaining in suspension in water environments. Perhaps the authors should consider particles that deposit to a clay or soil and then remain stable for decades as persistent. Perhaps use this structure: 4.1.1.1. Persistence, 4.1.1.1.1. Dissolution, 4.1.1.1.2. Particle Aggregation, Agglomeration, and Deposition, 4.1.1.1.3. Biological/Organism Transformation and Transport. (see comment below)

Section 4.1.1.2, Particle Aggregation, Agglomeration, and Deposition. "Cluster" is not used here

until line 14, which is okay but contradicts footnote 9.

After Liu & Hurt dissolution discussion, should also include reduction of Ag⁺ by humic acids to form AgNPs (Akaiige et al., *Environmental Science & Technology*, 2011, 45(9), 3895) or fulvic acids (Sal'nikov, et al., "Silver ion reduction with peat fulvic acid" *Russian J. Appl. Chem.* 2009, 82(4), 545). It is possible a cycle of oxidation/dissolution and reduction/formation could exist, increasing persistence. This cycle's equilibrium distribution of Ag⁺ and AgNPs will vary as environmental conditions (dissolved O₂, humic acid concentration, etc.) vary. As ions and nanoparticles transport through various conditions, fewer or greater numbers of particles may be found.

Section 4.1.1.1.3, Biological/Organism Transformation and Transport. If particles are present in the air, water or soil, they could adsorb onto the skin/feathers/exoskeletons/outer surfaces of reptiles/birds/insects/etc., which may then migrate and shed their skin/feathers/etc. and deposit silver in new areas. If organisms consume contaminated waters, their digestive systems may process/transform/bioaccumulate the nanoparticles, such as was observed for gold nanoparticles with filter-feeding clams (Hull et al., *Environmental Science & Technology*, 2011, 45, 6592). The reviewer recognizes this overlap with Chapter 5, and that little specific data is available. However, high-level concepts and potential scenarios should be presented, much as they are in Chapter 3 for product life cycle scenarios.

Section 4.1.1.5, Transformation, page 4-5. As written is merely an introductory sentence, and should be move to the general introduction in Section 4.1. Photochemical transformations from solar irradiance should be considered.

Section 4.1.2.1. Chinnapongse, et al., *Science of the Total Environment*, 2011 showed humic and fulvic acid provide varying degrees of colloidal stability to silver nanoparticles, slowing or preventing agglomeration likely through adsorption onto the nanoparticle surface. In section 4.4.2.2., the Chinnapongse et al. reference also applies.

Sections 4.2 and later read much better than Section 4.1.

Peter R. McClure

The rambling text between the introduction and Section 4.5 is poorly written and does not provide a clear and concise account of what is known (and not known) about the fate of silver nanoparticles in air, water, soil, and sediments. Many passages left me more confused than enlightened. Chapter 4 needs to be rewritten to present a high quality evaluation of information on this topic, similar to the evaluations presented in Chapters 1, 3, 5, and 6. See specific suggestions below for condensing and sharpening the focus of the text in Sections 4.1, 4.2, 4.3, and 4.4.

Specific observations:

Chapter 4. Chapter 4 needs to be rewritten and condensed to focus on what is known and not known about the environmental fate of silver nanoparticles and silver in aquatic systems. Sections 4.2, 4.3, 4.4 are overly wordy and are more confusing than enlightening.

Page 4-1 to 4-8, Section 4.1. Chapter 4 could be improved by deleting Section 4.1 and incorporating nanosilver specific information described or cited in this section in the subsections on the fate of Ag nanoparticles in specific environmental media: air (currently 4.2), terrestrial systems (4.3), and aquatic systems (4.4). Much of the generic information on possible effects of nanoparticle characteristics (presented in Section 4.1) on environmental fate and biological processes have already been discussed adequately in Section 2.3. The current section makes it difficult to discern what is known (and unknown) about the fate of silver nanoparticles in the environment and does not provide a concise rationale for what kinds of information need to be generated from new research.

Page 4-2, L22. Replace “as described detail in Section 2.3” with “as described in detail in Section 2.3.” But see suggestion about deleting Section 4.1.

Page 4-3, L30-32. Include a sentence or two describing data (presumably from Luoma, 2008) supporting the contention that nanoparticles having surface coatings to keep them dispersed have “greater persistence in the environment” than uncoated particles. Was this demonstrated under laboratory conditions with silver nanoparticles or some other type of nanoparticle? What was the difference in rate of dissolution of the coated nanoparticles versus uncoated nanoparticle? But see suggestion about deleting Section 4.1.

Page 4-4, Section 4.1.1.2. A poorly written section that does not add much from what was discussed in Section 2.3. See next two comments, as well. The text does not discern which statements about clustering and deposition are supported by observations of silver nanoparticles versus those of other types of nanoparticles. The first paragraph states that clusters of nanoparticles are less mobile than individual nanoparticles, but does not relate “translocation” or “mobility” to “deposition.” The text does not clearly or concisely state the expectation that clusters of nanoparticles are less mobile in environmental media because they deposit faster and to a greater extent than individual nanoparticles when suspended in air, in water, or in aqueous phases of soil. This expectation appears to be the main point of this section. See suggestion about deleting Section 4.1.

Page 4-4, L13-14. Do you mean that by reducing clusters you can increase surface area of Ag₀ nanoparticles interacting with an environmental media and increase the rate of release of ionic silver? The phrase, “thereby exploit the high surface reactivity of ionic silver” does not accurately portray these processes and ionic silver does not have a surface reactivity. What data are presented by Kandlikar et al. (2007) to support this contention? What data are presented by Tiede et al. (2009), Lowry and Casman (2009) and Handy et al. (2008b) to indicate that environmental conditions (what conditions? pH?, temp?, media?) influence clustering of silver nanoparticles in aqueous systems? Do these references provide specific data that should be discussed in Section 4.4 (i.e., environmental fate in aquatic systems)? See suggestion about deleting Section 4.1.

Page 4-4, L18-28. In this paragraph, the explanation of how particle clustering and deposition in the environment are related is garbled, and sprinkled with references without referring to the data that may support the statements. Deposition onto a water surface or land is first discussed, presumably referring to deposition from particles suspended in air, and then the discussion

switches without transition to deposition (i.e., sedimentation) in water columns. See suggestion about deleting Section 4.1.

Page 4-8 to 4-11, Section 4.2 (4.2.1 through 4.2.5). A better title would be: 4.2. Fate in Air. This is a poorly written section that uses too many words to try to convey the important points that (1) Ag nanoparticles can be released to indoor and outdoor air at various stages of the life cycle of nano-Ag disinfection sprays (manufacturing, use, disposal); (2) the magnitudes of releases into these air sources with current and expected future uses of nano-Ag sprays are unknown; (3) and although there is limited specific data about the fate of silver nanoparticles in air, deposition of nanoparticles from air is expected to be influenced by particle size (smaller nanoparticles stay suspended longer and diffuse more widely and rapidly than larger microparticles) and degree of clustering (larger clusters deposit more rapidly than smaller clusters or individual particles). Other important points to briefly mention are that nanoparticle coatings can reduce the degree of clustering of airborne nanoparticles (and thereby increase their resident time in air) and that individual nanoparticles can bind to other larger airborne particles which are subject to faster deposition. Subsections for Section 4.2 seem superfluous, since there really are not a lot of data to discuss.

The section could be additionally improved by a very brief discussion of the type of data that could be collected to increase quantitative understanding of the fate in air of silver nanoparticles from disinfection sprays. For example, one could collect air samples at various times after using a disinfection spray in an indoor space and characterize particle concentrations and characteristics (e.g., size distributions and degree of clustering) in the samples. This type of data would be helpful in developing and improving the environmental fate models discussed in Section 4.5.

Page 4-10 to 4-13, Section 4.3 Terrestrial Systems. Suggested title: Fate in Terrestrial Systems. This section also needs to be rewritten and condensed to emphasize the important types of information that are known and not known about the fate of silver nanoparticles in soil. For example, no studies are available on the rates of leaching or dissociation of silver nanoparticles through soil columns and how the rates are influenced by particle characteristics (e.g., coatings, particle size and clustering) and soil characteristics (e.g., soil pH, soil texture, and mineral and organic matter content) or on the temporal distribution of silver nanoparticles in soil systems among plants and other soil organisms, the solid soil matrix, and the aqueous phase. Quantitative data from these types of studies would be helpful in developing and improving the environmental fate models discussed in Section 4.5.

As with Section 4.2, I think that the two subsections are superfluous in the absence of pertinent data on the fate of silver nanoparticles in soils. Also, superfluous to the topic at hand are the discussions of airborne nanoparticles depositing on aerial plant surfaces and the study of plant uptake of aqueous silver nitrate by plants (Harris and Bali, 2008). Speculation about phytoremediation of silver-contaminated soil is altogether out of the focus that should be maintained in this section.

Page 4-13 to 4-22, Section 4.4 Aquatic Systems. Suggested title: Fate in Aquatic Systems. This section also needs to be rewritten and condensed to focus on what is known and not known about the environmental fate of silver in aquatic systems. The revised text should focus on the

available silver nanoparticle data pertinent to fate in aquatic systems [e.g., observations on sedimentation by Gao et al. (2009); Tiede et al. (2010); Kiser et al. (2010); Kim et al. (2010a)] and the general lack of information on the temporal distribution of silver nanoparticles among all components of aquatic systems (e.g., water column, sediments, and bioaccumulation in aquatic organisms) and how they are quantitatively influenced by particle characteristics and characteristics of the aquatic systems. As suggested for Sections 4.2 and 4.3, the revised text should note that this type of quantitative information will improve the environmental fate models discussed in Section 4.5.

The various pieces of text in this section on toxicity of silver ions and silver nanoparticles are distracting from what should be the main focus of this section (e.g., large sections of Section 4.4.2.1) and should be revised. Chapter 5 does an excellent job of concisely making inferences from toxicity data about environmental fate properties as it relates to biotic exposure.

Bernd Nowack

This chapter provides a good overview on the reactions of nano-Ag in the environment.

The most important reaction of nano-Ag in wastewater, the formation of silver sulfides, is not discussed in enough detail. There is only one study cited and presented. This reaction is extremely important as it transforms nano-Ag into another form, a silver sulfide. This results in the disappearance of the original nanoparticle and forms a silver-species that is almost completely insoluble. There is an article by Kaegi et al., 2011 (Kaegi, R., Voegelin, A., Sinn, B. et al., 2011. Behavior of Metallic Silver Nanoparticles in a Pilot Wastewater Treatment Plant. *Environmental Science and Technology* 45:3902-3908.) that describes the fate of nano-Ag during wastewater treatment and identifies and quantifies silver sulfides in sludge. This paper definitely needs to be considered. This reaction is again discussed in Chapter 5 (which is a problem of the document). The same reaction is presented and discussed in different chapters. Another example would be dissolution, which is discussed in three chapters (2, 3, and 4). See also the comments to Chapter 5.

Modeling: The Gottschalk et al. study not only modeled in Switzerland, but also in the EU and the US – the latter data are most relevant for this case study. These authors provided estimates of nano-Ag concentrations in wastewater, water, sediments, soils, and sludge-treated soils. These values – the only quantitative estimates of environmental concentrations so far – should be presented in detail as they provide the only available environmental exposure quantification.

Stig I. Olsen

This is not my expertise, but from what I have seen in papers and conferences it is an accurate reflection of the current knowledge. The information is well structured and provides a good overview and informative background for planning future research.

Specific observations:

Page 4-13, lines 16-28. It seems to me that this study does not investigate the uptake of nano-Ag, but the uptake of silver ion from AgNO₃-solution. It is however an interesting finding that silver taken up is stored as nano-particles, so formed by the plant. The mechanism of nature

forming silver nanoparticles is seen elsewhere, e.g., <http://www.nanowerk.com/news/newsid=21309.php> and Loeschner et al. *Particle and Fibre Toxicology* 2011, 8:18

Nonetheless, it cannot be concluded that plants can be used for sequestering nano-Ag for phytoremediation of nano-Ag!

Page 4-14, lines 3-10. As mentioned previously, I believe that most of the spray-disinfectant in the use stage will eventually end up in water due to washing of surfaces.

Page 4-24, Figure 4-1. The figure seems to miss an arrow from “water/soil” to ecological receptor. The figure also does not reflect the life cycle of a nano-Ag spray disinfectant where both production and disposal are potential releases to ambient air.

James F. Ranville

This chapter lays out an extensive list of processes likely to be important for nano-Ag behavior in the environment. All of the possible exposure routes are discussed and as such the chapter is quite complete. It is not quite as clear as to what direction future research should take when looking at nano-Ag transport in all the possible environmental media. Without knowing what process will be most important for the first step in the process, namely the release of nano-Ag from surfaces that have been sprayed, one cannot be sure what media should be studied in order to prioritize future research. Despite this, I do not see any need for improvement of the chapter. With respect the discussion of soils (4.3.1), no mention of the role of bioturbation of the upper zone of the soil is made. Earthworms can cause vertical migration of insoluble contaminants, such as uranium and plutonium, despite their very high soil K_d values. This process would also act on nano-Ag that reaches the soil surface.

Specific observations:

Page 4-16, Table 4-1. Solubility product constants refer to the dissolution of minerals (solids) whereas formation constants describe complexes. Thus, the title is incorrect and should read “Solubility product constants for various silver solids.”

Charge Question 5

Chapter 5 provides information on exposure, dose, and translocation of nano-Ag in humans and other biota. Please comment on the extent to which this chapter accurately and sufficiently characterizes this information and forms a basis for considering the health and ecological impacts of nano-Ag. To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

Paul M. Bertsch

The chapter represents a comprehensive examination of exposure, uptake, and dose of a wide range of ecoreceptors as well as a discussion of exposure pathways for humans. Again, as in the previous chapters, major knowledge gaps and research needs are not concisely articulated. There is once again a good deal of repetition and redundancy from earlier sections, and the scope and level of detail are sometimes not consistent as pointed out below.

Page 5-1, lines 16-21. It is important to point out here the point made on Page 5-3, lines 5-11, and again on Page 5-7, lines 4-8 that, unlike many conventional contaminants, internal dose is not necessarily required for toxicity. The nano cluster tridecameric Al species was shown in the 1980's to be highly toxic to plants, algae, and fish and the site of intoxication was cell wall or cell membranes. A similar picture is emerging with nanomaterials.

Page 5-5, lines 18-19. Unless I missed something, this statement is not consistent with either the discussion in Chapter 3 or that on page 5-4, i.e. there was no evidence presented that aquatic systems would predominate over terrestrial.

Page 5-5, lines 24-25. Where is the evidence that ingestion by ecoreceptors would be low? If the current biosolid concentrations are estimated to be ~6 mg/kg than land application could result in uptake by plants along with the transfer to herbivores (see Judy et al., 2011) or from re-suspension of soil onto plant tissue and then ingestion by herbivores.

Page 5-6, lines 1-8. There are a number of recent studies on plant and earthworm uptake of Ag NPs as well as on the uptake of other noble metal NPs (Cu and Au) which are reasonable proxies for Ag.

Page 5-7, lines 8-12. See Unrine et al., 2008; and Judy et al., 2011.

Page 5-7, lines 13-23. It might be worth characterizing these into intrinsic and extrinsic factors.

Page 5-8, lines 16-19. Suggest deleting this sentence.

Page 5-9, lines 1-3. Some studies have shown that humic substances interacting with NP surfaces can actually induce charge stabilization. NOM does play a dual role, i.e., it can promote aggregation/agglomeration, depending on the exact composition and chemistries (as discussed on

Page 5-10, lines 29-33. Also, as stated earlier, the conceptually complexation and precipitation (solubility) are distinct processes.

Page 5-9, lines 13-17. This is not entirely accurate. Most of the discussion in section 4 was focused on K_{sp}s and very little on soluble complexes. It was suggested previously that complexation constants for environmentally relevant ligands be presented in that section.

Page 5-9, line 25. Again, the terminology between complexation and solid phase formation is blurred.

Page 5-10, lines 9-13. Same comment here as above. Is the AgCl referred to here the solid phase AgCl_s or a neutral complex (AgCl⁰)? Also, working should be "...that at circumneutral pH" and delete parenthesis.

Page 5-11, lines 12-27. This discussion amplifies an earlier comment about the transformations of Ag NP in the environment, especially from the standpoint of sulfidation reactions.

Page 5-11, lines 33-36. This is inconsistent with prior discussions. While AgCl_s is very insoluble, soluble anionic chloro Ag complexes can form at high Cl concentrations. So the issue is how quickly oxidative dissolution (and then subsequent formation of anionic chloro-Ag complexes) would occur as a competing process to aggregation due to other processes.

Page 5-12, lines 14-16. There are several key new publications on the Ag in WWTP (e.g., see Kaegi et al., 2011).

Page 5-13, lines 28-30. See papers by Shoults-Wilson et al., 2011 a,b,c and Unrine et al., 2010 a&b.

Page 5-14, lines 21-26. Xu et al., 2004, reported that Ag nanoparticles up to 80 nm were transported across the outer and inner membrane of *Pseudomonas aeruginosa* and they provided strong evidence for the involvement of the MexAB-OperM extrusion pump in transmembrane transport of the Ag nanoparticles.

Page 5-17, line 31. What is being referred to hereAgCl_s or AgCl₂⁻?

Page 5-19, lines 20-26. See previous comment to this statement made earlier.

Page 5-27, lines 1-13. Finally a discussion of chloro Ag complexes! The influence of water chemistry is repeated multiple times and the message is not consistent. This needs to be integrated with most of the earlier discussions and repetition and redundancy eliminated.

Page 5-28, lines 1-9. See Ferry et al., 2009 for Au NP in estuarine mesocosms. This study is very relevant to the Ag NP story.

Page 5-30, 5.2.4.1. See Rico et al., 2011; Judy et al., 2011; Hawthorne et al., 2012; Yin, et al., 2011; Sabo-Atwood, et al., 2012.

Page 5-32, lines 1-4. See Unrine et al., 2008; 2010, 2011 a&b, Shoults-Wilson 2011a, b&c.

Page 5-32, lines 23-31. See Judy et al., 2011.

Page 5-33, lines 6-31. See Rico et al., 2011; Judy et al., 2011; Hawthorne et al., 2012; Yin, et al., 2011.

Jaclyn Cañas

Chapter 5 was very well written and organized given the amount of information that was presented. As an environmental toxicologist, the information provided was accurately presented and terminology was used correctly. The definitions at the beginning were useful and will be good for non-toxicologists that might read the document. The information included in Chapter 5 definitely provides extensive support for planning of future research as the text covers what is currently known regarding the impacts of nano-Ag and conventional Ag. In addition, the text also identifies some specific data gaps. However, as a toxicologist, even when gaps were not clearly identified, the text included is definitely sufficient enough to inspire future research questions. The summary of the chapter adequately highlighted the contents of Chapter 5.

There are a few things that could improve Chapter 5. First, a summary table or tables would be useful for all the biotic data presented, especially since there was data discussed for a few forms of Ag in a wide variety of species. Such a summary table(s) would provide the reader with a quick overview. Another improvement would be to include, perhaps in the summary, a list of questions or statements highlighting the most important research needs as brought forth by the text provided in Chapter 5. Several of these statements or questions are scattered throughout the Chapter.

Specific observations:

Page 5-23, line 25. Space is needed between 100 and nm.

Page 5-23, line 28. Space is needed between 10 and nm.

Page 5-59, line 19. The i is missing in the “as high as n males.”

Robert I. MacCuspie

Chapter 5 is very good, well written, and the material is effectively organized. Sufficient information is provided to the extent that literature reports are available. General population exposure (Section 5.3.1) nicely included consideration of susceptible populations such as children. Section 5.2.3.5, the zebrafish embryo section was very nice.

Specific suggestions for improvement:

Section 5.5.1.By-Products...Transformations, there are no transformations discussed here, just byproducts. Transformations really belong in the Chapter 4 appropriate chemistry sections, with just a reference to that here in Chapter 5.

Section 5.6, Models. Compared to other parts of Chapter 5, this seems short. Discussion of the shortcomings of the models, identifying knowledge gained from other sections or gaps that need to be incorporated into next-generation nanoAg-specific models needs to be added. For example, Liu & Hurt have shown AgNPs can dissolve and release ions; others have shown

humic acids reduce silver ions to form new nanoparticles. How could or should this knowledge be built into new models?

An additional reference on modeling exposure is by Musee, in *Hum Exp Toxicol* September 2011 vol. 30 no. 9 1181-1195.

Peter R. McClure

Chapter 5 is well organized and written and presents a logical and comprehensive evaluation of information on exposure, dose and translocation of silver nanoparticles in biota and humans. It is an excellent document to support planning for future research. See specific questions and suggestions below for improvement.

Specific observations:

Page 5-1 to 5-34, Sections 5.1 and 5.2. These sections are well written and organized. Integrated smoothly in this comprehensive evaluation of information on the biotic exposure to and uptake of silver nanoparticles is discussion of important issues about the fate of silver nanoparticles and silver ions in environmental media (water, sediments and soil) that is much clearer and succinct than the tortuous text in Chapter 4. A job well done.

Page 5-8, L2. Replace “adsorb” with “absorb.”

Page 5-12, L14-16. “Although almost no studies of nano-Ag removal in wastewater treatment were identified, a study of nanoscale titanium dioxide (nano-TiO₂) removal provides some indication of likely efficacy of nano-Ag removal during treatment.” Chapter 4 mentions a study by Tiede et al. (2010) that should be described here. The abstract of the paper (I could not access the full paper through HERO) noted that, following 6-hour incubations of a range of silver nanoparticle concentrations in sewage sludge supernatant, >90% of the silver partitioned to the sewage sludge, and that a portion of the remaining silver in the supernatant was in nanoparticle form.

Page 5-32, L31. At the end of this sentence, add “species” after herbivorous, or replace “herbivorous” with “herbivores.”

Page 5-35 to 5-48, Sections 5.3. to 5.6. Just as well written, comprehensive and informative as Sections 5.1 and 5.2. See suggestions for minor improvements below.

Page 5-36, L16-19. “The characteristics of nano-Ag sprays, such as the spray form of the product, in which particles are free and not fixed, the potential for direct exposure to nanomaterials in the product through application, and the potential for exposure through multiple routes, indicate that nano-Ag disinfectant sprays would be categorized as “high-potential-exposure” products.” I needed to read this complex sentence a few times to understand its structure. Perhaps separating it in two sentences would make it easier to read. “The characteristics of nano-Ag sprays indicated that they would be categorized as “high-potential-exposure” products. These characteristics include: the spray form of the product, in which particles are free and not fixed; the potential for direct exposure through application; and the potential for exposure through multiple indirect routes.”

Page 5-47, L12-14. “Baun et al. (2008b) evaluated the potential effects of C60 nanoparticles (Buckminster fullerenes, or 12 buckyballs) on the bioavailability of this substance, as measured by the toxicity of various organic 13 toxicants to the green alga *Pseudokirchneriella subcapitata* and the freshwater invertebrate *D. magna*.”

Suggested rewrite: Baun et al. (2008b) evaluated the potential effects of C60 nanoparticles (Buckminster fullerenes, or 12 buckyballs) on the bioavailability of 13 organic toxicants, as measured by their toxicity to the green alga *Pseudokirchneriella subcapitata* and the freshwater invertebrate *D. magna*.”

Section 5.7. Well organized and written. See specific questions and suggestions for improvements below.

Page 5-51, L28-29. “Sung et al. (2009), systemic distribution of silver in rats was reported for animals exposed via inhalation to aerosolized nano-Ag averaging approximately 18–19 nm in diameter.” Clarify if 18-19 nm refers to the particles from which the aerosols were generated or to the diameter of the aerosols.

Page 5-52, L24-25. “the investigators found that nano-Ag was rapidly distributed out of the blood to the liver, spleen.....” Replace “nano-Ag” with “silver.”

Page 5-54, L23-24. “demonstrate that the body cannot completely clear silver from all organs.” I prefer “demonstrate that there are limits to the clearance of silver from certain organs or tissues in the body”

Page 5-55 to 5-56, (p5-55 L21-32 to p5-56 L1-5). This passage is confusing and leads to some questions and comments. “The model predicted that for a person breathing through the nose, nanoparticles of 100, 10, and 1 nm in diameter had deposition probabilities in the nasopharyngeal region of 0.05, 0.2, and 0.8, respectively (Mark, 2007).” Are all of the cited predictions from Mark’s use of the ICRP model for nose breathing only? Are a combination of nose and mouth breathing expected to produce significant differences in deposition patterns?

“In other words, as the nanoparticles become smaller, they more effectively diffuse into the mucous layers where they can move farther along the respiratory tract (Witschi et al., 2008).” “Farther along” – do you mean lower in the respiratory tract? If an inhaled particle escapes deposition in the nasopharyngeal region, wouldn’t the airstream path to lower regions facilitate faster and easier transport (whether by bulk air movement of Brownian motion and diffusion) than through mucous layers?

“Ninety percent of 1-nm particles were predicted to be deposited in the nasopharyngeal region of the lung, with the remaining 10% deposited in the tracheobronchial region.” I would replace of the lung with of the respiratory tract. Are these percentages of inhaled particles? How does 0.8 probability of deposition in the nasopharyngeal region get translated into 90% deposited in the nasopharyngeal region? If 90% and 10% of 1 nm particles get deposited in the nasopharyngeal and tracheobronchial region, does this mean that 0% of 1 nm particles get deposited in the alveolar region?

“Nanoparticle deposition, especially for particle sizes of 20 nm and smaller, is governed by Brownian motion and diffusion, which allows movement of particles into the alveolar region of the lung, where larger particles (which are transported via bulk air flow) generally are not deposited (Elder et al., 2009).” Do you mean particles larger than 20 nm? Are larger particles not deposited in or not transported to the alveolar region?

“For nanoparticles between 20 and 100 nm in size, deposition probability dropped for all three regions of the respiratory tract (Mark, 2007).” Dropped compared to what? As particle diameter increased?

“Nonetheless, the ICRP model indicated that for nanoparticles measuring between 10 and 100 nm, the highest fractional deposition would occur in the alveolar region (Lynch and Elder, 2009).” Do you mean fraction of inhaled particles that are deposited in the alveolar region or fraction of particles reaching the alveolar region that are deposited?

So, is this the important bottom line (is it only for nose breathing?) -- Citing the predicted fractional deposition of inhaled particles of differing diameters-- e.g., 10, 20, 50, and 100 nm -- and comparing these to predicted fractional deposition for 1 to 2.5 μm particles in the alveolar region might be illustrative.

The following is my understanding of the predicted deposition pattern of inhaled nanoparticles in the respiratory tract, but I cannot discern this pattern in your account of information in Marks (2007), Elder et al. (2009), and Lynch and Elder (2009).

In contrast to fine particles with diameters in the 1–2.5 μm range, which are deposited mainly in the peripheral lung, inhaled nanoparticles (with at least one dimension <100 nm) can be deposited in the oral and nasal cavities, the tracheal/bronchiole region of the lung, and the alveolar region of the lung (Kreyling et al., 2002). Nanoparticles deposited in the alveolar region of the respiratory tract may be cleared from the alveolar region by: (1) macrophage phagocytosis and mucociliary transport along the tracheobronchial tree to the gastrointestinal tract; (2) translocation into interstitial tissue; (3) translocation to the lymphatic system; (4) particle dissolution with subsequent absorption into lung cells and transport into the blood; and (5) translocation of the particles into lung cells from lung surfaces and possible transport into the blood (Chen and Schluesener, 2008; Geiser et al., 2008; Kreyling et al., 2002; Oberdorster, 1988). Clearance from tracheal/bronchiole regions may occur by similar pathways. Nanoparticles deposited in the nasal mucosa also may be subject to particle dissolution and absorption into the blood or direct translocation of silver ions or elemental silver nanoparticles into the olfactory bulb of the brain via the olfactory nerve (Oberdorster et al., 2004).

Chen, X; Schluesener, HJ. (2008) Nanosilver: a nanoproduct in medical application. *Toxicol Lett* 176(1):1–12.

Geiser, M; Casaulta, M; Kupferschmid, B; et al. (2008) The role of macrophages in the clearance of inhaled ultrafine titanium dioxide particles. *Am J Respir Cell Mol Biol* 38(3):371–376.

Kreyling, WC; Semmier, M; Erbe, F; et al. (2002) Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. *J Toxicol Environ Health A* 65:1513–1530.

Oberdorster, G. (1988) Lung clearance of inhaled insoluble and soluble particles. *J Aerosol Sci* 1(4):289–332.

Oberdorster, G; Sharp, Z; Atudorei, V; et al. (2004) Translocation of inhaled ultrafine particles to the brain. *Inhal Toxicol* 16:437-445.

Page 5-58, L24-28. “Larese et al. (2009) reported that nano-Ag can pass through normal human skin (i.e., full-thickness abdominal skin) in vitro at a rate of 0.46 nanograms per square centimeter (ng/cm²) and through damaged skin at a rate five times higher. Nano-Ag particles between 7 and 20 nm can penetrate into the hair follicle, and nano-Ag particles less than 30 nm can passively penetrate the deepest skin layers, probably through the intercellular route (Larese et al., 2009).”

This description is misleading. Larese et al. did not demonstrate transport of silver nanoparticles across intact skin. Rather, they demonstrated silver transport across human skin exposed to silver nanoparticles. Their comments about possible translocation of particles through hair follicles and “intercellular” routes come from observations from experiments with other nanoparticles, not silver nanoparticles as suggested by the second sentence above.

The following contains a more accurate and comprehensive description of the data collected.

Silver absorption was detected across intact human skin samples mounted in Franz static diffusion cells and exposed to silver nanoparticles, but when the skin was abraded, rates were about five fold higher (Larese et al., 2009). The silver nanoparticles used in this experiment were coated with polyvinylpyrrolidone to prevent aggregation in an aqueous suspension. Intact and abraded human abdominal skin samples (stored for <4 months at -25°C) were exposed to 70 µg/cm² silver nanoparticles in aqueous 0.14% (w/w) ethanol diluted 1:10 with synthetic sweat for up to 24 hours. TEM examination indicated that particle diameters ranged from 9.8 to 48.8 nm, with a median of 25 ± 7.1 nm and 25th and 75th percentiles of 19.5 and 29.3 nm, respectively. Samples of receptor solutions were analyzed for silver concentration by atomic absorption spectrophotometry. With intact skin samples were exposed for 24 hours, silver was detected in the receptor solution at a median level above the detection limit (median = 0.46 ng/cm², range = less than the detection limit [0.1 µg/L] to 2.23 ng/cm²); absorption rates of silver through abraded skin were about fivefold higher (median = 2.32 ng/cm²; range = 0.43–11.6 ng/cm²). TEM of skin samples following exposure were reported to show silver nanoparticles in the stratum corneum and the upper layers of the epidermis “in some slices”.

Page 5-58, L28-32. “Samberg et al. (2010) applied nano-Ag particles 20 and 50 nm in size to the backs of pigs in solutions ranging from 0.34 to 34.0 micrograms per milliliter (µg/mL) for 14 days. TEM demonstrated the presence of nano-Ag within the superficial layers of the stratum corneum for the 50-nm particles and on the top layer of the stratum corneum for the 20-nm particles.”

It is important to emphasize that, regardless of size of silver nanoparticle applied, particles were detected only on the top or in the upper layers of the stratum corneum. Evidence for particle penetration to lower epidermal layers (some of which showed focal inflammation and edema) was not found. The latter observation led the investigators to hypothesize that silver ions, dissolved from particles in the stratum corneum, may translocate to the lower layers and cause the observed lesions.

Page 5-62, L8-12. “Deposition of nano-Ag in the human lung differs from that of conventional silver. Nano-Ag is more likely to enter the alveolar region and translocate to other tissues, while conventional silver is more likely to be taken up by macrophages in the lung and excreted (Elder et al., 2009; Lynch and Elder, 2009). Following inhalation, nano-Ag can translocate from the lung to the bloodstream via the mucociliary escalator and subsequent ingestion, through passage into the lymph nodes, via alveolar epithelial cells, or via absorption in the olfactory bulb (Ji et al., 2007).”

As a summary statement of the information on this topic, I prefer the following:

Deposition of silver nanoparticles in the human respiratory tract is expected to differ from that of silver particles in the 1-2.5- μm size range, but the degree to which this difference in lung deposition may quantitatively influence distribution to other tissues is unclear. In animal studies (Sung et al., 2009; Ji et al., 2007; Takenaka et al., 2001), the finding of elevated silver concentrations in extrapulmonary tissues and blood following inhalation exposure to aerosols of silver nanoparticles provides qualitative evidence of absorption of silver by the respiratory tract, followed by distribution to other tissues. The finding of very high silver concentrations in the lungs following exposure, compared with other organs, however, suggests that, at the tested concentrations, translocation to other tissues is not extensive. Possible routes of translocation to other tissues following deposition of silver nanoparticles in the respiratory tract include direct translocation to the brain olfactory bulb from the nasal olfactory epithelium via the olfactory nerve, translocation (of particles and silver ions) to lymph nodes and blood following alveolar deposition, and translocation via mucociliary clearance to the digestive tract following macrophage engulfment of alveolar deposited particles.

Page 5-62, L16-17. “Healthy skin exposed to nano-Ag resulted in dermal absorption, which appears to depend on exposure conditions, particle size, and other factors. Conventional silver is not expected to cross the skin barrier.”

These sentences imply that silver nanoparticles may lead to more absorption than conventional silver following dermal exposure, but I know of no direct empirical data to support this.

It is more accurate to say: Silver absorption has been demonstrated across healthy human skin samples exposed to suspensions of silver nanoparticles, but the degree to which this was due to transport of silver nanoparticles or silver ions released from particles in the stratum corneum is unknown. Rates of silver absorption were five-fold higher in skin samples damaged by abrasion.

Bernd Nowack

This chapter (and even more so chapter 6) contains a lot of text that is nicely grouped into sections. However, what is almost completely lost is the general overview – the reader is buried in a lot of information that is not equally important. It is necessary to add tables, figures and schemes that highlight the important aspects. An example could be that on page 5-18, where a scheme is added showing the different interactions with algae and summarizing the current state of knowledge. Also, figures taken from some of the key papers would help to identify important results. In the current version, important and marginal results are presented side-by-side.

Page 5-12: The TiO₂-data in wastewater should be removed and replaced by a description of the study of Kaegi (2011) that was mentioned above. When a specific Ag-study is available, it is not necessary to present data of a nanoparticle with completely different reactivity.

Page 5-12: It is important to note that the nano-silver sulfide that Kim et al. identified was the only Ag-containing solid that was identified. Because the majority of the silver flow in a wastewater treatment plant comes from non-nano sources, all silver forms must have been transformed naturally into nano silver sulfide. Kaegi et al. have also shown that nano-Ag is transformed into the same silver sulfide that is then discharged to some extent into natural water. This means that the fate of silver in the environment is the same for conventional silver and nanosilver because the wastewater treatment plant transforms all silver forms into the same silver sulfide. This has enormous implications for the risk assessment of nano-silver that reaches the environment through wastewater treatment plants. It is not possible to separate the discussion of nano-Ag from total silver flows.

Consumer exposure: A study that needs to be considered is “Size-fractionated characterization and quantification of nanoparticle release rates from a consumer spray product containing engineered nanoparticles” (J Nanopart Res (2010) 12:2481–2494). It provides information on the form of nano-silver that is released during spraying.

Page 5-40: The study by Demou et al. (Ann. Occup. Hyg., Vol. 52, No. 8, pp. 695–706, 2008) on occupational exposure during production of nano-silver should be used here.

Stig I. Olsen

The chapter is very comprehensive and sums up as far as I can tell the available information. Due to the vast amount of info mixed with the unknowns, it is a bit difficult to read. The summary at the end does provide a good overview.

It is surprising that the potential increase of silver concentration estimated on page 5-5 is so high, given that a large percentage of nano-Ag in waste water should be estimated to be removed in the sludge.

Most of the text (this goes also for many of the other chapters) present in a comprehensive way a good summary of individual studies. It would be worthwhile to sum up/conclude in each subchapter. Also, summing up actual knowledge, e.g., exposure vs. internal dose, which are now referred in the text would be good to present in tables.

I find that there is a considerable amount of information that is repeated several times, reflecting that the structure is perhaps not entirely clear.

James F. Ranville

The chapter does a good job of presenting all of the available information on exposure issues with respect to nano-Ag in general. Again the problem is that no information specific to nano-Ag containing disinfectant sprays is available. So in this respect, it makes it rather obvious where future research is needed. However, given the extreme breadth of possible exposure routes, it is hard to see what area of exposure research will be most fruitful. The chapter is well organized and needs no improvement.

Charge Question 6

Chapter 6 characterizes factors that influence ecological and health impacts of nano-Ag and discusses the currently available scientific evidence regarding these impacts. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of the science. To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

Paul M. Bertsch

As with Chapter 5, this chapter provides a comprehensive examination of information on Ag NP effects to ecoreceptors and various mammalian cell lines. It is well written and overall captures the literature well. Many of the references pointed out in the comments of the previous section are relevant to this chapter as well. As stated previously, it is important that the key knowledge gap and research needs be specifically articulated and summarized.

A lot of the information in 6.1.1 is repetitive with previous sections, even though a good review of toxicity related to varying parameters are covered. Perhaps this overall is unavoidable.

Page 6-3, lines 1-18; lines 26-34. Somewhere in this introduction it might be pointed out how difficult it often is to conduct systematic studies varying particle size, morphology, or surface coating chemistries. Certain coatings are often not available in the same sizes/morphologies as others and then there is the relative stability of the particles as a function of size/morphology that varies depending on the surface coating. Solubility of the particles can also co-vary depending on size x surface coating interactions. This is important to put much of the subsequent discussion in context.

Page 6-8, lines 3-7. Oxidative dissolution is finally introduced!

Page 6-8, lines 13-33. Generally, positively charged and hydrophobic coatings on a number of nanoparticle types have been shown to induce much greater toxicity than more negatively charged or more hydrophilic particles in a number of receptors and cell lines as mentioned on page 6-9.

Page 6-14, lines 5-22. Sulfidation needs to be introduced as a critical transformation process in WWTP.

Page 6-15, lines 1-6. See references suggested earlier.

Page 6-21, lines 5-18. See Dubchak, et al., 2012

Page 6-23, lines 26-29. This is not consistent with the discussion concerning size exclusion limits in Chapter 5, where I pointed out this reference.

Page 6-24, line 2. I believe this reference was just Neal.

Page 6-41, lines 1-12. See Unrine et al., 2008 concerning the development of a conceptual

particle biotic receptor model.

Page 6-4, lines 13-16. See references suggested above for terrestrial organisms.

Jaclyn Cañas

In my opinion, the information provided in Chapter 6 is accurately presented and sufficiently characterizes the state of the science regarding nano-Ag. Chapter 6 is very well organized. The content is presented in a clear and logical manner. The sections on Mode of Action at each organism level were especially useful to summarize all the toxicity data presented in the previous section and highlight significant findings of studies. These sections, in particular, are very useful to support planning for future research as synthesis information was provided that highlighted major overarching trends observed related to nano-Ag toxicity (especially when compared to conventional silver or with regards to effects of size on toxicity). Overall, the logical presentation of data by organisms (microorganisms, algae, aquatic, etc.) in conjunction with the Mode of Action data is extremely useful to support planning of future research as data gaps are evident. The only improvements identified for Chapter 6 are related to grammar and formatting issues which are listed below as Specific Observations.

Specific observations:

Page 6-21, line 2. There is an extra space before the period that needs to be removed.

Page 6-40, line 16. It should be “estimate” instead of “estimated.”

Page 6-50, line 2. The reference year 1954 appears to be a larger font.

Page 6-50, lines 5 & 6. The 3 in AgNO₃ needs to be a subscript.

Page 6-50, line 31. The reference year 2010a appears to be a larger font.

Page 6-51, lines 8-17. Why not include the nano-Ag study by Li et al., 2010a in the next section on nano-Ag effects?

Page 6-53, line 3. The 2 in nano-TiO₂ needs to be a subscript.

Page 6-54, line 26. Only one ‘also’ is needed.

Page 6-68, lines 6-7. There is some sort of spacing or font issue between the two paragraphs.

Robert I. MacCuspie

Overall, Chapter 6 is very good and well written. It is nice to see that comparisons to photography industry wastewater emissions were included. Nice comparisons of Nano-Ag specific to conventional silver throughout chapter.

Specific suggestions for improvement:

At end of Section 6.1.1.1, Size. Discussion on how agglomeration/aggregation within size

distribution has been shown to affect toxicity tests such as hemolysis, where both total silver concentration and agglomerate size affect results (Zook et al., *Nanotoxicology*, 2011). Also, page 6-8, line 24, ...prevent formation of clusters in solution, OR, can control the size of cluster formation (Zook et al., *Nanotoxicology*, 2011).

Page 6-8, line 26. When used as a bactericide in water... Or when used as a bactericide attached to ceramic point-of-use water filters, Oyanedel-Carver et al., *Environmental Science & Technology*, 2008.

Page 6-10, lines 22-24. Sonication can be highly irreproducible if details are not reported, see Taurozzi et al., *Nanotoxicology*, 2011.

Page 6-57, Line 5. Units should be mg kg⁻¹ d⁻¹, or mg/kg·d (no dash)

General comments:

Much of the historical effects of colloidal Ag are ignored.

- Early 1900's medical treatments with colloidal Ag, "Argyrol" (Schack W (1960) *Art and argyrol. The life and career of Dr. Albert C. Barnes*. Thomas Yoseloff Press, New York).
- Pool treatments with colloidal Ag, "Silver Algaeden" (Height MJ (2009) *Evaluation of hazard and exposure associated with nanosilver and other nanometal oxide pesticide products*.

<http://www.regulations.gov/search/Regs/contentStreamer?objectId=0900006480a52512&disposition=attachment&contentType=pdf>

There is a general issue regarding the use of "uncoated" ...it is not possible for Ag(0) metal NPs to be stable in solution without having some colloidal stabilizing agent (small molecule for electrostatic repulsion, adsorbed protein/NOM for steric). No "naked" or "uncoated" singly-dispersed particles exist in solution. Using uncoated typically refers to "citrate stabilized" before adding an intended coating (polysaccharide, PVP, etc.). While authors use this to convey differences efficiently when comparing surface coating effects, it leaves out the critical control information of what the uncoated particles were coated with. The reviewer recognizes that the authors of the document were transmitting the language that the authors of the citations used, however in the broader context of this document it can create an issue.

Peter R. McClure

Chapter 6 is well organized and written and presents a logical and comprehensive evaluation of information on factors that may influence ecological and health impacts of silver nanoparticles and the state of knowledge on possible impacts on ecological and human health. It is an excellent document to support planning for future research. See comments below for specific questions and suggestions for improvement.

Specific observations:

Chapter 6. These sections are well organized, well written, and present a logical and comprehensive evaluation of what is known and not known about the ecological and human health effects of silver nanoparticles. Some comments follow on a few of the cited effects in laboratory animals; a few minor editorial changes are also included.

Page 6-31, L24-25. Replace “Authors did not investigate the effects of silver ions on *C. riparius*,...” with “The authors did not”

Page 6-32, L27. “authors observed delayed hatching” suggested replacement “delayed hatching was observed.”

Page 6-40, L16. Replace “are used to estimated predicted” with “are used to estimate predicted.”

Page 6-58, L22-25. “Decreased tidal volume, minute volume, and peak inspiration flow and incidences of alveolaritis, granulomatous lesions, alveolar wall thickening, and alveolar macrophage accumulation were observed in both male and female rats exposed to the highest dose of nano-Ag in whole-body exposure chambers for 90 days, though not all effects were statistically significant.”

You may want to examine and cite a later report by Sung et al. of the histopathology from the same study:

Sung, JH; Ji, JH; Park, JD; et al. (2009) Subchronic inhalation toxicity of silver nanoparticles. *Toxicol Sci* 108(2):452–461.

I think the following more accurately describes these effects reported by Sung et al. (2009, 2008):

Decreases in tidal volume, minute volume, and peak inspiration flow in males and increases in incidences of alveolar macrophage inflammation, chronic alveolar inflammation, and mixed cell perivascular infiltrate in males and females were statistically significant in the high-dose group, compared with the control group.

Page 6-60, L14-16. “These findings support the conclusions of the subchronic oral study conducted by Kim et al. (2008) and the subchronic inhalation study conducted by Sung et al. (2009) that the liver and bile ducts are targets for nano-Ag toxicity.”

I agree with this conclusion, but the important findings for increased incidences of bile duct hyperplasia in high-dose males and females in the 90-day inhalation study of rats (Sung et al., 2009, 2008) are not cited in this section. They should be mentioned.

Page 6-61, L3-6. “Although macroscopic observations revealed no gross edema or erythema at any tested dose, microscopic observations showed dose-dependent increases in morphological changes. Common morphological changes observed at the highest dose of nano-Ag were edema, focal inflammation, and epidermal hyperplasia with rete pegs extending below into the dermis.”

You might want to amend this with some of the following:

- 1) the lesions were observed in skin layers under the stratum corneum;
- 2) the effect was dose-related, with slight intracellular and intercellular epidermal edema at 0.34 µg/mL, moderate epidermal edema and focal epidermal and dermal inflammation at 3.4 µg/mL, and severe epidermal edema with severe focal dermal inflammation, epidermal hyperplasia, and parakeratosis at 34 µg/mL.

- 3) particle size or washing did not affect the observed skin responses;
- 4) Samberg et al. (2010) hypothesized that the lesions in layers under the stratum corneum were caused by silver ion flux into the lower layers from the particles in the stratum corneum. TEM examination of skin detected no particles in the skin layers showing lesions; particles were found only on the surface of the stratum corneum or with superficial layers of the stratum corneum; and
- 5) no examinations of nondermal tissues were conducted.

Page 6-61, L8-10. "Similar results in pigs exposed dermally were also reported by Nadwory et al. (2008), with some increased inflammatory cell apoptosis, decreased expression of pro-inflammatory cytokines, and decreased gelatinase activity observed."

The abstract of this study does not reflect the effects you describe (see below). I do not have access to the full report and a more in depth description of this study does not appear to be in Appendix C. Please check the accuracy of your description against the full report.

The anti-inflammatory activity of nanocrystalline silver was examined using a porcine model of contact dermatitis. Inflammation was induced with dinitrochlorobenzene and then treated daily with nanocrystalline silver dressings, 0.5% silver nitrate, or saline. Erythema, edema, and histological data showed that nanocrystalline silver-treated pigs had near-normal skin after 72 hours, while other treatment groups remained inflamed.

Bernd Nowack

Same problem here as with Chapter 5, just more severe: The main problem with this chapter is that it contains a lot of text that is nicely grouped into sections. However, what is lost is the general overview – the reader is buried in a lot of information that is not equally important. There are 68 pages and only a single table and no figure! It is absolutely necessary to add tables, figures and schemes that highlight the important aspects. Figures taken from some of the key papers would help to identify important results. In the current version, important and marginal results are presented side-by-side. Tables could be used to combine the key results from different sections and to help keep the overview. In the current version, this chapter is almost impossible to read.

Page 6-40. The authors use the PNEC value given by Mueller and Nowack in 2008. However, it would be better to use the newer value from the Gottschalk et al. (2009) paper. Why did the authors not try to derive their own PNEC value by taking advantage of the fact that in the last few years a lot of new data were published that would allow the derivation of a much better PNEC than the values from 2008 or 2009? This is a main problem of the whole review, it remains very qualitative and does not aim to quantitatively evaluate the data.

Section 6.3.3. It is written: "Nano-Ag, although not purified and produced extensively until recently, has long been present as a fraction in conventional silver, and particularly in colloidal silver, though not as intentionally engineered particles." As discussed above, this is not correct. The historically used colloidal silver was always in the nano-form and was deliberately produced and engineered in nanosized form. Most of the silver exposure data that are discussed in the following therefore correspond not to "conventional silver," but in fact to nanosilver, e.g., Collargol, Agyrol and so on.

Section 6.3.3.1. Medical Use Studies: The first of these studies were performed around 1900 and in 1939 a book was published summarizing the effects of silver and nano-silver (Hill, W.R.; Pillsbury, D.M. *Argyria, the Pharmacology of Silver*; The Williams & Wilkins Co.: Baltimore, MD, 1939). This section definitely needs to consider this old literature.

Stig I. Olsen

The chapter provides a very comprehensive overview of current knowledge of toxic effect and actions. It is good that for ROS the known mechanisms is described. I also appreciate the subchapters summing up and providing the hypothesized mode of action – this is something I missed in the previous chapters. One aspect that has received some attention is the possible resistance development towards antibiotics as cross-resistance developing. This is not at all mentioned here – I am not sure if it is important (again this is not really my expertise), but I do believe that it deserves attention in the text. The chapter is fairly well structured.

Specific observations:

Page 6-7, several lines. Some strange {...} with number inside occurred?

Page 6-13, Table 6.1. While I appreciate seeing a table, I miss an explanation of the toxicity values (or unit if applicable).

James F. Ranville

My comments for this chapter are the same as for chapter five. The chapter seems sufficient in its current state to direct future research.

One thing that seems lacking is a discussion of photochemical effects. Given that the nano-Ag will be deposited on surfaces, unlike nano-Ag in textiles or other consumer products, it will have direct exposure to light. What is known about the extent to which reactants such as ROS can be formed from interaction of light and nano-Ag?

Charge Question 7

Chapter 7 summarizes the information and research questions presented in the nano-Ag case study, as well as discusses the role of case studies in the refinement of research strategies and potential future assessment efforts. We would appreciate comment from the peer reviewers on the integration of evidence in this chapter and its usefulness in supporting future development of research strategies and assessments. How might this chapter be improved?

Paul M. Bertsch

While this is a workshop summary report, it is quite repetitive and not particularly effective at distilling down to a specific, concise, prioritized list. Section 7.3.1 comes the closest, but after wading through this massive document the overall recommendations are very broad, general, and at a pretty high level. As stated previously, the distillation of the key knowledge gaps and research questions for each chapter might be a more useful approach.

Jaclyn Cañas

Chapter 7 is an excellent and very useful synthesis of what was presented throughout the whole document. The appropriate information was selected for inclusion in this summary chapter to support future development of research strategies and assessments. The inclusion of the CEA workshop findings was also useful to further support future development of research strategies and assessments. One could read this chapter alone and gain an understanding of the current state of the science regarding nano-Ag. The research questions (or needs) were more clearly identified in this chapter than throughout the previous chapters.

Specific observations:

Page 7-13, Heading. Needs to be in bold.

Page 7-16, line 3. There is an underscore or a space underlined between uptake and is.

Robert I. MacCuspie

As this is the chapter many people will choose to read if they do not have time to read the entire document, it warrants most attention. It is indeed quite a useful summary of the entire document as written. It would be useful to have a section that specifically identifies key knowledge gaps that the scientific research community and funding agencies could focus future efforts on to achieve maximum effectiveness of those investments. A minor suggestion for improvement would be to match the order of topics in the outline with the order of topics in the flow of the CEA Figure 7-1, even though this was easy to read with good transitions between sections.

In regards to Figure 7-1, the workshop report draft 3 (as cited in this document) in Section 3.2.2.2 "PowerPoint slide" on page 3-35, provides more details than Fig 7-1 of this document, which is a reproduction from Figure 1-1 earlier in the document. As this chapter intends to be a detailed summary, a detailed pictorial should be presented.

Overall, Chapter 7 is very nicely done. For example, in Section 7.2.1.3, page 7-7, a nice

example of sophisticated research instrumentation solving real world problems is provided with FFF-ICP-MS.

Peter R. McClure

This chapter is generally well written. It is somewhat repetitive of summaries and conclusions from the previous chapters, but serves effectively as a comprehensive, detailed “executive summary.” See below for a few suggestions for improvement.

Specific observations:

Chapter 7. This chapter is an overview prepared from the generally well written summaries and conclusions of the preceding chapters. It is repetitive, but serves effectively as a comprehensive, detailed “executive summary.” See previous comments about certain summary statements in Chapters 5 and 6.

Page 7-13, L11-12. “In fact, some plant species take up and accumulate silver in nanoparticle form after silver exposure, suggesting that metal-tolerant plant species could be used for phytoremediation.”

Speculation of phytoremediation seems out of focus for this document. What is more important to note is the possibility of ingestion of silver-contaminated plant materials by humans and other herbivorous species.

Page 7-14, L15-16. “One study shows that most of the nano-Ag removed during treatment processes ends up in sludge soils;” Replace sludge soils with sewage sludge.

Page 7-17, L25-29. “In both consumer and occupational populations, exposure can also occur through hand-to-mouth contact from touching or handling treated surfaces, a behavior that is particularly prevalent in children. Higher metabolic rates and greater consumption of food and water per body weight also indicate that children could be a susceptible population to nano-Ag spray use.”

For silver (nano or otherwise), I suspect that higher metabolic rate per body weight is not as pertinent as the other factors noted here.

Page 7-18, L10-11. “Studies show that nano-Ag is more likely to enter the alveolar region and translocate to other tissues than conventional silver.”

See my previous comments about the differences in deposition patterns between nanoparticles and um-sized particles.

Page 7-18, L13-14. “Although conventional silver is likely not taken up after dermal exposure, data indicate that nano-Ag can cross the dermal layer under some circumstances.”

See my previous comments about this issue in Chapter 5.

Page 7-21, L30-32. “Findings indicate that nano-Ag exposure via one of several routes (e.g., oral, intravenous) can lead to gene expression changes, inflammatory response in the liver and kidney, and adverse functional effects in the lungs, heart, intestine, and spleen.”

This discussion should be expanded to note that toxicity studies in rats repeatedly exposed to certain silver nanoparticles by the oral and inhalation routes are available.

Bernd Nowack

This chapter is useful for all of those readers that don't want to go through 200 pages, but want a concise overview of the most important results. The main problem is that no references are given and therefore the usefulness of this summary is greatly reduced, in fact, it's almost useless as a stand-alone summary. For a normal few-page summary it is okay not to cite any references, but for this extended summary of more than 20 pages it is absolutely necessary that the main messages are underlined with references. It is even more important that this summary be used alone, as the text in some of the chapters is so long and will be hardly read at all.

Stig I. Olsen

The chapter summarizes the information and integrates the evidence presented in the preceding chapter very well. I don't think the chapter can stand alone, but having gone through the preceding chapters it gives a very good overview for support of research strategies.

I fully agree on the research priorities derived from the workshop and mentioned in the subchapters of each main theme (life cycle stages, exposure, effect), but it lacks a summary of the overall priority in chapter 7.3.1.

Page 7-1, line 12-13. The “extended cradle-to-grave life cycle approach” is mentioned and I agree that such an approach is necessary to be able to evaluate the potential risks and identify in which life cycle stages the highest risks may occur. This approach has been used in the European Risk Assessment for decades.

Page 7-19, line 20. I don't understand the term “lifestages” and how it is applied in this context. Is it lifestages of biota and humans?

Page 7-23, line 7. Would be valuable information to know how many participants in the workshop.

James F. Ranville

As I have discussed previously, I believe chapter 7 could be significantly improved by not restating so much of the material presented in the other preceding chapters. Using a case study approach is a good strategy in general. In this case, the issue is that none of the case studies actually involved the disinfectant sprays under consideration. Although the studies presented are probably all very relevant, they do not directly address the material of concern. This leads to the obvious conclusion that a direct exposure and effects studies of disinfectant sprays will be helpful.

Specific observations:

Page 7-7, line 20. It is stated that one analytical limitation is the inability to differentiate natural nano-Ag from engineered nano-Ag. I would argue that it is highly unlikely in most systems there is any appreciable natural nano-Ag. It is likely that the nano-Ag present in a system, especially in wastewater, could be an altered form of engineered nano-Ag or the result of anthropogenically introduced dissolved Ag.

Charge Question 8

For the document as a whole, are there ways to improve the structure, scope or presentation of information to better support the identification and prioritization of research needs by diverse stakeholders?

Paul M. Bertsch

The document could be improved by reducing the duplication and redundancy and reducing its length. Furthermore, it is important that the various chapters that cover the same material communicate consistent information. A more specific life-cycle inspired conceptual model should be developed with a detailed figure illustrating the key knowledge gaps to accompany the more general CEA diagram in the first chapter. This figure could then be referred to in subsequent chapters to tie together the relevant information from the review of the literature. Furthermore, a research need or knowledge gap summary section for each of the chapters with a prioritized list of knowledge gaps and research needs would be extremely helpful. As currently constructed, the document is very long and cumbersome and the reader has to search to identify important research needs.

Jaclyn Cañas

Overall, the document is well written and the structure, scope and presentation of information presented is excellent. One possible change (which would be major), would be to separate Chapters 5 and 6 into smaller chapters that can have exposure, dose, and effects information for each study presented in one place. For example, separate them out into 3 chapters: 1) Aquatic, 2) Terrestrial, and 3) Human. This is only a suggestion that would improve the overall structure and eliminate some duplication. However, it is not necessary to better support the identification and prioritization of research needs by diverse stakeholders.

Chapter 7 identified the top priority research questions, needs, and data gaps more clearly than what was previously done in the Chapters 2-6. Adding a section at the end of each chapter (perhaps even in the summary) that clearly identifies the most pressing research needs. Using 1,2,3 or A,B,C would clearly define these research needs. This addition in each chapter would strengthen the support for identification and prioritization of research needs by diverse stakeholders.

Robert I. MacCuspie

This document clearly summarizes that significant progress has been made regarding research into the nanoEHS hazards and exposures of nano-Ag. This document is quite useful and should accelerate further the iterative process of CEA through this summary and the identification of key knowledge gaps and research needs.

The Appendices are very useful references, and add significant value to the document.

It would be useful to have a more succinct summary, perhaps in Chapter 7 or perhaps in one of the appendices, such as a table listing (in common units) the exposure level and biological response of all studies. The results should be ordered by increasing exposures, with a column

stating "toxic/sub-lethal/ no effect", so readers can easily compare at what levels the responses begin to occur to which nanoparticle sizes/surface coatings/etc. and in which organisms. While the desire certainly exists to test every specific formulation of nano-Ag exhaustively for safety and toxicity, this must be balanced with the hurdle this places on bringing novel competitive products to market that benefit the consumer and society. Perhaps it is worth considering how does this balance compare to other chemicals or materials evaluated in the CEA context?

General comments:

There is a very heavy reliance on the PEN reports, compared to other non-profits, NGO's, or think-tanks.

More focus should be placed on the analytical methods and metrology discussions. For example, key knowledge gaps and research priorities should be identified. How to measure dissolved silver? Challenges on size? Extraction techniques from complex environmental media?

General comment prompted by a statement in Section 7.2.1.5 on page 7-8, line 12. Analytical methods are a high priority area for accelerating the development of nanomaterial EHS risk assessment and should continue to receive attention. Few affordable, rapid techniques exist for qualitatively and quantitatively identifying low concentrations of silver nanoparticles in complex environmental or biological samples, and efforts should be made to overcome this challenge. However, Appendix A provides an overwhelming selection of analytical methods for the many types of characterizations that are desired, and careful scientific judgment can often lead to acceptable answers given modern instrumentation constraints. Additionally, better biological testing is just as needed as analytical methods. There is no standardized cell culture, in-vitro or in-vivo test, or even series of tests that can provide the information needed to determine hazards. Much like for size characterization, where multiple orthogonal measurements providing size distributions tells the total picture of the size of the material, and is often missing from most reports, for biological testing multiple orthogonal and thorough measurements are required. There is no one cell culture test that can definitively tell if a small molecule chemical drug or pesticide molecule is safe to use and non-toxic. For example, research papers often report one kind of test (cell death) for one specific cell line (such as kidney cells or liver cells). The interactions between types of cells, organs, and tissues can often only be determined through in-vivo testing, but is not always directly applicable to humans. As much pressure should be put onto the biological community to fundamentally improve their screening tools as is put onto the analytical methods community. It is equally challenging to assure absolutely no small (say less than 2 nm diameter) silver nanoparticles exist in a sample, as it is to assure a given silver nanoparticle formulation is completely safe at a given level for humans or the environment.

The combination of rigorous physical, physicochemical, and biological characterization is not often seen in the literature, although this challenge has become more recognized in recent years. Yet, too often, those entering the field from one background or the other do not have the understanding or technical ability to achieve all of these physicochemical and biological characterizations.

Specific observations:

General. *in vivo* and *in vitro* are typically italicized.

Appendix A comments. Very good reference, great detail. A.2, A.12, include Small Angle X-ray Scattering (SAXS). A.2, A.9, A.12, include nanoparticle tracking analysis (NTA)

Peter R. McClure

See my overall impressions above.

Bernd Nowack

Any remarks to the general structure have been made under the other charge questions.

Stig I. Olsen

In several comments above I mentioned the organization of the available data in tables or the like in order to get a better overview. Prioritizing and planning future research requires not just the identification of available information, but also the interpretation of current knowledge in terms of where research would be most profitable (and a better overview of the data in terms of tables or figures would improve this). I think this is a bit lacking in the overall document. I do understand that the aim is to provide an objective “state of the art” for current knowledge, but I think it would improve the document if the information was “digested” a little bit more.

James F. Ranville

The document is extensive and in some respects suffers from this. I do not believe that Chapter 7 works very well as a summary. I suggest a 2-3 page executive summary be added to very succinctly state the most future research directions based on the available information.

Charge Question 9

The case study follows the CEA framework, which combines a product life-cycle perspective with the risk assessment paradigm to support subsequent steps in the CEA process. Please comment on aspects of the CEA framework and process that can be improved in future applications of CEA. We would appreciate input on the overall structure and scope of the framework and process and the extent to which they support the development and refinement of research directions for future CEAs of nano-Ag in particular and nanomaterials in general.

Paul M. Bertsch

The CEA framework is sufficiently comprehensive at a high level. As indicated above, it would have been very useful to have a detailed diagram of a life-cycle inspired risk analysis for nano-Ag sprays with all detailed pathways and feedbacks incorporated with some indication of those processes with the greatest uncertainty. Perhaps question marks scaled to different sizes could be used.

Jaclyn Cañas

Overall, the use of the CEA framework for this case study and future case studies of other nanomaterials was an excellent choice, especially to drive and support future research to be able to complete a comprehensive environmental and human health risk assessment. The CEA framework definitely accounts for all the information that needs to be assembled or collected to do such an assessment and gain an understanding of future research needs due to data gaps that are identified as a result of the CEA framework. The process of collective judgment to evaluate the data collected as a result of the CEA framework is very valuable. Research regarding the fate and effects of nanomaterials is challenging and requires the expertise of various types of scientists and engineers to determine the true toxicity and impact of these materials. As more nanotoxicology research is conducted, it is becoming more evident that traditional toxicology methods may not be applicable to truly assessing the risk posed by nanomaterials. Thus, the CEA process brings together experts to work through and identify the most urgent research needs. In my limited experience with the CEA approach (primarily as a reviewer of this document, the CEA framework and process seem to be extremely valuable and useful when assessing the risks posed by nanomaterials. The CEA approach is also highly effective at supporting the development and refinement of future research directions for not only nano-Ag, but other nanomaterials as well. I do not have any suggestions on how to improve the CEA approach, as I feel it is effective in its current format.

Robert I. MacCuspie

The overall scope and structure of the document was quite useful, and provided a logical way of systematically working through the CEA framework (which at first glance in Figure 1-1 seems potentially challenging as so many things are interconnected).

One area for improvement could be to identify where the delineation between AgNPs's nano-specific effects is, and once they transform/dissolve into Ag⁺ such that they are no longer a "nanoAg" effect? Are there scenarios where one could consider that AgNPs are merely dosing

Ag⁺, and previously known information on "bulk" or "ionic" silver could then be applied? How could the need for such a demarcation be confirmed/ruled out? This also ties into the tiered approach many are proposing for hazard assessment of nanomaterials (*e.g.*, red/orange/yellow/green), and reducing the uncertainty of hazard assessments.

Better and more specific identification of knowledge gaps and research priorities could help the iterative process by guiding funding agencies and researchers, and instructing future CEA participants.

This appears to be an excellent example of a successful CEA iteration.

Peter R. McClure

I have no further comments.

Bernd Nowack

Chapter 5 is on exposure, Chapter 6 on the effect, but what is missing is a chapter on risk (risk = exposure x effect). I would suggest creating a new chapter on risk and summarize the few studies that have already tried to perform an environmental risk assessment of nano-silver. Even if the data quality and quantity about exposure and effect is still sparse, it is possible to make some first conclusions about the risk, using established assessment factors to cope with the uncertainty. It is my opinion, a serious omission is that nothing is said about risk assessment in the whole document, although it is described in Chapter 1 that the CEA-approach is used.

Stig I. Olsen

As the name indicates, I think the CEA framework is very comprehensive both in terms of the data compilation and the process. The only improvement I see is, as I mention above, to present the data compilation in a way that provides a better overview and input for prioritization. The scope is very wide, but apparently it can be narrowed a bit down depending on the subject of investigation, as has been done here for nano-Ag. I find that the current document with its comprehensive overview of the current knowledge supports the development of research direction very well.

James F. Ranville

I believe the document makes a strong case for the value of a CEA process in the hazard assessment of nanotechnology. The CEA approach is comprehensive which is its strength and its weakness. In being so comprehensive, the CEA approach identifies all relevant aspects of the problem. The weakness is that it is unlikely that information exists on all these aspects, leaving obvious knowledge gaps. The CEA approach does provide useful guidance for research to fill these gaps.

VI. INDIVIDUAL REVIEWER COMMENTS

Review By:
Paul M. Bertsch, Ph.D.

Peer Review Comments on EPA's Draft Document *Nanomaterial Case Study: Nanoscale Silver in Disinfectant Spray*

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January 27, 2012

I. GENERAL IMPRESSIONS

The document purports to be a “case study” of nanoscale silver in disinfectant spray. However, the document contains a good deal of information and material both on nano silver and nanomaterials, generally that extend well beyond what might be anticipated in a “case study.” While much of the discussion surrounding what is known about nano Ag and its environmental fate, transport, bioavailability, and toxicity might be considered pertinent to disinfectant sprays containing nano Ag, the presentation is broad and comprehensive relative to Ag and nano-Ag in the environment quite generally and elements related to the actual focus of the case study seem to become lost. Chapter 7 does not help bring the case study back into focus, as much of what was covered previously in the document relative to a broad discussion of Ag and nano-Ag is repeated and there are some lists of research gaps interspersed with larger research themes, again making it difficult to wade through the material to identify those areas critical to moving a risk analysis forward.

The failure to identify what major stakeholder or stakeholders were the primary target audience for this document made it difficult to evaluate the appropriateness of the depth and breadth of much of the information presented.

Identifying key knowledge gaps and defining risk trade-offs leading to prioritized research and adaptive risk management plans is certainly an important goal; however the document is very long, covering a lot of material with significant overlap and redundancy throughout (sometimes inconsistently presented) and a concise prioritized list aimed at advancing research and adaptive risk management plans never really emerges, although section 7.3.1 is the most useful in this regard.

Overall the document is well written and the material is presented clearly. The redundancy in the document might be justified if the intention is to have most of the chapters read as stand-alone contributions; however this is not a stated goal. One major problem with the repetition and redundancy is that the information covered multiple times is not always internally consistent. For the most part, the information presented appears to be accurate and conclusions sound, albeit most conclusions posed are rather general in nature. There are a number of specific areas needing attention (with respect to clarity or accuracy) are indicated below.

II. RESPONSE TO CHARGE QUESTIONS

1. Chapter 1 provides introductory material regarding the CEA approach used in these case studies along with other background information and a discussion of terminology. Is this information accurately and clearly presented? Please comment on the utility of the chapter in providing background and support for the remainder of the document. In particular, are the figures summarizing the CEA framework and process clear? How might this chapter be improved?

The chapter attempts to introduce the document and provide background information relative to the motivation for generating the document. In terms of an overall introduction, the chapter is adequate and appears accurate and reasonably clear. I would suggest moving the discussion of naturally occurring, incidental, and engineered nanoparticles (nanomaterials?) from the “Terminology section” to page 1-1, first paragraph, since this is touched upon in this section and this distinction represents more of a higher level definition/classification of nanoscale materials.

The discussion relative to how the nano-Ag in disinfectant spray was chosen for a case study is not particularly clear or compelling. It would be useful if a more specific conceptual model for nano-Ag in disinfectant sprays be developed to illustrate a life-cycle inspired risk analysis for these products. Much of the subsequent discussion (later chapters) seems to amplify the relative relevance/importance and existing data for nano-Ag in such products as fabrics, personal care products, and food storage devices, etc.

The CEA framework is fairly well presented. The captions for figures 1-1 and 1-2 need to be expanded. I do not understand the abiotic resources component in the exposure-dose facet of the CEA. It seems to me that these would be part of the product life cycle or transport/transformation/fate facets. While there is a statement concerning the relative simplicity of the CEA not capturing the real-life complexity (Page 1-2, lines 30-31), this discussion could be expanded a bit to introduce the notion of coupled processes and feedbacks between elements listed in environmental conditions, environmental media, and between each of the facets of the CEA framework. I believe this is what the authors were attempting to articulate with the comment “...numerous linkages and transfers...”

Section 1.3 could be beefed up. It seems that the chapters are organized to introduce the production and use of Ag and nano-Ag, and provide background on important characteristics of nano-Ag (Chapter 2) and then describe the facets of the CEA framework, i.e., life cycle (Chapter 3), transport/transformations, and fate (Chapter 4), exposure-dose (Chapter 5), and then impacts (Chapter 6). Outlining this in section 1.3 might help. I would also suggest having the terminology section (1.4) prior to section 1.3.

2. Chapter 2 presents basic information on conventional silver, including data on usage and historic environmental levels. Information on the physical-chemical properties of nanoscale silver and analytic methods makes up the rest of the chapter. Is this information clear and accurate? How might this chapter be improved?

Overall, the information is clearly presented and for the most part accurate. Some specific items that should be addressed for clarity and accuracy:

Page 2-3, lines 13-19. The Hornberger et al. (2000) study is discussed in detail with the suggestion that Ag was the primary cause of reproductive failure. The authors themselves indicate that Cu co-varied with Ag in the field site under question and they acknowledge that other co-contaminants not accounted for could have been involved in the observed effects. This is probably not a good example for Ag effects given the confounding variables.

Page 2-4, lines 22-32. I realize there is another section on Nanoscale silver (2.2), but neither discussion includes estimates of the quantities of nano Ag produced or how this compares to the information here (not mentioned until the life cycle 3.1). There are several papers that attempt to estimate nano Ag production, beyond the number of products discussed as part of the Woodrow Wilson Center's inventory (e.g., see Hendren et al., 2011 ES&T). Also, related to the point above about the method and rationale for choosing spray disinfectants for this case study.....how will the use of Ag in these products compare to others in terms of overall mass? Even back-of-the-envelope estimates would be useful to gain a perspective of the relative importance of this scenario.

Page 2-10, lines 1-2. In recent years, synthesis methods have been developed to produce nanoparticles, ~~and~~ including silver nanoparticles ~~in particular~~, of various shape and size distributions.

Page 2-10, line 21. ~~dissociate~~-dissolve

Page 2-10, line 26. Suggest silver ions (Ag^+), ~~sometimes referred to as Ag+ ions,~~

Page 2-10, line 27. From the particle and **influence** the particle's behavior in the environment --- **this is not clear**

Section 2.3.2. While morphology is an important parameter of nanoparticles, the discussion of crystal structure is important but not especially relevant to the particle morphology. Many particles having different morphologies can have similar crystal structures. Nanoparticles, such as TiO_2 , which can have different crystal structures (anatase vs. rutile), can have similar spherical morphologies but much different toxicities. This should be the primary point of this discussion. Furthermore, Figure 2-2 does not offer any relevant information to the discussion and I suggest deleting.

Page 2-14, lines 21-25. Charge is not the only way particles are stabilized as implied in this discussion. Amphiphilic polymers are also used as a stabilizer, as discussed on p 2-15. Table 2-2 would be more useful if the different coatings were put into some sort of perspective in terms of commercial production. For instance, PVP stabilized nano-Ag seems to be the most readily available product commercially available in large quantities. Many of the others in the table are rather exotic and either available in small quantities with special order or are not commercially available at all.

Page 2-15, lines 10-14. This is an important point that should be expanded here, i.e., the importance of the coating. It is especially important since not all components of coatings are revealed by the manufacturers or certain mixtures of materials remain on the particle surfaces following synthesis and not recognized or revealed by the manufacturers.

Section 2.3.6. The discussion on solubility needs to be beefed up. The solubility of nano Ag is a critical part of its efficacy as a biocide. While there exists emerging evidence that there are particle specific effects, many observed effects are a result of particle dissolution and release of Ag⁺. It is important that the reader realize that oxidation of Ag (0) at the surface is critical reaction prior to dissolution, since Ag(0) is very insoluble.

Page 2-18, line 18. Ensemble methods are certainly important, but the authors need to define what they mean by “preferred”, as there are differences between hydrodynamic radii and crystalline radii that are typically obtained by ensemble vs. individual particle methods.

Page 2-18, line 29. salt precipitation?

Page 2-18; 2-19. Isolating and measuring nanoparticles from/in environmental media is not only challenging, it represents a grand challenge that needs to be urgently addressed if environmental nanotechnology and risk assessment is to advance. This needs to be stressed in this section.

Table 2-3. It would be good to have representative references in this table.

Page 2-20, lines 3-8. TEM is not an ensemble method, but rather a single particle method. Is the intent to indicate multiple or orthogonal methods? You may want to include examples where micro-XRF imaging coupled to XANES and EXAFS as well as TEM have been used.

3. Chapter 3 summarizes information on the lifecycle stages of nano-Ag disinfectant spray products, including potential releases to the environment of nano-Ag and by-products. To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-Ag as it might be used in disinfectant spray products? To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

The chapter does a decent job of summarizing the life-cycle stages of nano-Ag. The big challenge is that there is so little information. As indicated below, there needs to be greater discussion concerning by-products and multiple surface stabilizers that are not revealed by the manufacturer. In terms of disinfectant spray products, there is even less information for fabrics, food storage devices, personal care products, etc. The organization of the chapter is reasonable. There are parts of the discussion that seem irrelevant to the disinfectant spray story. However, in terms of informing planning for future research.....I do not think that the major gaps and issues are adequately defined and prioritized.

Page 3-2, lines 5-11. As indicated above, there are several papers that attempt to estimate nano Ag production in the U.S., beyond the number of products discussed as part of the Woodrow Wilson Center's inventory (e.g., see Hendren et al., 2011 ES&T).

Page 3-2, lines 12-18. This section seems out of place. As stated before, the process of oxidative dissolution of Ag (0) is important in terms of Ag⁺ release and the efficacy of many nano-Ag products as biocides and is not introduced until 4-3, and even then not clearly. The discussion on AgNO₃ and halides does not seem to fit here or at least the relevance is not clear.

Page 3-6, line 3. One problem with evaluating the particles is related to the removal of surface active agents as stated. However, this is a very common problem and our experience indicates that many manufacturers are not sure what the composition of the surface active agents are and they simply report what they believe to be the primary agent. This seems even more complicated for nano-Ag sprays, as described on page 3-8 and 3-9/10. The possible presence of multiple surface stabilizers and other by-products has many implications for evaluating materials in a systematic way and should be discussed.

Page 3-10, lines 1-20. It is not clear how this discussion relates to the nano-Ag spray solutions.

Page 3-11, lines 1-8. What about Ag speciation and Ag⁺ release?

4. Information on the transport, transformation, and fate of nano-Ag in air, water, sediment, and soil is discussed in Chapter 4. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of understanding regarding the known and anticipated behavior of nano-Ag in the environment. To what extent is this information presented in a manner that would inform consideration of likely exposure routes relevant to biota and human health? For each of the environmental media discussed, to what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

The chapter is a comprehensive list of the major factors that are known to or thought to influence the behavior, transport, bioavailability and toxicity of manufactured nanomaterials. While it is correctly stated that there are relatively few studies on many of the processes and pathways discussed, there are a number of key publications on nano-Ag that have emerged in the past few years that are not included (indicated below). While some areas needing additional research are at least eluded to in the discussion, there needs to be a distilled prioritized list of research needs at the end of the chapter that links back to the life-cycle inspired CEA and knowledge gaps so that one does not need to wade through the voluminous material (this is true for all subsequent chapters as well). This chapter could be organized more efficiently. The environmental factors that influence nano Ag behavior are largely the same for both terrestrial and aquatic systems so repeating these in each subsection is redundant and is also confusing.

Page 4-3, lines 5-29. As mentioned previously, it is the oxidation of Ag (0) to Ag₂O that is the critical intermediate step in dissolution.

Page 4-5, lines 19-25. There are a number of papers published in 2011 that examine the transformation of Ag NPs as influenced by sulfidation as well as one that provides evidence for Ag₂S nanoparticles in biosolids from waste water treatment plants. These should definitely be included in this discussion.

Page 4-5, line 27. Abiotic factors.....what about biotic factors? Biota in soil and sediments drive many geochemical reactions that are discussed subsequently and can also have a direct influence via exudates, biofilms, etc.

Page 4-6, lines 15-18. The sulfides are very insoluble ($K_{sp} \sim 10^{-51}$) which leads to their immobilization potential. The discussion here should focus on soluble complexes.

Page 4-7, lines 1-4. As mentioned several times previously, the dissolution of Ag (0) is largely dependent on the formation of Ag₂O and the pH dependence of Ag₂O solubility (that is why dissolved oxygen is an important player).

Page 4-7, lines 14-32. It should be noted that the CCC is highly dependent on the characteristics of the surface coating, thus presenting specific concentrations (Page 4-8, lines 1-2) is only useful in this context.

Page 4-8, lines 3-18. See comments above about Ag (0) oxidation. A discussion of sulfidation of Ag (0) NPs should be included since the transformation to Ag₂S has recently been demonstrated to be a dominant transformation product and this is, of course, redox sensitive reaction.

Page 4-11, lines 28-34. There are several newer references that point to the importance of biosolids and biosolid applications to agricultural lands as an important vector for nanomaterials to be introduced to the environment.

Page 4-12, lines 10-24. There is a good deal of literature on colloid facilitated transport of contaminants that involves naturally occurring or incidental nano sized mineral phases being transported through porous media that would be relevant to this discussion.

Page 4-13, lines 6-28. There are a few more recent references on plant uptake of nano Ag and Au that are be relevant to this discussion. There also have been many studies published recently examining the uptake and effects of Au, Cu, and Ag NPs from soil to earthworms and a few on *C. elegans* (soil nematode) that should be included in 4.3.

Section 4.4.2.1, line 17. Water chemistry vs. nature of the water? Lines 17-21, Table 4-1. This is very confusing as the verbiage mentions Ag complexes, but the data is on solubility products. Complexation and solubility need to be discussed separately and association constants for Ag complexes should also be presented. Many of the solid phases listed in Table 4-1 are not environmentally relevant.

Sections 4.4.2.2/4.4.2.3. This information is largely redundant with the earlier discussion. Suggest reorganizing the chapter to cover important environmental factors that are common to nano Ag in both aquatic and terrestrial systems and then discuss specific life-cycle inspired pathways for the terrestrial and aquatic systems separately.

Page 4-18, lines 1-5. Again, this discussion seems to be mixing up solubility and complexation. AgCl is very insoluble, but in the presence of high Cl⁻ concentrations, soluble negatively charged Ag chloro complexes are formed.

Page 4-18, lines 14-18. It should be clear what media was/were used to derive K_d's.

Section 4.4.3. This section covers data and studies focused on the biosolids produced in the WWTP, which is more relevant to the terrestrial section. Also, this is the first mention of Ag₂S formation, which as described earlier is emerging as a critical transformation process (sulfidation).

Page 4-20, lines 9-15. This section needs to be re-written.

Page 4-20, lines 24-31 and Page 4-21, lines 1-12. There is a rich literature dealing with colloid facilitated transport that considers transport through porous media as a three phase system.

Pages 4-21 and 4-22. It is noteworthy that the Gottshalk study estimated biosolid Ag concentrations from nano Ag use to be ~5-6 mg/kg *currently*. There mixing scenario for biosolids in soil was extremely conservative. The EPA 503 document on risk of biosolids uses a 1:1 biosolid to soil ratio to estimate metal loading from long term biosolid application to agricultural land. Thus, the concentrations using this approach would be orders-of-magnitude higher than their estimates.

5. Chapter 5 provides information on exposure, dose, and translocation of nano-Ag in humans and other biota. Please comment on the extent to which this chapter accurately and sufficiently characterizes this information and forms a basis for considering the health and ecological impacts of nano-Ag. To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

The chapter represents a comprehensive examination of exposure, uptake, and dose of a wide range of ecoreceptors as well as a discussion of exposure pathways for humans. Again, as in the previous chapters, major knowledge gaps and research needs are not concisely articulated. There is once again a good deal of repetition and redundancy from earlier sections, and the scope and level of detail are sometimes not consistent as pointed out below.

Page 5-1, lines 16-21. It is important to point out here the point made on Page 5-3, lines 5-11, and again on Page 5-7, lines 4-8 that, unlike many conventional contaminants, internal dose is not necessarily required for toxicity. The nano cluster tridecameric Al species was shown in the 1980's to be highly toxic to plants, algae, and fish and the site of intoxication was cell wall or cell membranes. A similar picture is emerging with nanomaterials.

Page 5-5, lines 18-19. Unless I missed something, this statement is not consistent with either the discussion in Chapter 3 or that on page 5-4, i.e. there was no evidence presented that aquatic systems would predominate over terrestrial.

Page 5-5, lines 24-25. Where is the evidence that ingestion by ecoreceptors would be low? If the current biosolid concentrations are estimated to be ~6 mg/kg than land application could result in uptake by plants along with the transfer to herbivores (see Judy et al., 2011) or from re-suspension of soil onto plant tissue and then ingestion by herbivores.

Page 5-6, lines 1-8. There are a number of recent studies on plant and earthworm uptake of Ag NPs as well as on the uptake of other noble metal NPs (Cu and Au) which are reasonable proxies for Ag.

Page 5-7, lines 8-12. See Unrine et al., 2008; and Judy et al., 2011.

Page 5-7, lines 13-23. It might be worth characterizing these into intrinsic and extrinsic factors.

Page 5-8, lines 16-19. Suggest deleting this sentence.

Page 5-9, lines 1-3. Some studies have shown that humic substances interacting with NP surfaces can actually induce charge stabilization. NOM does play a dual role, i.e., it can promote aggregation/agglomeration, depending on the exact composition and chemistries (as discussed on Page 5-10, lines 29-33. Also, as stated earlier, the conceptually complexation and precipitation (solubility) are distinct processes.

Page 5-9, lines 13-17. This is not entirely accurate. Most of the discussion in section 4 was focused on K_{sp} s and very little on soluble complexes. It was suggested previously that complexation constants for environmentally relevant ligands be presented in that section.

Page 5-9, line 25. Again, the terminology between complexation and solid phase formation is blurred.

Page 5-10, lines 9-13. Same comment here as above. Is the AgCl referred to here the solid phase $AgCl_s$ or a neutral complex ($AgCl^0$)? Also, working should be "...that at circumneutral pH" and delete parenthesis.

Page 5-11, lines 12-27. This discussion amplifies an earlier comment about the transformations of Ag NP in the environment, especially from the standpoint of sulfidation reactions.

Page 5-11, lines 33-36. This is inconsistent with prior discussions. While $AgCl_s$ is very insoluble, soluble anionic chloro Ag complexes can form at high Cl concentrations. So the issue is how quickly oxidative dissolution (and then subsequent formation of anionic chloro-Ag complexes) would occur as a competing process to aggregation due to other processes.

Page 5-12, lines 14-16. There are several key new publications on the Ag in WWTP (e.g., see Kaegi et al., 2011).

Page 5-13, lines 28-30. See papers by Shoults-Wilson et al., 2011 a,b,c and Unrine et al., 2010 a&b.

Page 5-14, lines 21-26. Xu et al., 2004, reported that Ag nanoparticles up to 80 nm were transported across the outer and inner membrane of *Pseudomonas aeruginosa* and they provided strong evidence for the involvement of the MexAB-OperM extrusion pump in transmembrane transport of the Ag nanoparticles.

Page 5-17, line 31. What is being referred to here $AgCl_s$ or $AgCl_2^-$?

Page 5-19, lines 20-26. See previous comment to this statement made earlier.

Page 5-27, lines 1-13. Finally a discussion of chloro Ag complexes! The influence of water chemistry is repeated multiple times and the message is not consistent. This needs to be integrated with most of the earlier discussions and repetition and redundancy eliminated.

Page 5-28, lines 1-9. See Ferry et al., 2009 for Au NP in estuarine mesocosms. This study is

very relevant to the Ag NP story.

Page 5-30, 5.2.4.1. See Rico et al., 2011; Judy et al., 2011; Hawthorne et al., 2012; Yin, et al., 2011; Sabo-Atwood, et al., 2012.

Page 5-32, lines 1-4. See Unrine et al., 2008; 2010, 2011 a&b, Shoultz-Wilson 2011a, b&c.

Page 5-32, lines 23-31. See Judy et al., 2011.

Page 5-33, lines 6-31. See Rico et al., 2011; Judy et al., 2011; Hawthorne et al., 2012; Yin, et al., 2011

6. Chapter 6 characterizes factors that influence ecological and health impacts of nano-Ag and discusses the currently available scientific evidence regarding these impacts. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of the science. To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

As with Chapter 5, this chapter provides a comprehensive examination of information on Ag NP effects to ecoreceptors and various mammalian cell lines. It is well written and overall captures the literature well. Many of the references pointed out in the comments of the previous section are relevant to this chapter as well. As stated previously, it is important that the key knowledge gap and research needs be specifically articulated and summarized.

A lot of the information in 6.1.1 is repetitive with previous sections, even though a good review of toxicity related to varying parameters are covered. Perhaps this overall is unavoidable.

Page 6-3, lines 1-18; lines 26-34. Somewhere in this introduction it might be pointed out how difficult it often is to conduct systematic studies varying particle size, morphology, or surface coating chemistries. Certain coatings are often not available in the same sizes/morphologies as others and then there is the relative stability of the particles as a function of size/morphology that varies depending on the surface coating. Solubility of the particles can also co-vary depending on size x surface coating interactions. This is important to put much of the subsequent discussion in context.

Page 6-8, lines 3-7. Oxidative dissolution is finally introduced!

Page 6-8, lines 13-33. Generally, positively charged and hydrophobic coatings on a number of nanoparticle types have been shown to induce much greater toxicity than more negatively charged or more hydrophilic particles in a number of receptors and cell lines as mentioned on page 6-9.

Page 6-14, lines 5-22. Sulfidation needs to be introduced as a critical transformation process in WWTP.

Page 6-15, lines 1-6. See references suggested earlier.

Page 6-21; lines 5-18. See Dubchak, et al., 2012

Page 6-23, lines 26-29. This is not consistent with the discussion concerning size exclusion limits in Chapter 5, where I pointed out this reference.

Page 6-24, line 2. I believe this reference was just Neal.

Page 6-41, lines 1-12. See Unrine et al., 2008 concerning the development of a conceptual particle biotic receptor model.

Page 6-4, lines 13-16. See references suggested above for terrestrial organisms.

7. Chapter 7 summarizes the information and research questions presented in the nano-Ag case study, as well as discusses the role of case studies in the refinement of research strategies and potential future assessment efforts. We would appreciate comment from the peer reviewers on the integration of evidence in this chapter and its usefulness in supporting future development of research strategies and assessments. How might this chapter be improved?

While this is a workshop summary report, it is quite repetitive and not particularly effective at distilling down to a specific, concise, prioritized list. Section 7.3.1 comes the closest, but after wading through this massive document the overall recommendations are very broad, general, and at a pretty high level. As stated previously, the distillation of the key knowledge gaps and research questions for each chapter might be a more useful approach.

8. For the document as a whole, are there ways to improve the structure, scope or presentation of information to better support the identification and prioritization of research needs by diverse stakeholders?

The document could be improved by reducing the duplication and redundancy and reducing its length. Furthermore, it is important that the various chapters that cover the same material communicate consistent information. A more specific life-cycle inspired conceptual model should be developed with a detailed figure illustrating the key knowledge gaps to accompany the more general CEA diagram in the first chapter. This figure could then be referred to in subsequent chapters to tie together the relevant information from the review of the literature. Furthermore, a research need or knowledge gap summary section for each of the chapters with a prioritized list of knowledge gaps and research needs would be extremely helpful. As currently constructed, the document is very long and cumbersome and the reader has to search to identify important research needs.

9. The case study follows the CEA framework, which combines a product life-cycle perspective with the risk assessment paradigm to support subsequent steps in the CEA process. Please comment on aspects of the CEA framework and process that can be improved in future applications of CEA. We would appreciate input on the overall structure and scope of the framework and process and the extent to which they support the development and refinement of research directions for future CEAs of nano-Ag in particular and nanomaterials in general.

The CEA framework is sufficiently comprehensive at a high level. As indicated above, it would

have been very useful to have a detailed diagram of a life-cycle inspired risk analysis for nano-Ag sprays with all detailed pathways and feedbacks incorporated with some indication of those processes with the greatest uncertainty. Perhaps question marks scaled to different sizes could be used.

III. SPECIFIC OBSERVATIONS

See comments above.

Review By:
Jaclyn Cañas, Ph.D.

Peer Review Comments on EPA's Draft Document *Nanomaterial Case Study: Nanoscale Silver in Disinfectant Spray*

Jaclyn Cañas, Ph.D.

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January 9, 2012

I. GENERAL IMPRESSIONS

Overall, the case study document is very well written, organized, and very informative. To my knowledge, although limited with regards to nano-Ag, the information presented is heavily cited with the most current nano-Ag literature. I can, however, confirm that the way data/information was interpreted based on the literature and with regards to general fate, toxicity, and nanotoxicology principles was definitely accurate. Sufficient evidence was provided to fully support any conclusions outlined in the case study. The CEA approach was effective at gathering all the relevant nano-Ag information to assess the state of the science and direct future research planning. The document is not only useful with regards to nano-Ag research, but there are several overarching themes or questions related to nanomaterials in general that are raised throughout. Therefore, this document will be of use to nano-Ag researchers as well as to those working with other nanomaterials.

With regards to presentation of the document, it is well organized and presented in a very logical manner. The introductory chapter does an excellent at setting the stage for the remainder of the document. The extensive use of headings and subheadings is very useful to the reader. The use of summaries at the end of each chapter is also very useful. Overall, the document is easy to read and is presented in a clear and logical manner.

II. RESPONSE TO CHARGE QUESTIONS

1. Chapter 1 provides introductory material regarding the CEA approach used in these case studies along with other background information and a discussion of terminology. Is this information accurately and clearly presented? Please comment on the utility of the chapter in providing background and support for the remainder of the document. In particular, are the figures summarizing the CEA framework and process clear? How might this chapter be improved?

The information presented in Chapter 1 is very clear and appropriately presented. The chapter is extremely helpful in laying the foundation for and providing the context in which the remainder of the document is presented. I found both the figures and the text regarding the figures to be extremely clear. The text was helpful and easy to read and follow the steps/compartments in the figures. I also believe the inclusion of the Purpose of the Document to be useful in setting the stage and expectations for the remainder of the document. As an experienced nanotoxicology researcher of carbon nanotubes, I appreciated the Terminology section as it clearly outlined the definitions of terms that often have different meanings depending on their use. Overall, I think Chapter 1 was well written and served as a good introduction to the document. I do not have any

suggestions with regards to how the chapter might be improved.

2. Chapter 2 presents basic information on conventional silver, including data on usage and historic environmental levels. Information on the physical-chemical properties of nanoscale silver and analytic methods makes up the rest of the chapter. Is this information clear and accurate? How might this chapter be improved?

I am not knowledgeable regarding the uses and environmental levels of conventional silver and thus, cannot comment on the accuracy of the information presented. However, what was presented was definitely presented in a clear and concise manner. While I am not an expert in the area of nano-Ag, I have experience with other nanomaterials, how physical-chemical properties dictate fate and transport of contaminants, and analytical chemistry. The information regarding nano-Ag physical-chemical properties and analytical methods was clearly and accurately presented. The information provided a good introduction to topics that will be discussed in the remaining chapters with regards to physical-chemical properties and analytical methods. The text accurately reflected the current issues and challenges in working with nanomaterials, especially with regards to lack of appropriate or feasible analytical methods and characterization. Therefore, I do not have any suggestions with regards to how the chapter might be improved.

3. Chapter 3 summarizes information on the lifecycle stages of nano-Ag disinfectant spray products, including potential releases to the environment of nano-Ag and by-products. To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-Ag as it might be used in disinfectant spray products? To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

Chapter 3 does an excellent job at describing what is known and what is unknown regarding the life-cycle stages of nano-Ag. It is very clear what information is lacking, especially with regards to manufacturing and synthesis of nano-Ag in general as well in the manufacturing of nano-Ag for disinfectant sprays.

The material presented in Chapter 3 is very well organized and presented in a logical manner starting with the extraction or mining of conventional silver to synthesis of nano-Ag to potential uses of disinfectant sprays with nano-Ag to disposal (both proper and improper) of disinfectant sprays with nano-Ag. From Section 3.2.1 to the end of the chapter, the information provided is definitely informative to support planning of future research. Section 3.4 is especially informative and raises numerous needed areas of research to identify the true risk associated with using disinfectant sprays with nano-Ag. The whole chapter, from a toxicologist's perspective, is informative and raised numerous research questions to assess exposure and fate of nano-Ag used in disinfectant sprays.

The chapter could be improved by the inclusion of tables and figures. The inclusion of a summary table of the synthesis techniques with references would be useful. In addition, a figure that illustrates the life-cycle of nano-Ag from mining of conventional silver to disposal (both proper and improper) would be useful for the reader to follow along. Perhaps a table of potential uses of disinfectant sprays with nano-Ag would also be useful. Adding tables and/or figures will

really add to the chapter.

4. Information on the transport, transformation, and fate of nano-Ag in air, water, sediment, and soil is discussed in Chapter 4. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of understanding regarding the known and anticipated behavior of nano-Ag in the environment. To what extent is this information presented in a manner that would inform consideration of likely exposure routes relevant to biota and human health? For each of the environmental media discussed, to what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

Chapter 4 most definitely, accurately, and sufficiently (perhaps with exception of fate of nano-Ag in soil – see below) characterizes what is known regarding the fate of nano-Ag in the environment. The Chapter also does a good job at including expectations of anticipated behavior of nano-Ag based on conventional Ag, other nanoparticles, or the properties of nano-Ag in general. This chapter is a great resource to spark and guide future research planning. As presented, this chapter definitely has enough good information, related to both general fate principles as well as to nano-Ag specifically, to seriously inform considerations of likely exposure routes and drive future research directions in fate and toxicity of nano-Ag.

Air: The text related to the fate of nano-Ag in air is well organized and presented in a logical manner. The necessary and relevant information is present to support planning of future research. Even though there is limited fate and transport data for nano-Ag in air, the section provides sufficient information to begin to develop research questions and identifies research gaps related to nano-Ag fate in air. The only suggestion to improve this section would be to use bullets for the paragraphs that identify how nano-Ag might be released into the air, as is done for the other two environmental media sections.

Terrestrial: The text related to the fate of nano-Ag in soil was brief but informative enough to at least start to think about future research. The text presented seems very basic soil fate material that is taught in environmental chemistry courses with a few sentences specific to nanoparticles (rarely to nano-Ag). Researchers would definitely need to consult other sources to fully develop a research question related to fate of nano-Ag in soil. Perhaps the brevity of this section is due to the lack of research in this area.

Aquatic: The text related to the fate of nano-Ag in water was well organized and logically presented. Additionally, the aquatic text was very thorough and sufficiently informative to fully support planning of future research. Inclusion of conventional Ag fate in water is excellent and useful to begin to plan future research with nano-Ag instead.

5. Chapter 5 provides information on exposure, dose, and translocation of nano-Ag in humans and other biota. Please comment on the extent to which this chapter accurately and sufficiently characterizes this information and forms a basis for considering the health and ecological impacts of nano-Ag. To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

Chapter 5 was very well written and organized given the amount of information that was presented. As an environmental toxicologist, the information provided was accurately presented and terminology was used correctly. The definitions at the beginning were useful and will be good for non-toxicologists that might read the document. The information included in Chapter 5 definitely provides extensive support for planning of future research as the text covers what is currently known regarding the impacts of nano-Ag and conventional Ag. In addition, the text also identifies some specific data gaps. However, as a toxicologist, even when gaps were not clearly identified, the text included is definitely sufficient enough to inspire future research questions. The summary of the chapter adequately highlighted the contents of Chapter 5.

There are a few things that could improve Chapter 5. First, a summary table or tables would be useful for all the biotic data presented, especially since there was data discussed for a few forms of Ag in a wide variety of species. Such a summary table(s) would provide the reader with a quick overview. Another improvement would be to include, perhaps in the summary, a list of questions or statements highlighting the most important research needs as brought forth by the text provided in Chapter 5. Several of these statements or questions are scattered throughout the Chapter.

6. Chapter 6 characterizes factors that influence ecological and health impacts of nano-Ag and discusses the currently available scientific evidence regarding these impacts. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of the science. To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

In my opinion, the information provided in Chapter 6 is accurately presented and sufficiently characterizes the state of the science regarding nano-Ag. Chapter 6 is very well organized. The content is presented in a clear and logical manner. The sections on Mode of Action at each organism level were especially useful to summarize all the toxicity data presented in the previous section and highlight significant findings of studies. These sections, in particular, are very useful to support planning for future research as synthesis information was provided that highlighted major overarching trends observed related to nano-Ag toxicity (especially when compared to conventional silver or with regards to effects of size on toxicity). Overall, the logical presentation of data by organisms (microorganisms, algae, aquatic, etc.) in conjunction with the Mode of Action data is extremely useful to support planning of future research as data gaps are evident. The only improvements identified for Chapter 6 are related to grammar and formatting issues which are listed below as Specific Observations.

7. Chapter 7 summarizes the information and research questions presented in the nano-Ag case study, as well as discusses the role of case studies in the refinement of research strategies and potential future assessment efforts. We would appreciate comment from the peer reviewers on the integration of evidence in this chapter and its usefulness in supporting future development of research strategies and assessments. How might this chapter be improved?

Chapter 7 is an excellent and very useful synthesis of what was presented throughout the whole document. The appropriate information was selected for inclusion in this summary chapter to support future development of research strategies and assessments. The inclusion of the CEA workshop findings was also useful to further support future development of research strategies

and assessments. One could read this chapter alone and gain an understanding of the current state of the science regarding nano-Ag. The research questions (or needs) were more clearly identified in this chapter than throughout the previous chapters.

8. For the document as a whole, are there ways to improve the structure, scope or presentation of information to better support the identification and prioritization of research needs by diverse stakeholders?

Overall, the document is well written and the structure, scope and presentation of information presented is excellent. One possible change (which would be major), would be to separate Chapters 5 and 6 into smaller chapters that can have exposure, dose, and effects information for each study presented in one place. For example, separate them out into 3 chapters: 1) Aquatic, 2) Terrestrial, and 3) Human. This is only a suggestion that would improve the overall structure and eliminate some duplication. However, it is not necessary to better support the identification and prioritization of research needs by diverse stakeholders.

Chapter 7 identified the top priority research questions, needs, and data gaps more clearly than what was previously done in the Chapters 2-6. Adding a section at the end of each chapter (perhaps even in the summary) that clearly identifies the most pressing research needs. Using 1,2,3 or A,B,C would clearly define these research needs. This addition in each chapter would strengthen the support for identification and prioritization of research needs by diverse stakeholders.

9. The case study follows the CEA framework, which combines a product life-cycle perspective with the risk assessment paradigm to support subsequent steps in the CEA process. Please comment on aspects of the CEA framework and process that can be improved in future applications of CEA. We would appreciate input on the overall structure and scope of the framework and process and the extent to which they support the development and refinement of research directions for future CEAs of nano-Ag in particular and nanomaterials in general.

Overall, the use of the CEA framework for this case study and future case studies of other nanomaterials was an excellent choice, especially to drive and support future research to be able to complete a comprehensive environmental and human health risk assessment. The CEA framework definitely accounts for all the information that needs to be assembled or collected to do such an assessment and gain an understanding of future research needs due to data gaps that are identified as a result of the CEA framework. The process of collective judgment to evaluate the data collected as a result of the CEA framework is very valuable. Research regarding the fate and effects of nanomaterials is challenging and requires the expertise of various types of scientists and engineers to determine the true toxicity and impact of these materials. As more nanotoxicology research is conducted, it is becoming more evident that traditional toxicology methods may not be applicable to truly assessing the risk posed by nanomaterials. Thus, the CEA process brings together experts to work through and identify the most urgent research needs. In my limited experience with the CEA approach (primarily as a reviewer of this document, the CEA framework and process seem to be extremely valuable and useful when assessing the risks posed by nanomaterials. The CEA approach is also highly effective at supporting the development and refinement of future research directions for not only nano-Ag, but other nanomaterials as well. I do not have any suggestions on how to improve the CEA approach, as I

feel it is effective in its current format.

III. SPECIFIC OBSERVATIONS

Page	Line # or Paragraph	Comment or Question
xv	14	Why is this reference used when U.S. EPA 2010a is used on pg 1-1 line 15 and both lines refer to the same case study document?
1-2	18	Same comment as the previous comment. It is not clear why sometimes the 2010a reference is used and other times the 2009d (which is the external review) reference is used. It should be consistent or made clear why the different stages of the document are referenced.
1-2	19	CEA is first written in line 2 on the same page and therefore the acronym should be in line 2 not 19.
1-2	26	Extra space before the period.
3-1	14	There appear to be at least 2 different font sizes.
4-6	8-14	The last half of the paragraph seems a little disjointed from the first part of the paragraph. Perhaps a transition sentence to tie the two sections of the paragraph together might help it to flow better.
4-8	26	Also appears twice in 3 words. Only one is needed.
4-11	27	Groundwater is presented as two separate words when it is one word.
4-12	1-5	The two sentences presented here seem contradictory. If nanoparticles are unlikely to resuspend, then why even include it as a possible route of release into the terrestrial environment?
4-12	23 and 24	Groundwater is presented as two separate words when it is one word.
4-12	16	Groundwater is presented as two separate words when it is one word.
4-18	27	Groundwater is presented as two separate words when it is one word.
4-19	21	Groundwater is presented as two separate words when it is one word.
4-21	18	Groundwater is presented as two separate words when it is one word.
4-23	25	Groundwater is presented as two separate words when it is one word.
5-23	25	Space is needed between 100 and nm.
5-23	28	Space is needed between 10 and nm.
5-59	19	The i is missing in the "as high as n males."
6-21	2	There is an extra space before the period that needs to be removed.
6-40	16	It should be "estimate" instead of "estimated."
6-50	2	The reference year 1954 appears to be a larger font.
6-50	5 & 6	The 3 in AgNO ₃ needs to be a subscript.
6-50	31	The reference year 2010a appears to be a larger font.
6-51	8-17	Why not include the nano-Ag study by Li et al., 2010a in the next section on nano-Ag effects?
6-53	3	The 2 in nano-TiO ₂ needs to be a subscript.
6-54	26	Only one 'also' is needed.
6-68	6-7	There is some sort of spacing or font issue between the two paragraphs.
7-13	Heading	Needs to be in bold.
7-16	3	There is an underscore or a space underlined between uptake and is.

Review By:
Robert I. MacCuspie, Ph.D.

Peer Review Comments on EPA's Draft Document *Nanomaterial Case Study: Nanoscale Silver in Disinfectant Spray*

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National Institute of Standards and Technology
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January 11, 2012

I. GENERAL IMPRESSIONS

See comments below.

II. RESPONSE TO CHARGE QUESTIONS

1. Chapter 1 provides introductory material regarding the CEA approach used in these case studies along with other background information and a discussion of terminology. Is this information accurately and clearly presented? Please comment on the utility of the chapter in providing background and support for the remainder of the document. In particular, are the figures summarizing the CEA framework and process clear? How might this chapter be improved?

Chapter 1 provides a concise discussion of the relevant terminology involved. The information is presented accurately and clearly with good citations. Chapter 1 clearly states what this document intends and does not intend to accomplish.

Specific suggestions for improvement:

Figure 1-1. Summarizing the CEA framework is probably the best that can be achieved given the vast complexity of this approach. Does the color gradient from left to right signify anything beyond visual appeal?

Figure 1-2. It was quite clear CEA is meant to be an iterative process.

Page 1-9, lines 1-10. ASTM & ISO also have similar definitions to the BSI definitions of aggregate and agglomerate. Lines 10-27 make an excellent point.

2. Chapter 2 presents basic information on conventional silver, including data on usage and historic environmental levels. Information on the physical-chemical properties of nanoscale silver and analytic methods makes up the rest of the chapter. Is this information clear and accurate? How might this chapter be improved?

Chapter 2 overall presents the information in a clear and accurate fashion. Specific points are outlined below. The authors need to make sure the point of multiple, orthogonal techniques employed to provide size distribution plots for initial stock characterization is driven home more emphatically. Many of their citations reach this conclusion as well, and it is essential for research papers to be effective and intercomparable. Further, reporting all details is critical, including those beyond the "Minimum Characterization" lists, timing of measurements,

processing history such as sonication parameters (see for example Taurozzi et al., *Nanotoxicology*, 2011), dilution orders of addition, and age of stocks before use in experiments.

Specific suggestions for improvement:

Section 2.1. It would be nice to know the forms of silver most commonly found in soils, waters, etc., and the relative distribution of the most abundant forms (i.e., does Ag₂S make up 40% of all silver compounds or 98%?) across environmental compartments, if this information is known. If not, it should be identified as a critical knowledge gap.

Page 2-2, line 1. Silver nitrate solution is also used in modern medicine, for example to cauterize and/or sterilize wounds, from nosebleeds to infant bellybuttons.

Page 2-6, lines 4-5. It would appear 3.2 ppb concentrations of silver, regulated through discharge permits, is orders of magnitude lower than the 0.1ppm (100ppb) drinking water concentration ceiling. Please discuss why is there such a significant difference, and if one regulation makes the other unnecessary.

Section 2.3.1, Size. The document needs to discuss how the measurement methods used affect the reported size distribution, for example see MacCusprie, Rogers, et al., *Journal of Environmental Monitoring*, 2011, 13, 1212. The discussion probably belongs here, but may also be mentioned in a 2.4 Analytical Methods section. Additionally, this section should discuss the importance of reporting the handling/processing history of the materials, along with the time elapsed at each step. Silver nanoparticles are inherently unstable, releasing silver ions into solution, thereby potentially altering the size and size distribution over time. Also, recommend pointing to later discussions on metrologies of measuring size, and point to Appendix A.

Page 2-13, lines 3-4. Auffan's estimates seem off. In the reference, this claim is made in the second paragraph of the introduction, with no explanation or subsequent reference for the methodology of determining these values. A more analytical way to solve this would be as follows:

- Determine the number of silver atoms in the sphere of diameter dNP.
- Determine the number of silver atoms in a sphere of diameter (dNP - 2dAg), assuming only one layer of silver atoms is on the surface (for silver metal particles dAg = 0.288 nm, which may be much less than the diameter to subtract for metal oxides, which Auffan tended to focus on in the reference).
- The difference of these two values is the number of silver atoms on the surface, and the fraction on the surface can be determined by dividing by the number of silver atoms in the sphere of diameter dNP.

See Leff, et al., *J. Phys. Chem.*, Vol. 99, No. 18, 1995, Page7036 for further details of how this was implemented (they used gold nanoparticles, but the same method can be extended to silver). By this method, a 10nm silver nanoparticle would have 16.3% of its atoms on the surface, while a 30nm silver nanoparticle would have only 5.65% of its atoms on the surface.

Sections 2.3.4, 2.3.5. Suggest all surface coating issues should be in one spot, and point to that section elsewhere as needed. Surface chemistry makes most sense to place lengthy discussion.

Section 2.3.5. No reactivity is discussed in this section, thus suggest changing section title to Surface Chemistry and Coatings.

Sections 2.3.5, 2.3.6. Please discuss how formation of silver oxide or silver sulfide on surface can affect solubility, for example, Liu & Hurt's three papers in 2010-2011 in Environmental Science & Technology and ACS Nano.

Section 2.4.1, page 2-18, lines 3-13. TEM and SEM are important tools. The limitation of SEM, unable to see particles smaller than 10 nm, in most instrumental implementations, could pose a significant gap in the size analysis.

Section 2.4.1. Also, atomic force microscopy (AFM) should be considered here as well. In Appendix A, it is identified as a frequently or commonly used size technique.

Page 2-18, lines 18-19. "Ensemble methods are preferred" should be removed, as relying upon DLS alone could underrepresent or entirely miss small nanoparticles in polydisperse samples. The light hitting the photodetector in a DLS instrument is due to Rayleigh scattering, which is proportional to radius to the sixth power, r^6 . Thus, one particle (silver nanoparticle or dust) or cluster that is 10 times larger than the primary particle size will provide half of the intensity on the photodetector. DLS provides an advantage at identifying early signs of clustering. It is unrealistic to expect a TEM/SEM/AFM microscopist to count well over one million particles to identify these clusters or large particles. However, microscopies will identify small nanoparticles in polydisperse samples that DLS may never resolve. Line 23 should be amplified. The best approach is multiple and orthogonal measurement techniques (such as pairing DLS and TEM).

Section 2.4.1. TEM/SEM/AFM sample prep - Timing is critical! Glover et al., ACS Nano, 2011, 5(11), 8950 identify that high humidity and a few weeks between prep and analysis leads to artifacts of small nanoparticles.

Chapter 2 may not be the best place for this discussion, but after such attention to definitions of agglomerates and clusters, a discussion about measuring clusters and reporting them in the size distribution is needed. Additionally, suggest discussing methods of making controlled agglomerates, such as Zook et al., Nanotoxicology 2011, 5(4), 517.

It is worth discussing in the metrology sections the Kennedy et al., Environmental Science & Technology 2010 article "Fractioning Nanosilver-Importance for Determining Toxicity to Aquatic Test Organisms" (referenced in later chapters), which demonstrates an easily transferrable method for measuring dissolved silver fractions and particle fractions.

3. Chapter 3 summarizes information on the lifecycle stages of nano-Ag disinfectant spray products, including potential releases to the environment of nano-Ag and by-products. To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-Ag as it might be used in disinfectant spray products? To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

Chapter 3 is a clear and complete description of the life cycle analysis of nano-Ag disinfecting sprays. The information is presented concisely, and with as thorough of a literature citation as possible.

Specific suggestions for improvement:

Section 3.2.1, lines 4-5. Some nutritional supplements claim electrolysis generates their nano-Ag.

Section 3.2.1, line 17. After Faraday, Carey Lea reported in 1889 on the reduction of silver nitrate in the presence of trisodium citrate. Carey Lea M. (1889) On allotropic forms of silver. *Am J Sci* 37:476–491.

Section 3.2.1, lines 18-19. Revise to: Subsequently, many nanoscientists now view the Carey Lea method as the Turkevich gold method extended to silver.

Page 3-3, lines 5-9. This is true, as recently reported by Takesue et al., *Journal of the American Chemical Society*, 2011, 133, 14164.

4. Information on the transport, transformation, and fate of nano-Ag in air, water, sediment, and soil is discussed in Chapter 4. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of understanding regarding the known and anticipated behavior of nano-Ag in the environment. To what extent is this information presented in a manner that would inform consideration of likely exposure routes relevant to biota and human health? For each of the environmental media discussed, to what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

Chapter 4 is less effectively organized. The outline is so much more subdivided compared to previous chapters; it gives some sections a stunted and incomplete feeling, and leads to much repetition. However, this does make the table of contents easier to navigate.

Specific suggestions for improvement:

General Comment. Suggest changing "throughout this document" to "throughout this chapter."

Page 4-1, line 2, Footnote 7. This important point is presented too late in the document; this should be introduced at the first usage of nano-Ag.

Page 4-1, line 10, Footnote 8. It is the opinion of this reviewer that trying to extend trends to all "nanoparticles" is too broad, much the same way extending trends to all "chemicals" is too broad. Classes of chemicals such as "cationic surfactants" have general trends, while still requiring careful analysis of specific chemicals (quaternary vs. tertiary cations, lengths and/or branching of alkyl chains, etc.); classes of nanoparticles such as "silver nanoparticles" may be more appropriate for general trends, while still requiring careful analysis of specific nano-Ag particles (surface coating, size, shape, etc.)

Page 4-2, line 12, Footnote 9. Excellent point, worth mentioning again. Please ensure this terminology is in fact used consistently throughout the entire document (not just Ch.4), to avoid

propagating the confusion.

Section 4.1.1.1, Persistence page 4-3. Here, persistence only talks about dissolution into Ag⁺. Title should be dissolution. Persistence should include both dissolution (and reformation from ions, see comment below), and colloidal stability. It seems to this reviewer that persistence in 4.1.1.1 only refers to particles remaining in suspension in water environments. Perhaps the authors should consider particles that deposit to a clay or soil and then remain stable for decades as persistent. Perhaps use this structure: 4.1.1.1. Persistence, 4.1.1.1.1. Dissolution, 4.1.1.1.2. Particle Aggregation, Agglomeration, and Deposition, 4.1.1.1.3. Biological/Organism Transformation and Transport. (see comment below)

Section 4.1.1.2, Particle Aggregation, Agglomeration, and Deposition. "Cluster" is not used here until line 14, which is okay but contradicts footnote 9.

After Liu & Hurt dissolution discussion, should also include reduction of Ag⁺ by humic acids to form AgNPs (Akaighe et al., *Environmental Science & Technology*, 2011, 45(9), 3895) or fulvic acids (Sal'nikov, et al., "Silver ion reduction with peat fulvic acid" *Russian J. Appl. Chem.* 2009, 82(4), 545). It is possible a cycle of oxidation/dissolution and reduction/formation could exist, increasing persistence. This cycle's equilibrium distribution of Ag⁺ and AgNPs will vary as environmental conditions (dissolved O₂, humic acid concentration, etc.) vary. As ions and nanoparticles transport through various conditions, fewer or greater numbers of particles may be found.

Section 4.1.1.1.3, Biological/Organism Transformation and Transport. If particles are present in the air, water or soil, they could adsorb onto the skin/feathers/exoskeletons/outer surfaces of reptiles/birds/insects/etc., which may then migrate and shed their skin/feathers/etc. and deposit silver in new areas. If organisms consume contaminated waters, their digestive systems may process/transform/bioaccumulate the nanoparticles, such as was observed for gold nanoparticles with filter-feeding clams (Hull et al., *Environmental Science & Technology*, 2011, 45, 6592). The reviewer recognizes this overlap with Chapter 5, and that little specific data is available. However, high-level concepts and potential scenarios should be presented, much as they are in Chapter 3 for product life cycle scenarios.

Section 4.1.1.5, Transformation, page 4-5. As written is merely an introductory sentence, and should be move to the general introduction in Section 4.1. Photochemical transformations from solar irradiance should be considered.

Section 4.1.2.1. Chinnapongse, et al., *Science of the Total Environment*, 2011 showed humic and fulvic acid provide varying degrees of colloidal stability to silver nanoparticles, slowing or preventing agglomeration likely through adsorption onto the nanoparticle surface. In section 4.4.2.2., the Chinnapongse et al. reference also applies.

Sections 4.2 and later read much better than Section 4.1.

5. Chapter 5 provides information on exposure, dose, and translocation of nano-Ag in humans and other biota. Please comment on the extent to which this chapter accurately and sufficiently characterizes this information and forms a basis for considering the health and ecological impacts of nano-Ag. To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

Chapter 5 is very good, well written, and the material is effectively organized. Sufficient information is provided to the extent that literature reports are available. General population exposure (Section 5.3.1) nicely included consideration of susceptible populations such as children. Section 5.2.3.5, the zebrafish embryo section was very nice.

Specific suggestions for improvement:

Section 5.5.1.By-Products...Transformations, there are no transformations discussed here, just byproducts. Transformations really belong in the Chapter 4 appropriate chemistry sections, with just a reference to that here in Chapter 5.

Section 5.6, Models. Compared to other parts of Chapter 5, this seems short. Discussion of the shortcomings of the models, identifying knowledge gained from other sections or gaps that need to be incorporated into next-generation nanoAg-specific models needs to be added. For example, Liu & Hurt have shown AgNPs can dissolve and release ions; others have shown humic acids reduce silver ions to form new nanoparticles. How could or should this knowledge be built into new models?

An additional reference on modeling exposure is by Musee, in Hum Exp Toxicol September 2011 vol. 30 no. 9 1181-1195.

6. Chapter 6 characterizes factors that influence ecological and health impacts of nano-Ag and discusses the currently available scientific evidence regarding these impacts. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of the science. To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

Overall, Chapter 6 is very good and well written. It is nice to see that comparisons to photography industry wastewater emissions were included. Nice comparisons of Nano-Ag specific to conventional silver throughout chapter.

Specific suggestions for improvement:

At end of Section 6.1.1.1, Size. Discussion on how agglomeration/aggregation within size distribution has been show to affect toxicity tests such as hemolysis, where both total silver concentration and agglomerate size affect results (Zook et al., Nanotoxicology, 2011). Also, page 6-8, line 24, ...prevent formation of clusters in solution, OR, can control the size of cluster formation (Zook et al., Nanotoxicology, 2011).

Page 6-8, line 26. When used as a bactericide in water... Or when used as a bactericide attached to ceramic point-of-use water filters, Oyanedel-Carver et al., Environmental Science & Technology, 2008.

Page 6-10, lines 22-24. Sonication can be highly irreproducible if details are not reported, see Taurozzi et al., *Nanotoxicology*, 2011.

General comments:

Much of the historical effects of colloidal Ag are ignored.

- Early 1900's medical treatments with colloidal Ag, "Argyrol" (Schack W (1960) *Art and argyrol. The life and career of Dr. Albert C. Barnes.* Thomas Yoseloff Press, New York).
- Pool treatments with colloidal Ag, "Silver Algaeden" (Height MJ (2009) *Evaluation of hazard and exposure associated with nanosilver and other nanometal oxide pesticide products.*
<http://www.regulations.gov/search/Regs/contentStreamer?objectId=0900006480a52512&disposition=attachment&contentType=pdf>

There is a general issue regarding the use of "uncoated"...it is not possible for Ag(0) metal NPs to be stable in solution without having some colloidal stabilizing agent (small molecule for electrostatic repulsion, adsorbed protein/NOM for steric). No "naked" or "uncoated" singly-dispersed particles exist in solution. Using uncoated typically refers to "citrate stabilized" before adding an intended coating (polysaccharide, PVP, etc.). While authors use this to convey differences efficiently when comparing surface coating effects, it leaves out the critical control information of what the uncoated particles were coated with. The reviewer recognizes that the authors of the document were transmitting the language that the authors of the citations used, however in the broader context of this document it can create an issue.

7. Chapter 7 summarizes the information and research questions presented in the nano-Ag case study, as well as discusses the role of case studies in the refinement of research strategies and potential future assessment efforts. We would appreciate comment from the peer reviewers on the integration of evidence in this chapter and its usefulness in supporting future development of research strategies and assessments. How might this chapter be improved?

As this is the chapter many people will choose to read if they do not have time to read the entire document, it warrants most attention. It is indeed quite a useful summary of the entire document as written. It would be useful to have a section that specifically identifies key knowledge gaps that the scientific research community and funding agencies could focus future efforts on to achieve maximum effectiveness of those investments. A minor suggestion for improvement would be to match the order of topics in the outline with the order of topics in the flow of the CEA Figure 7-1, even though this was easy to read with good transitions between sections.

In regards to Figure 7-1, the workshop report draft 3 (as cited in this document) in Section 3.2.2.2 "PowerPoint slide" on page 3-35, provides more details than Fig 7-1 of this document, which is a reproduction from Figure 1-1 earlier in the document. As this chapter intends to be a detailed summary, a detailed pictorial should be presented.

Overall, Chapter 7 is very nicely done. For example, in Section 7.2.1.3, page 7-7, a nice example of sophisticated research instrumentation solving real world problems is provided with FFF-ICP-MS.

8. For the document as a whole, are there ways to improve the structure, scope or presentation of information to better support the identification and prioritization of research needs by diverse stakeholders?

This document clearly summarizes that significant progress has been made regarding research into the nanoEHS hazards and exposures of nano-Ag. This document is quite useful and should accelerate further the iterative process of CEA through this summary and the identification of key knowledge gaps and research needs.

The Appendices are very useful references, and add significant value to the document.

It would be useful to have a more succinct summary, perhaps in Chapter 7 or perhaps in one of the appendices, such as a table listing (in common units) the exposure level and biological response of all studies. The results should be ordered by increasing exposures, with a column stating "toxic/sub-lethal/ no effect", so readers can easily compare at what levels the responses begin to occur to which nanoparticle sizes/surface coatings/etc. and in which organisms. While the desire certainly exists to test every specific formulation of nano-Ag exhaustively for safety and toxicity, this must be balanced with the hurdle this places on bringing novel competitive products to market that benefit the consumer and society. Perhaps it is worth considering how does this balance compare to other chemicals or materials evaluated in the CEA context?

General comments:

There is a very heavy reliance on the PEN reports, compared to other non-profits, NGO's, or think-tanks.

More focus should be placed on the analytical methods and metrology discussions. For example, key knowledge gaps and research priorities should be identified. How to measure dissolved silver? Challenges on size? Extraction techniques from complex environmental media?

General comment prompted by a statement in Section 7.2.1.5 on page 7-8, line 12. Analytical methods are a high priority area for accelerating the development of nanomaterial EHS risk assessment and should continue to receive attention. Few affordable, rapid techniques exist for qualitatively and quantitatively identifying low concentrations of silver nanoparticles in complex environmental or biological samples, and efforts should be made to overcome this challenge. However, Appendix A provides an overwhelming selection of analytical methods for the many types of characterizations that are desired, and careful scientific judgment can often lead to acceptable answers given modern instrumentation constraints. Additionally, better biological testing is just as needed as analytical methods. There is no standardized cell culture, in-vitro or in-vivo test, or even series of tests that can provide the information needed to determine hazards. Much like for size characterization, where multiple orthogonal measurements providing size distributions tells the total picture of the size of the material, and is often missing from most reports, for biological testing multiple orthogonal and thorough measurements are required. There is no one cell culture test that can definitively tell if a small molecule chemical drug or pesticide molecule is safe to use and non-toxic. For example, research papers often report one kind of test (cell death) for one specific cell line (such as kidney cells or liver cells). The interactions between types of cells, organs, and tissues can often only be determined through in-

vivo testing, but is not always directly applicable to humans. As much pressure should be put onto the biological community to fundamentally improve their screening tools as is put onto the analytical methods community. It is equally challenging to assure absolutely no small (say less than 2 nm diameter) silver nanoparticles exist in a sample, as it is to assure a given silver nanoparticle formulation is completely safe at a given level for humans or the environment.

The combination of rigorous physical, physicochemical, and biological characterization is not often seen in the literature, although this challenge has become more recognized in recent years. Yet, too often, those entering the field from one background or the other do not have the understanding or technical ability to achieve all of these physicochemical and biological characterizations.

9. The case study follows the CEA framework, which combines a product life-cycle perspective with the risk assessment paradigm to support subsequent steps in the CEA process. Please comment on aspects of the CEA framework and process that can be improved in future applications of CEA. We would appreciate input on the overall structure and scope of the framework and process and the extent to which they support the development and refinement of research directions for future CEAs of nano-Ag in particular and nanomaterials in general.

The overall scope and structure of the document was quite useful, and provided a logical way of systematically working through the CEA framework (which at first glance in Figure 1-1 seems potentially challenging as so many things are interconnected).

One area for improvement could be to identify where the delineation between AgNPs's nano-specific effects is, and once they transform/dissolve into Ag⁺ such that they are no longer a "nanoAg" effect? Are there scenarios where one could consider that AgNPs are merely dosing Ag⁺, and previously known information on "bulk" or "ionic" silver could then be applied? How could the need for such a demarcation be confirmed/ruled out? This also ties into the tiered approach many are proposing for hazard assessment of nanomaterials (*e.g.*, red/orange/yellow/green), and reducing the uncertainty of hazard assessments.

Better and more specific identification of knowledge gaps and research priorities could help the iterative process by guiding funding agencies and researchers, and instructing future CEA participants.

This appears to be an excellent example of a successful CEA iteration.

III. SPECIFIC OBSERVATIONS

Page	Line # or Paragraph	Comment or Question
-	-	General: <i>in vivo</i> and <i>in vitro</i> are typically italicized.
6-57	Line 5	Units should be $\text{mg kg}^{-1} \text{d}^{-1}$, or $\text{mg/kg}\cdot\text{d}$ (no dash)
2-3	Table 2-1	Suggest to also include mid 1990's study on Texas estuaries, Wen, L.; Santschi, P. H.; Gill, G. A.; Paternostro, C. L.; Lehman, R. D. Colloidal and particulate silver in river and estuarine waters of Texas <i>Environ. Sci. Technol.</i> 1997 , 31,723– 731. While outside the US, this study of silver mines shows nanometric silver in mine tailings, Gomez-Caballero, J. A.; Villasenor-Cabral, M. G.; Santiago-Jacinto, P.; Ponce-Abad, F. Hypogene Ba-rich todorokite and associated nanometric native silver in The San Miguel Tenango mining area, zacatlan, puebla, Mexico <i>Can. Mineral</i> 2010 , 48, 1237–1253.
-	-	Appendix A comments: Very good reference, great detail. A.2, A.12, include Small Angle X-ray Scattering (SAXS) A.2, A.9, A.12, include nanoparticle tracking analysis (NTA)

Review By:
Peter R. McClure, Ph.D., DABT

Peer Review Comments on EPA's Draft Document *Nanomaterial Case Study: Nanoscale Silver in Disinfectant Spray*

Peter R McClure, Ph.D., DABT
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North Syracuse, NY, USA

January 9, 2012

I. GENERAL IMPRESSIONS

The accuracy of information presented and the clarity of presentation in Chapters 1, 3, 5, 6 and 7 of this document are excellent, and the conclusions reached are sound. The document should be an excellent resource for planning for future research on ecological and human health impacts of silver nanoparticles in disinfection sprays. Unfortunately, much of Chapters 2 and 4 are poorly written. While I essentially agree with the conclusions for these chapters, I think they should be rewritten to reflect the high quality of organization, presentation, and evaluation demonstrated in the other chapters. Specific suggestions for improvement of the document are presented in Sections II and III of this review.

II. RESPONSE TO CHARGE QUESTIONS

1. Chapter 1 provides introductory material regarding the CEA approach used in these case studies along with other background information and a discussion of terminology. Is this information accurately and clearly presented? Please comment on the utility of the chapter in providing background and support for the remainder of the document. In particular, are the figures summarizing the CEA framework and process clear? How might this chapter be improved?

The background information presented in this chapter is clearly presented. The information on the CEA framework and process is important for the reader to understand the purpose of the document. The terminology information presented in Section 1.4 (on silver nanoparticles; conventional silver; agglomeration/aggregation/cluster and colloid; and naturally occurring, incidental, and engineered nanoparticles) is important to frame the analyses presented in subsequent chapters. I have no general suggestions for improvement, but see a few specific comments in Section III.

2. Chapter 2 presents basic information on conventional silver, including data on usage and historic environmental levels. Information on the physical-chemical properties of nanoscale silver and analytic methods makes up the rest of the chapter. Is this information clear and accurate? How might this chapter be improved?

The chapter is difficult to read and should be rewritten. It does not reflect the high quality of organization, writing, and evaluation displayed in Chapters 1, 3, 5, and 6.

The chapter could be improved by preparing more accurately descriptive titles for the various subsections. I suggest the following:

Chapter 2. Introduction to Silver and Nanoscale Silver

- 2.1. Conventional Silver: Uses, Occurrence in the Environment, and U.S. Standards
 - 2.1.1. Uses of Silver and Silver Compounds
 - 2.1.2. Occurrence of Silver in the Environment
 - 2.1.3. U.S. Standards for Environmental Silver (*Use the text starting on p 2-4, L28 and extending through p 2-6, L18*)
- 2.2. Historical and Emerging Uses of Nanoscale Silver
- 2.3. Physical and Chemical Properties of Nanoscale Silver
 - 2.3.1. Size
 - 2.3.2. Morphology
 - 2.3.3. Surface Area
 - 2.3.4. Chemical Composition
 - 2.3.5. Surface Chemistry and Reactivity
 - 2.3.6. Solubility
 - 2.3.7. Conductive, Magnetic, and Optical Properties
- 2.4. Analytical Methods to Characterize Nanoscale Silver
 - 2.4.1. Methods for Laboratory Research
 - 2.4.2. Methods to Assess Environmental Occurrence
 - 2.4.3. Methods to Assess Workplace Occurrence
 - 2.4.4. Methods for Assessing Dose and Dose Metrics
- 2.5. Summary of Physicochemical Properties and Analytical Methods

See Section III for other size specific comments for improving the writing in Chapter 2.

3. Chapter 3 summarizes information on the lifecycle stages of nano-Ag disinfectant spray products, including potential releases to the environment of nano-Ag and by-products. To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-Ag as it might be used in disinfectant spray products? To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

I found this chapter informative and easy to read. See Section III for suggestions for improvement.

4. Information on the transport, transformation, and fate of nano-Ag in air, water, sediment, and soil is discussed in Chapter 4. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of understanding regarding the known and anticipated behavior of nano-Ag in the environment. To what extent is this information presented in a manner that would inform consideration of likely exposure routes relevant to biota and human health? For each of the environmental media discussed, to what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

The rambling text between the introduction and Section 4.5 is poorly written and does not provide a clear and concise account of what is known (and not known) about the fate of silver nanoparticles in air, water, soil, and sediments. Many passages left me more confused than

enlightened. Chapter 4 needs to be rewritten to present a high quality evaluation of information on this topic, similar to the evaluations presented in Chapters 1, 3, 5, and 6. See specific suggestions for condensing and sharpening the focus of the text in Sections 4.1, 4.2, 4.3, and 4.4 in Section III.

5. Chapter 5 provides information on exposure, dose, and translocation of nano-Ag in humans and other biota. Please comment on the extent to which this chapter accurately and sufficiently characterizes this information and forms a basis for considering the health and ecological impacts of nano-Ag. To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

Chapter 5 is well organized and written and presents a logical and comprehensive evaluation of information on exposure, dose and translocation of silver nanoparticles in biota and humans. It is an excellent document to support planning for future research. See Section III for specific questions and suggestions for improvement.

6. Chapter 6 characterizes factors that influence ecological and health impacts of nano-Ag and discusses the currently available scientific evidence regarding these impacts. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of the science. To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

Chapter 6 is well organized and written and presents a logical and comprehensive evaluation of information on factors that may influence ecological and health impacts of silver nanoparticles and the state of knowledge on possible impacts on ecological and human health. It is an excellent document to support planning for future research. See Section III for specific questions and suggestions for improvement.

7. Chapter 7 summarizes the information and research questions presented in the nano-Ag case study, as well as discusses the role of case studies in the refinement of research strategies and potential future assessment efforts. We would appreciate comment from the peer reviewers on the integration of evidence in this chapter and its usefulness in supporting future development of research strategies and assessments. How might this chapter be improved?

This chapter is generally well written. It is somewhat repetitive of summaries and conclusions from the previous chapters, but serves effectively as a comprehensive, detailed “executive summary.” See Section III for a few suggestions for improvement.

8. For the document as a whole, are there ways to improve the structure, scope or presentation of information to better support the identification and prioritization of research needs by diverse stakeholders?

See my overall impressions above.

9. The case study follows the CEA framework, which combines a product life-cycle perspective with the risk assessment paradigm to support subsequent steps in the CEA process. Please comment on aspects of the CEA framework and process that can be improved in future applications of CEA. We would appreciate input on the overall structure and scope of the framework and process and the extent to which they support the development and refinement of research directions for future CEAs of nano-Ag in particular and nanomaterials in general.

I have no further comments.

III. SPECIFIC OBSERVATIONS

Page	Line # or Paragraph	Comment or Question
1-2	L8-10	The framework indicates that impacts other than human health and ecological impacts can be accommodated. The last sentence in this paragraph states that "... the focus in this case study is limited to environmental impacts." Does "environmental impacts" refer only to health and ecological impacts or some subset of "other" impacts in addition to health and ecological impacts?
1-4	L14-18	This text clearly and importantly restricts the focus of the present case study to identifying and prioritizing information gaps.
	Chapter 2	Difficult to read and often confusing. Needs rewriting.
2-1	L10-13	References for these statements about background levels should be added.
2-9	L8-9	Replace "as well as disperse into smaller particles or ions" with "as well as disperse into smaller particles or dissociate into ionic forms of silver."
2-10	L18-33	This information about influences of nanosilver size is repeated in Section 4.1.1. Leave here and discard Section 4.1.
2-15	L12-14	Consider replacing "Their findings imply that the surface coating on the particles in a nano-Ag spray disinfectant, and not necessarily the nano-Ag per se, might be the key to product effectiveness" with "Their findings imply that surface coating characteristics can impact bactericidal effectiveness of nano-Ag sprays."
2-15 to 2-16	2 paragraphs on solubility	These paragraphs on solubility should be rewritten to: (1) note that silver nanoparticles are essentially insoluble in water (since they are composed of Ag ⁰); (2) describe evidence that Ag ⁰ nanoparticles can remain suspended longer in aqueous solutions (depending on particle and solution characteristics) than larger sized Ag ⁰ particles; and (3) describe evidence that dissociation of Ag ions into aqueous solutions may be greater or faster from Ag ⁰ nanoparticles (depending on particle and solution characteristics) than from larger-sized Ag ⁰ particles. The current descriptions of observations by Cumberland and Lead (2009) and Griffett et al. (2009) are more confusing than enlightening. For example, did Cumberland and Lead (2009) show that dissociation from silver nanoparticles in aqueous systems was greater when human substances and sodium and calcium were added at environmentally relevant concentrations (1% "dissolved" versus what percentage in absence)?

Page	Line # or Paragraph	Comment or Question
		Did they use methods that could adequately discern “dissolved” Ag (i.e., Ag ⁺) from suspended Ag ⁰ nanoparticles? See next row for comments on the Griffett et al. (2009) citation.
2-16	L3-6	Wording and citation of this finding from Griffett et al. (2009) is confusing and misleading here. They added 1000 µg/L of silver nanoparticles to 2L of 0.02-µm filtered water, noticed that most of the material sedimented within 15 minutes, characterized the suspended material as showing a range of sizes from single nanoparticles to aggregates of several hundred nm size, determined that Ag concentrations in water samples taken at 2, 24 and 48 hours after addition of the silver were constant at about 50 µg/L, and determined that Ag concentrations in samples filtered through 0.02 µm filters were about 10 times lower than Ag concentrations in unfiltered samples. Griffett et al. (2009) called the filtrate Ag “soluble silver,” but their methods could not determine what fraction of the silver in the filtrates was suspended Ag ⁰ nanoparticles with diameters < 20 nm versus dissociated silver ions. I could not access the Cumberland and Lead (2009) paper through HERO, so I could not determine what methods these authors used to determine “dissolved” Ag.
2-18	L3-4	Replace “atomic adsorption” with “atomic absorption.”
2-23	L28	Replace “e.g. in solution or in air” with “e.g., in water, air, sediments, or soil.”
	Chapter 3	Well written and informative.
3-3	L7-10	Please clarify here the chemical state of the silver spherical particles produced – elemental, not ionic, silver Ag ⁰ .
3-3	Paragraph L10-24	The opening topical sentence should be amended to note whether or not data on air concentrations in, or emissions from, nano-Ag spray manufacturing facilities are available. I suspect data are not available, otherwise I would expect it to be cited and described in this section.
3-2	L17-18	It would be informative to describe some of the results of the Park et al. (2009) study. What was the range of concentrations and the characteristics of the particles (i.e., size distribution, degree of clustering) detected in workplace air samples?
3-2	L20-21	Replace “Available data on exposures are described in Section 5.3.2.” with “Available data on occupational exposure are described in Section 5.3.2.”
3-9	L12-14	Replace “including the humans, pets....” with “including humans, pets....”
3-11 to 3-12	Summary paragraph	A statement should be added stating whether or not data on air concentrations in, or emissions from, nano-Ag spray manufacturing facilities are available.
3-12	L5-6	“Results of bench-scale syntheses of nano-Ag suggest that wet chemical processing is more efficient than other production processes; wet chemical processing is likely to result in lower inhalation exposures during the manufacturing stage than solid- or vapor-phase processes.”

Page	Line # or Paragraph	Comment or Question
		What is the evidence for the latter contention in this summary sentence? It is not mentioned, but should be, in Section 3.2.
	Chapter 4	Chapter 4 needs to be rewritten and condensed to focus on what is known and not known about the environmental fate of silver nanoparticles and silver in aquatic systems. Sections 4.2, 4.3, 4.4 are overly wordy and are more confusing than enlightening.
4-1 to 4-8	Section 4.1	Chapter 4 could be improved by deleting Section 4.1 and incorporating nanosilver specific information described or cited in this section in the subsections on the fate of Ag nanoparticles in specific environmental media: air (currently 4.2), terrestrial systems (4.3), and aquatic systems (4.4). Much of the generic information on possible effects of nanoparticle characteristics (presented in Section 4.1) on environmental fate and biological processes have already been discussed adequately in Section 2.3. The current section makes it difficult to discern what is known (and unknown) about the fate of silver nanoparticles in the environment and does not provide a concise rationale for what kinds of information need to be generated from new research.
4-2	L22	Replace “as described detail in Section 2.3” with “as described in detail in Section 2.3.” <i>But see suggestion about deleting Section 4.1.</i>
4-3	L30-32	Include a sentence or two describing data (presumably from Luoma, 2008) supporting the contention that nanoparticles having surface coatings to keep them dispersed have “greater persistence in the environment” than uncoated particles. Was this demonstrated under laboratory conditions with silver nanoparticles or some other type of nanoparticle? What was the difference in rate of dissolution of the coated nanoparticles versus uncoated nanoparticle? <i>But see suggestion about deleting Section 4.1.</i>
4-4	Section 4.1.1.2	A poorly written section that does not add much from what was discussed in Section 2.3. See next two comments, as well. The text does not discern which statements about clustering and deposition are supported by observations of silver nanoparticles versus those of other types of nanoparticles. The first paragraph states that clusters of nanoparticles are less mobile than individual nanoparticles, but does not relate “translocation” or “mobility” to “deposition.” The text does not clearly or concisely state the expectation that clusters of nanoparticles are less mobile in environmental media because they deposit faster and to a greater extent than individual nanoparticles when suspended in air, in water, or in aqueous phases of soil. This expectation appears to be the main point of this section. <i>See suggestion about deleting Section 4.1.</i>
4-4	L13-14	Do you mean that by reducing clusters you can increase surface area of Ag ⁰ nanoparticles interacting with an environmental media and increase the rate of release of ionic silver? The phrase, “thereby exploit the high surface reactivity of ionic silver” does not accurately portray these processes and ionic silver does not have a surface reactivity. What data are presented by Kandlikar et al. (2007) to support this contention? What

Page	Line # or Paragraph	Comment or Question
		<p>data are presented by Tiede et al. (2009), Lowry and Casman (2009) and Handy et al. (2008b) to indicate that environmental conditions (what conditions? pH?, temp?, media?) influence clustering of silver nanoparticles in aqueous systems? Do these references provide specific data that should be discussed in Section 4.4 (i.e., environmental fate in aquatic systems)? <i>See suggestion about deleting Section 4.1.</i></p>
4-4	L18-28	<p>In this paragraph, the explanation of how particle clustering and deposition in the environment are related is garbled, and sprinkled with references without referring to the data that may support the statements. Deposition onto a water surface or land is first discussed, presumably referring to deposition from particles suspended in air, and then the discussion switches without transition to deposition (i.e., sedimentation) in water columns. <i>See suggestion about deleting Section 4.1.</i></p>
4-8 to 4-11	Section 4.2 (4.2.1 through 4.2.5)	<p>A better title would be: 4.2. Fate in Air. This is a poorly written section that uses too many words to try to convey the important points that (1) Ag nanoparticles can be released to indoor and outdoor air at various stages of the life cycle of nano-Ag disinfection sprays (manufacturing, use, disposal); (2) the magnitudes of releases into these air sources with current and expected future uses of nano-Ag sprays are unknown; (3) and although there is limited specific data about the fate of silver nanoparticles in air, deposition of nanoparticles from air is expected to be influenced by particle size (smaller nanoparticles stay suspended longer and diffuse more widely and rapidly than larger microparticles) and degree of clustering (larger clusters deposit more rapidly than smaller clusters or individual particles). Other important points to briefly mention are that nanoparticle coatings can reduce the degree of clustering of airborne nanoparticles (and thereby increase their resident time in air) and that individual nanoparticles can bind to other larger airborne particles which are subject to faster deposition. Subsections for Section 4.2 seem superfluous, since there really are not a lot of data to discuss.</p> <p>The section could be additionally improved by a very brief discussion of the type of data that could be collected to increase quantitative understanding of the fate in air of silver nanoparticles from disinfection sprays. For example, one could collect air samples at various times after using a disinfection spray in an indoor space and characterize particle concentrations and characteristics (e.g., size distributions and degree of clustering) in the samples. This type of data would be helpful in developing and improving the environmental fate models discussed in Section 4.5.</p>
4-10 to 4-13	Section 4.3 Terrestrial Systems	<p>Suggested title: Fate in Terrestrial Systems. This section also needs to be rewritten and condensed to emphasize the important types of information that are known and not known about the fate of silver nanoparticles in soil. For example, no studies are available on the rates of leaching or</p>

Page	Line # or Paragraph	Comment or Question
		<p>dissociation of silver nanoparticles through soil columns and how the rates are influenced by particle characteristics (e.g., coatings, particle size and clustering) and soil characteristics (e.g., soil pH, soil texture, and mineral and organic matter content) or on the temporal distribution of silver nanoparticles in soil systems among plants and other soil organisms, the solid soil matrix, and the aqueous phase. Quantitative data from these types of studies would be helpful in developing and improving the environmental fate models discussed in Section 4.5.</p> <p>As with Section 4.2, I think that the two subsections are superfluous in the absence of pertinent data on the fate of silver nanoparticles in soils. Also, superfluous to the topic at hand are the discussions of airborne nanoparticles depositing on aerial plant surfaces and the study of plant uptake of aqueous silver nitrate by plants (Harris and Bali, 2008). Speculation about phytoremediation of silver-contaminated soil is altogether out of the focus that should be maintained in this section.</p>
4-13 to 4-22	Section 4.4 Aquatic Systems	<p>Suggested title: Fate in Aquatic Systems. This section also needs to be rewritten and condensed to focus on what is known and not known about the environmental fate of silver in aquatic systems. The revised text should focus on the available silver nanoparticle data pertinent to fate in aquatic systems [e.g., observations on sedimentation by Gao et al. (2009); Tiede et al. (2010); Kiser et al. (2010); Kim et al. (2010a)] and the general lack of information on the temporal distribution of silver nanoparticles among all components of aquatic systems (e.g., water column, sediments, and bioaccumulation in aquatic organisms) and how they are quantitatively influenced by particle characteristics and characteristics of the aquatic systems. As suggested for Sections 4.2 and 4.3, the revised text should note that this type of quantitative information will improve the environmental fate models discussed in Section 4.5.</p> <p>The various pieces of text in this section on toxicity of silver ions and silver nanoparticles are distracting from what should be the main focus of this section (e.g., large sections of Section 4.4.2.1) and should be revised. Chapter 5 does an excellent job of concisely making inferences from toxicity data about environmental fate properties as it relates to biotic exposure.</p>
5-1 to 5-34	Sections 5.1 and 5.2	<p>These sections are well written and organized. Integrated smoothly in this comprehensive evaluation of information on the biotic exposure to and uptake of silver nanoparticles is discussion of important issues about the fate of silver nanoparticles and silver ions in environmental media (water, sediments and soil) that is much clearer and succinct than the tortuous text in Chapter 4. A job well done.</p>
5-8	L2	<p>Replace “adsorb” with “absorb.”</p>
5-12	L14-16	<p>“Although almost no studies of nano-Ag removal in wastewater treatment were identified, a study of nanoscale titanium dioxide (nano-</p>

Page	Line # or Paragraph	Comment or Question
		TiO ₂) removal provides some indication of likely efficacy of nano-Ag removal during treatment.” Chapter 4 mentions a study by Tiede et al. (2010) that should be described here. The abstract of the paper (I could not access the full paper through HERO) noted that, following 6-hour incubations of a range of silver nanoparticle concentrations in sewage sludge supernatant, >90% of the silver partitioned to the sewage sludge, and that a portion of the remaining silver in the supernatant was in nanoparticle form.
5-32	L31	At the end of this sentence, add “ <i>species</i> ” after herbivorous, or replace “herbivorous” with “herbivores.”
5-35 to 5-48	Sections 5.3. to 5.6	Just as well written, comprehensive and informative as Sections 5.1 and 5.2. See suggestions for minor improvements below.
5-36	L16-19	“The characteristics of nano-Ag sprays, such as the spray form of the product, in which particles are free and not fixed, the potential for direct exposure to nanomaterials in the product through application, and the potential for exposure through multiple routes, indicate that nano-Ag disinfectant sprays would be categorized as “high-potential-exposure” products.” I needed to read this complex sentence a few times to understand its structure. Perhaps separating it in two sentences would make it easier to read. “The characteristics of nano-Ag sprays indicated that they would be categorized as “high-potential-exposure” products. These characteristics include: the spray form of the product, in which particles are free and not fixed; the potential for direct exposure through application; and the potential for exposure through multiple indirect routes.”
5-47	L12-14	“Baun et al. (2008b) evaluated the potential effects of C60 nanoparticles (Buckminster fullerenes, or 12 buckyballs) on the bioavailability of this substance, as measured by the toxicity of various organic 13 toxicants to the green alga <i>Pseudokirchneriella subcapitata</i> and the freshwater invertebrate <i>D. magna</i> .” Suggested rewrite: Baun et al. (2008b) evaluated the potential effects of C60 nanoparticles (Buckminster fullerenes, or 12 buckyballs) on the bioavailability of 13 organic toxicants, as measured by their toxicity to the green alga <i>Pseudokirchneriella subcapitata</i> and the freshwater invertebrate <i>D. magna</i> .”
	Section 5.7	Well organized and written. See specific questions and suggestions for improvements below.
5-51	L28-29	“Sung et al. (2009), systemic distribution of silver in rats was reported for animals exposed via inhalation to aerosolized nano-Ag averaging approximately 18–19 nm in diameter.” Clarify if 18-19 nm refers to the particles from which the aerosols were generated or to the diameter of the aerosols.
5-52	L24-25	“the investigators found that nano-Ag was rapidly distributed out of the blood to the liver, spleen.....” Replace “nano-Ag” with “silver.”

Page	Line # or Paragraph	Comment or Question
5-54	L23-24	“demonstrate that the body cannot completely clear silver from all organs.” I prefer “demonstrate that there are limits to the clearance of silver from certain organs or tissues in the body”
5-55 to 5-56	p55 L21-32 to p5-56 L1-5	<p>This passage is confusing and leads to some questions and comments. “The model predicted that for a person breathing through the nose, nanoparticles of 100, 10, and 1 nm in diameter had deposition probabilities in the nasopharyngeal region of 0.05, 0.2, and 0.8, respectively (Mark, 2007).” Are all of the cited predictions from Mark’s use of the ICRP model for nose breathing only? Are a combination of nose and mouth breathing expected to produce significant differences in deposition patterns?</p> <p>“In other words, as the nanoparticles become smaller, they more effectively diffuse into the mucous layers where they can move farther along the respiratory tract (Witschi et al., 2008).” “Farther along” – do you mean lower in the respiratory tract? If an inhaled particle escapes deposition in the nasopharyngeal region, wouldn’t the airstream path to lower regions facilitate faster and easier transport (whether by bulk air movement of Brownian motion and diffusion) than through mucous layers?</p> <p>“Ninety percent of 1-nm particles were predicted to be deposited in the nasopharyngeal region of the lung, with the remaining 10% deposited in the tracheobronchial region.” I would replace <i>of the lung</i> with <i>of the respiratory tract</i>. Are these percentages of inhaled particles? How does 0.8 probability of deposition in the nasopharyngeal region get translated into 90% deposited in the nasopharyngeal region? If 90% and 10% of 1 nm particles get deposited in the nasopharyngeal and tracheobronchial region, does this mean that 0% of 1 nm particles get deposited in the alveolar region?</p> <p>“Nanoparticle deposition, especially for particle sizes of 20 nm and smaller, is governed by Brownian motion and diffusion, which allows movement of particles into the alveolar region of the lung, where larger particles (which are transported via bulk air flow) generally are not deposited (Elder et al., 2009).” Do you mean particles larger than 20 nm? Are larger particles not deposited in or not transported to the alveolar region?</p> <p>“For nanoparticles between 20 and 100 nm in size, deposition probability dropped for all three regions of the respiratory tract (Mark, 2007).” <i>Dropped</i> compared to what? As particle diameter increased?</p> <p>“Nonetheless, the ICRP model indicated that for nanoparticles measuring between 10 and 100 nm, the highest fractional deposition would occur in</p>

Page	Line # or Paragraph	Comment or Question
		<p>the alveolar region (Lynch and Elder, 2009).” Do you mean fraction of inhaled particles that are deposited in the alveolar region or fraction of particles reaching the alveolar region that are deposited?</p> <p>So, is this the important bottom line (is it only for nose breathing?) -- Citing the predicted fractional deposition of inhaled particles of differing diameters-- e.g., 10, 20, 50, and 100 nm --and comparing these to predicted fractional deposition for 1 to 2.5 µm particles in the alveolar region might be illustrative.</p> <p>The following is my understanding of the predicted deposition pattern of inhaled nanoparticles in the respiratory tract, but I cannot discern this pattern in your account of information in Marks (2007), Elder et al. (2009), and Lynch and Elder (2009).</p> <p>In contrast to fine particles with diameters in the 1–2.5 µm range, which are deposited mainly in the peripheral lung, inhaled nanoparticles (with at least one dimension <100 nm) can be deposited in the oral and nasal cavities, the tracheal/bronchiole region of the lung, and the alveolar region of the lung (Kreyling et al., 2002). Nanoparticles deposited in the alveolar region of the respiratory tract may be cleared from the alveolar region by: (1) macrophage phagocytosis and mucociliary transport along the tracheobronchial tree to the gastrointestinal tract; (2) translocation into interstitial tissue; (3) translocation to the lymphatic system; (4) particle dissolution with subsequent absorption into lung cells and transport into the blood; and (5) translocation of the particles into lung cells from lung surfaces and possible transport into the blood (Chen and Schluesener, 2008; Geiser et al., 2008; Kreyling et al., 2002; Oberdorster, 1988). Clearance from tracheal/bronchiole regions may occur by similar pathways. Nanoparticles deposited in the nasal mucosa also may be subject to particle dissolution and absorption into the blood or direct translocation of silver ions or elemental silver nanoparticles into the olfactory bulb of the brain via the olfactory nerve (Oberdorster et al., 2004).</p> <p>Chen, X; Schluesener, HJ. (2008) Nanosilver: a nanoproduct in medical application. <i>Toxicol Lett</i> 176(1):1–12.</p> <p>Geiser, M; Casaulta, M; Kupferschmid, B; et al. (2008) The role of macrophages in the clearance of inhaled ultrafine titanium dioxide particles. <i>Am J Respir Cell Mol Biol</i> 38(3):371–376.</p> <p>Kreyling, WC; Semmier, M; Erbe, F; et al. (2002) Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. <i>J Toxicol</i></p>

Page	Line # or Paragraph	Comment or Question
		<p>Environ Health A 65:1513–1530.</p> <p>Oberdorster, G. (1988) Lung clearance of inhaled insoluble and soluble particles. <i>J Aerosol Sci</i> 1(4):289–332.</p> <p>Oberdorster, G; Sharp, Z; Atudorei, V; et al. (2004) Translocation of inhaled ultrafine particles to the brain. <i>Inhal Toxicol</i> 16:437-445.</p>
5-58	L24-28	<p>“Larese et al. (2009) reported that nano-Ag can pass through normal human skin (i.e., full-thickness abdominal skin) in vitro at a rate of 0.46 nanograms per square centimeter (ng/cm²) and through damaged skin at a rate five times higher. Nano-Ag particles between 7 and 20 nm can penetrate into the hair follicle, and nano-Ag particles less than 30 nm can passively penetrate the deepest skin layers, probably through the intercellular route (Larese et al., 2009).”</p> <p>This description is misleading. Larese et al. did not demonstrate transport of silver nanoparticles across intact skin. Rather, they demonstrated silver transport across human skin exposed to silver nanoparticles. Their comments about possible translocation of particles through hair follicles and “intercellular” routes come from observations from experiments with other nanoparticles, not silver nanoparticles as suggested by the second sentence above.</p> <p>The following contains a more accurate and comprehensive description of the data collected.</p> <p>Silver absorption was detected across intact human skin samples mounted in Franz static diffusion cells and exposed to silver nanoparticles, but when the skin was abraded, rates were about five fold higher (Larese et al., 2009). The silver nanoparticles used in this experiment were coated with polyvinylpyrrolidone to prevent aggregation in an aqueous suspension. Intact and abraded human abdominal skin samples (stored for <4 months at -25°C) were exposed to 70 µg/cm² silver nanoparticles in aqueous 0.14% (w/w) ethanol diluted 1:10 with synthetic sweat for up to 24 hours. TEM examination indicated that particle diameters ranged from 9.8 to 48.8 nm, with a median of 25 ± 7.1 nm and 25th and 75th percentiles of 19.5 and 29.3 nm, respectively. Samples of receptor solutions were analyzed for silver concentration by atomic absorption spectrophotometry. With intact skin samples were exposed for 24 hours, silver was detected in the receptor solution at a median level above the detection limit (median = 0.46 ng/cm², range = less than the detection limit [0.1 µg/L] to 2.23 ng/cm²); absorption rates of silver through abraded skin were about fivefold higher (median = 2.32 ng/cm²; range = 0.43–11.6 ng/cm²). TEM of skin samples following exposure were reported to show silver nanoparticles in the stratum</p>

Page	Line # or Paragraph	Comment or Question
		corneum and the upper layers of the epidermis “in some slices”.
5-58	L28-32	<p>“Samberg et al. (2010) applied nano-Ag particles 20 and 50 nm in size to the backs of pigs in solutions ranging from 0.34 to 34.0 micrograms per milliliter (µg/mL) for 14 days. TEM demonstrated the presence of nano-Ag within the superficial layers of the stratum corneum for the 50-nm particles and on the top layer of the stratum corneum for the 20-nm particles.”</p> <p>It is important to emphasize that, regardless of size of silver nanoparticle applied, particles were detected only on the top or in the upper layers of the stratum corneum. Evidence for particle penetration to lower epidermal layers (some of which showed focal inflammation and edema) was not found. The latter observation led the investigators to hypothesize that silver ions, dissolved from particles in the stratum corneum, may translocate to the lower layers and cause the observed lesions.</p>
5-62	L8-12	<p>“Deposition of nano-Ag in the human lung differs from that of conventional silver. Nano-Ag is more likely to enter the alveolar region and translocate to other tissues, while conventional silver is more likely to be taken up by macrophages in the lung and excreted (Elder et al., 2009; Lynch and Elder, 2009). Following inhalation, nano-Ag can translocate from the lung to the bloodstream via the mucociliary escalator and subsequent ingestion, through passage into the lymph nodes, via alveolar epithelial cells, or via absorption in the olfactory bulb (Ji et al., 2007).”</p> <p>As a summary statement of the information on this topic, I prefer the following:</p> <p>Deposition of silver nanoparticles in the human respiratory tract is expected to differ from that of silver particles in the 1-2.5-µm size range, but the degree to which this difference in lung deposition may quantitatively influence distribution to other tissues is unclear. In animal studies (Sung et al., 2009; Ji et al., 2007; Takenaka et al., 2001), the finding of elevated silver concentrations in extrapulmonary tissues and blood following inhalation exposure to aerosols of silver nanoparticles provides qualitative evidence of absorption of silver by the respiratory tract, followed by distribution to other tissues. The finding of very high silver concentrations in the lungs following exposure, compared with other organs, however, suggests that, at the tested concentrations, translocation to other tissues is not extensive. Possible routes of translocation to other tissues following deposition of silver nanoparticles in the respiratory tract include direct translocation to the brain olfactory bulb from the nasal olfactory epithelium via the olfactory nerve, translocation (of particles and silver ions) to lymph nodes and blood</p>

Page	Line # or Paragraph	Comment or Question
		following alveolar deposition, and translocation via mucociliary clearance to the digestive tract following macrophage engulfment of alveolar deposited particles.
5-62	L16-17	<p>“Healthy skin exposed to nano-Ag resulted in dermal absorption, which appears to depend on exposure conditions, particle size, and other factors. Conventional silver is not expected to cross the skin barrier.”</p> <p>These sentences imply that silver nanoparticles may lead to more absorption than conventional silver following dermal exposure, but I know of no direct empirical data to support this.</p> <p>It is more accurate to say: Silver absorption has been demonstrated across healthy human skin samples exposed to suspensions of silver nanoparticles, but the degree to which this was due to transport of silver nanoparticles or silver ions released from particles in the stratum corneum is unknown. Rates of silver absorption were five-fold higher in skin samples damaged by abrasion.</p>
	Chapter 6	These sections are well organized, well written, and present a logical and comprehensive evaluation of what is known and not known about the ecological and human health effects of silver nanoparticles. Some comments follow on a few of the cited effects in laboratory animals; a few minor editorial changes are also included.
6-31	L24-25	Replace “Authors did not investigate the effects of silver ions on <i>C. riparius</i> ,...” with “The authors did not”
6-32	L27	“authors observed delayed hatching” suggested replacement “delayed hatching was observed.”
6-40	L16	Replace “are used to estimated predicted” with “are used to estimate predicted.”
6-58	L22-25	<p>“Decreased tidal volume, minute volume, and peak inspiration flow and incidences of alveolitis, granulomatous lesions, alveolar wall thickening, and alveolar macrophage accumulation were observed in both male and female rats exposed to the highest dose of nano-Ag in whole-body exposure chambers for 90 days, though not all effects were statistically significant.”</p> <p>You may want to examine and cite a later report by Sung et al. of the histopathology from the same study:</p> <p>Sung, JH; Ji, JH; Park, JD; et al. (2009) Subchronic inhalation toxicity of silver nanoparticles. <i>Toxicol Sci</i> 108(2):452–461.</p> <p>I think the following more accurately describes these effects reported by Sung et al. (2009, 2008):</p> <p>Decreases in tidal volume, minute volume, and peak inspiration flow in</p>

Page	Line # or Paragraph	Comment or Question
		<p>males and increases in incidences of alveolar macrophage inflammation, chronic alveolar inflammation, and mixed cell perivascular infiltrate in males and females were statistically significant in the high-dose group, compared with the control group.</p>
6-60	L14-16	<p>“These findings support the conclusions of the subchronic oral study conducted by Kim et al. (2008) and the subchronic inhalation study conducted by Sung et al. (2009) that the liver and bile ducts are targets for nano-Ag toxicity.”</p> <p>I agree with this conclusion, but the important findings for increased incidences of bile duct hyperplasia in high-dose males and females in the 90-day inhalation study of rats (Sung et al., 2009, 2008) are not cited in this section. They should be mentioned.</p>
6-61	L3-6	<p>“Although macroscopic observations revealed no gross edema or erythema at any tested dose, microscopic observations showed dose-dependent increases in morphological changes. Common morphological changes observed at the highest dose of nano-Ag were edema, focal inflammation, and epidermal hyperplasia with rete pegs extending below into the dermis.”</p> <p>You might want to amend this with some of the following:</p> <ol style="list-style-type: none"> 1) the lesions were observed in skin layers under the stratum corneum; 2) the effect was dose-related, with slight intracellular and intercellular epidermal edema at 0.34 µg/mL, moderate epidermal edema and focal epidermal and dermal inflammation at 3.4 µg/mL, and severe epidermal edema with severe focal dermal inflammation, epidermal hyperplasia, and parakeratosis at 34 µg/mL. 3) particle size or washing did not affect the observed skin responses; 4) Samberg et al. (2010) hypothesized that the lesions in layers under the stratum corneum were caused by silver ion flux into the lower layers from the particles in the stratum corneum. TEM examination of skin detected no particles in the skin layers showing lesions; particles were found only on the surface of the stratum corneum or with superficial layers of the stratum corneum; and 5) no examinations of nondermal tissues were conducted.
6-61	L8-10	<p>“Similar results in pigs exposed dermally were also reported by Nadwory et al. (2008), with some increased inflammatory cell apoptosis, decreased expression of pro-inflammatory cytokines, and decreased gelatinase activity observed.”</p> <p>The abstract of this study does not reflect the effects you describe (see below). I do not have access to the full report and a more in depth description of this study does not appear to be in Appendix C. Please check the accuracy of your description against the full report.</p>

Page	Line # or Paragraph	Comment or Question
		The anti-inflammatory activity of nanocrystalline silver was examined using a porcine model of contact dermatitis. Inflammation was induced with dinitrochlorobenzene and then treated daily with nanocrystalline silver dressings, 0.5% silver nitrate, or saline. Erythema, edema, and histological data showed that nanocrystalline silver-treated pigs had near-normal skin after 72 hours, while other treatment groups remained inflamed.
	Chapter 7	This chapter is an overview prepared from the generally well written summaries and conclusions of the preceding chapters. It is repetitive, but serves effectively as a comprehensive, detailed "executive summary." See previous comments about certain summary statements in Chapters 5 and 6.
7-13	L11-12	<p>"In fact, some plant species take up and accumulate silver in nanoparticle form after silver exposure, suggesting that metal-tolerant plant species could be used for phytoremediation."</p> <p>Speculation of phytoremediation seems out of focus for this document. What is more important to note is the possibility of ingestion of silver-contaminated plant materials by humans and other herbivorous species.</p>
7-14	L15-16	<p>"One study shows that most of the nano-Ag removed during treatment processes ends up in sludge soils;"</p> <p>Replace sludge soils with sewage sludge.</p>
7-17	L25-29	<p>"In both consumer and occupational populations, exposure can also occur through hand-to-mouth contact from touching or handling treated surfaces, a behavior that is particularly prevalent in children. Higher metabolic rates and greater consumption of food and water per body weight also indicate that children could be a susceptible population to nano-Ag spray use."</p> <p>For silver (nano or otherwise), I suspect that higher metabolic rate per body weight is not as pertinent as the other factors noted here.</p>
7-18	L10-11	<p>"Studies show that nano-Ag is more likely to enter the alveolar region and translocate to other tissues than conventional silver."</p> <p>See my previous comments about the differences in deposition patterns between nanoparticles and um-sized particles.</p>
7-18	L13-14	<p>"Although conventional silver is likely not taken up after dermal exposure, data indicate that nano-Ag can cross the dermal layer under some circumstances."</p> <p>See my previous comments about this issue in Chapter 5.</p>
7-21	L30-32	<p>"Findings indicate that nano-Ag exposure via one of several routes (e.g., oral, intravenous) can lead to gene expression changes, inflammatory response in the liver and kidney, and adverse functional effects in the</p>

Page	Line # or Paragraph	Comment or Question
		<p>lungs, heart, intestine, and spleen.”</p> <p>This discussion should be expanded to note that toxicity studies in rats repeatedly exposed to certain silver nanoparticles by the oral and inhalation routes are available.</p>

Review By:
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Peer Review Comments on EPA's Draft Document *Nanomaterial Case Study: Nanoscale Silver in Disinfectant Spray*

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January 6, 2012

I. GENERAL IMPRESSIONS

The document is pretending to make a “case study;” however, it is rather using one application of nano-Ag to organize to some extent the information extracted from the scientific literature and to guide the questions. However, this is not a real case study. From a case study, I would expect that only the information that is pertinent to the case study is summarized. My expectation was that the document contains some general chapters that are then followed by the “case study,” where the information that is relevant for the case is listed based on the life cycle aspects of the application. At the moment, just the information about nano-Ag is listed and the case study information (that is in some chapters covered quite nicely) gets completely lost. In principle, this document is a normal review of nano-Ag with some additional focus on sprays. But it's not really a case study. This could be solved by writing an additional chapter that collects all of the case-specific information in a single place.

The document is extremely long and goes, especially in Chapters 5 and 6, into very small details. Chapter 6 is almost not readable due to the huge amount of information that is presented – what is missing are tables and figures that help to organize the information and provide a critical discussion. The document is a summary of the work performed so far rather than a critical review. This is what makes the document so difficult to read. The different pieces of information are never really connected to each other. This is especially true for exposure and effect concentrations that are never compared to each other – however, this is the most crucial information that is needed for any risk assessment.

It is also surprising that from the wealth of information on use and human health effects of the historic use of nanosilver, e.g., Collargol or Argyrol, almost nothing is cited and used. The basic book about the human health effects of silver should be acknowledged as it forms the basis for all human health standards up to today (Hill, W.R.; Pillsbury, D.M. *Argyria, the Pharmacology of Silver*; The Williams & Wilkins Co.: Baltimore, MD, 1939).

II. RESPONSE TO CHARGE QUESTIONS

1. Chapter 1 provides introductory material regarding the CEA approach used in these case studies along with other background information and a discussion of terminology. Is this information accurately and clearly presented? Please comment on the utility of the chapter in providing background and support for the remainder of the document. In particular, are the figures summarizing the CEA framework and process clear? How might this chapter be improved?

This chapter provides a good overview on what the review is about. However, what needs to be improved is the explanation for why the spray application was chosen. It is only very briefly covered that it was based on an expert process. What other products were under discussion? How was the evaluation carried out?

In Chapter 1.4, "Terminology: Colloidal Silver," more background discussion is needed. The only definition for colloids that should be used is that commonly given in textbooks on aquatic chemistry, so particles between 1 and 1000 nm (e.g., Stumm and Morgan, *Aquatic Chemistry*). The "colloidal silver" mentioned in historic publications and patents is often actually nanosilver and not particulate silver between 100 and 1000 nm, as the definition of colloids might suggest.

2. Chapter 2 presents basic information on conventional silver, including data on usage and historic environmental levels. Information on the physical-chemical properties of nanoscale silver and analytic methods makes up the rest of the chapter. Is this information clear and accurate? How might this chapter be improved?

In Chapter 2.2, more information on the historic use of nanosilver is needed: much of the "colloidal silver" used in the last 100 years is actually nano-silver. The paper by Nowack et al. in *ES&T (Environ. Sci. Technol.* 2011, 45, 1177–1183) has shown that before 1995 all EPA-registered silver-products are actually based on nano-silver and only after this date "conventional" Ag-products were registered. So one could also say that nano-Ag is the conventional form of silver!

It should also be made clear that nanosilver was produced by scientists and companies around 1900, so it's not a new compound but has just recently gotten a lot of attention because of the "nano-hype". A thorough review of nano-silver needs to consider more than 100 years of production and use. It is important to note that shortly after 1900, scientists knew that they were producing particulate silver with a size in the nano-range (and were able to determine the size even without TEM!). These early nano-silvers were therefore deliberately made and were not an unintentional and unrecognized formation of nanoparticles. Many patents, e.g., from the 1960s, make it clear that the companies knew that for certain functionality the silver needed to be in the nano-range (at that time called micro-micro). On page 2-8, it seems that the authors consider historic uses of colloidal silver (which actually is nano-silver) as "conventional silver". This of course is wrong. What matters is if there were particles present in the nano-range and then it needs to be considered as nano-Ag. It is not correct to name the same compound before 1990 "conventional Ag" and after that date "nano-silver" because then this term was invented. "Conventional silver" should be restricted to uses of dissolved silver in all forms and metals salts and metals in particle sizes larger than 100 nm.

Chapter 2.3.6 covers dissolution of nano-Ag. This chapter needs to be rewritten. Metallic Ag first needs to be oxidized to Ag⁺, which can then be released into solution and it is therefore different to AgCl or other silver salts that can release Ag without any oxidation step. The oxidation of metallic Ag is covered later in the review (Chapter 4), but it needs to be made clear here that "dissolution" of nano-Ag involves two coupled processes.

3. Chapter 3 summarizes information on the lifecycle stages of nano-Ag disinfectant spray products, including potential releases to the environment of nano-Ag and by-products. To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-Ag as it might be used in disinfectant spray products? To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

On a qualitative level this chapter lists what is already known about the lifecycle of nano-Ag and what information we have about sprays. However, what is missing is a quantitative evaluation. I expected from this chapter to get numbers of release to the environment during the different life cycle stages. The whole chapter remains very vague and again, it is not really a case study, but listing of qualitative data. From a case study, I expect to see quantitative data. For example, a material flow diagram showing the flows of Ag from production, formulation, use and disposal to the environment. Even if a lot of data is missing, we can still make some estimations and best guesses.

Chapter 3.1, production amount of nano-Ag. In the beginning of 2011, a paper was published that gave an estimate of the US nano-Ag production (Hendren et al. Environ. Sci. Technol., 2011, 45 (7), pp 2562–2569). Why is this information not used? It's a much better value than the estimate by Mueller and Nowack (2008) that is cited in this chapter. The value by Hendren is not an estimate, but a range based on different pieces of information about the companies producing nano-Ag.

In Chapter 3.2.1, why are flame and plasma-processes not mentioned in this part? See for example Sotiriou (Environ. Sci. Technol. 2010, 44, 5649–5654) that used a flame-synthesized nano-Ag or NanoGrade, a company that produces nano-Ag based on flame processes. Demou et al. (Ann. Occup. Hyg., Vol. 52, No. 8, pp. 695–706, 2008) measured particle exposure in a nano-Ag producing facility that uses flame-synthesis. At least some of the nano-Ag on the market is therefore produced by flame-methods and need to be covered in this review.

4. Information on the transport, transformation, and fate of nano-Ag in air, water, sediment, and soil is discussed in Chapter 4. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of understanding regarding the known and anticipated behavior of nano-Ag in the environment. To what extent is this information presented in a manner that would inform consideration of likely exposure routes relevant to biota and human health? For each of the environmental media discussed, to what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

This chapter provides a good overview on the reactions of nano-Ag in the environment.

The most important reaction of nano-Ag in wastewater, the formation of silver sulfides, is not discussed in enough detail. There is only one study cited and presented. This reaction is extremely important as it transforms nano-Ag into another form, a silver sulfide. This results in the disappearance of the original nanoparticle and forms a silver-species that is almost completely insoluble. There is an article by Kaegi et al., 2011 (Kaegi, R., Voegelin, A., Sinnet,

B. et al., 2011. Behavior of Metallic Silver Nanoparticles in a Pilot Wastewater Treatment Plant. *Environmental Science and Technology* 45:3902-3908.) that describes the fate of nano-Ag during wastewater treatment and identifies and quantifies silver sulfides in sludge. This paper definitely needs to be considered. This reaction is again discussed in Chapter 5 (which is a problem of the document). The same reaction is presented and discussed in different chapters. Another example would be dissolution, which is discussed in three chapters (2, 3, and 4). See also the comments to Chapter 5.

Modeling: The Gottschalk et al. study not only modeled in Switzerland, but also in the EU and the US – the latter data are most relevant for this case study. These authors provided estimates of nano-Ag concentrations in wastewater, water, sediments, soils, and sludge-treated soils. These values – the only quantitative estimates of environmental concentrations so far – should be presented in detail as they provide the only available environmental exposure quantification.

5. Chapter 5 provides information on exposure, dose, and translocation of nano-Ag in humans and other biota. Please comment on the extent to which this chapter accurately and sufficiently characterizes this information and forms a basis for considering the health and ecological impacts of nano-Ag. To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

This chapter (and even more so chapter 6) contains a lot of text that is nicely grouped into sections. However, what is almost completely lost is the general overview – the reader is buried in a lot of information that is not equally important. It is necessary to add tables, figures and schemes that highlight the important aspects. An example could be that on page 5-18, where a scheme is added showing the different interactions with algae and summarizing the current state of knowledge. Also, figures taken from some of the key papers would help to identify important results. In the current version, important and marginal results are presented side-by-side.

Page 5-12: The TiO₂-data in wastewater should be removed and replaced by a description of the study of Kaegi (2011) that was mentioned above. When a specific Ag-study is available, it is not necessary to present data of a nanoparticle with completely different reactivity.

Page 5-12: It is important to note that the nano-silver sulfide that Kim et al. identified was the only Ag-containing solid that was identified. Because the majority of the silver flow in a wastewater treatment plant comes from non-nano sources, all silver forms must have been transformed naturally into nano silver sulfide. Kaegi et al. have also shown that nano-Ag is transformed into the same silver sulfide that is then discharged to some extent into natural water. This means that the fate of silver in the environment is the same for conventional silver and nanosilver because the wastewater treatment plant transforms all silver forms into the same silver sulfide. This has enormous implications for the risk assessment of nano-silver that reaches the environment through wastewater treatment plants. It is not possible to separate the discussion of nano-Ag from total silver flows.

Consumer exposure: A study that needs to be considered is “Size-fractionated characterization and quantification of nanoparticle release rates from a consumer spray product containing

engineered nanoparticles” (J Nanopart Res (2010) 12:2481–2494). It provides information on the form of nano-silver that is released during spraying.

Page 5-40: The study by Demou et al. (Ann. Occup. Hyg., Vol. 52, No. 8, pp. 695–706, 2008) on occupational exposure during production of nano-silver should be used here.

6. Chapter 6 characterizes factors that influence ecological and health impacts of nano-Ag and discusses the currently available scientific evidence regarding these impacts. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of the science. To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

Same problem here as with Chapter 5, just more severe: The main problem with this chapter is that it contains a lot of text that is nicely grouped into sections. However, what is lost is the general overview – the reader is buried in a lot of information that is not equally important. There are 68 pages and only a single table and no figure! It is absolutely necessary to add tables, figures and schemes that highlight the important aspects. Figures taken from some of the key papers would help to identify important results. In the current version, important and marginal results are presented side-by-side. Tables could be used to combine the key results from different sections and to help keep the overview. In the current version, this chapter is almost impossible to read.

Page 6-40. The authors use the PNEC value given by Mueller and Nowack in 2008. However, it would be better to use the newer value from the Gottschalk et al. (2009) paper. Why did the authors not try to derive their own PNEC value by taking advantage of the fact that in the last few years a lot of new data were published that would allow the derivation of a much better PNEC than the values from 2008 or 2009? This is a main problem of the whole review, it remains very qualitative and does not aim to quantitatively evaluate the data.

Section 6.3.3. It is written: “Nano-Ag, although not purified and produced extensively until recently, has long been present as a fraction in conventional silver, and particularly in colloidal silver, though not as intentionally engineered particles.” As discussed above, this is not correct. The historically used colloidal silver was always in the nano-form and was deliberately produced and engineered in nanosized form. Most of the silver exposure data that are discussed in the following therefore correspond not to “conventional silver,” but in fact to nanosilver, e.g., Collargol, Agyrol and so on.

Section 6.3.3.1. Medical Use Studies: The first of these studies were performed around 1900 and in 1939 a book was published summarizing the effects of silver and nano-silver (Hill, W.R.; Pillsbury, D.M. *Argyria, the Pharmacology of Silver*; The Williams & Wilkins Co.: Baltimore, MD, 1939). This section definitely needs to consider this old literature.

7. Chapter 7 summarizes the information and research questions presented in the nano-Ag case study, as well as discusses the role of case studies in the refinement of research strategies and potential future assessment efforts. We would appreciate comment from the peer reviewers on the integration of evidence in this chapter and its usefulness in supporting future development of research strategies and assessments. How might this chapter be improved?

This chapter is useful for all of those readers that don't want to go through 200 pages, but want a concise overview of the most important results. The main problem is that no references are given and therefore the usefulness of this summary is greatly reduced, in fact, it's almost useless as a stand-alone summary. For a normal few-page summary it is okay not to cite any references, but for this extended summary of more than 20 pages it is absolutely necessary that the main messages are underlined with references. It is even more important that this summary be used alone, as the text in some of the chapters is so long and will be hardly read at all.

8. For the document as a whole, are there ways to improve the structure, scope or presentation of information to better support the identification and prioritization of research needs by diverse stakeholders?

Any remarks to the general structure have been made under the other charge questions.

9. The case study follows the CEA framework, which combines a product life-cycle perspective with the risk assessment paradigm to support subsequent steps in the CEA process. Please comment on aspects of the CEA framework and process that can be improved in future applications of CEA. We would appreciate input on the overall structure and scope of the framework and process and the extent to which they support the development and refinement of research directions for future CEAs of nano-Ag in particular and nanomaterials in general.

Chapter 5 is on exposure, Chapter 6 on the effect, but what is missing is a chapter on risk (risk = exposure x effect). I would suggest creating a new chapter on risk and summarize the few studies that have already tried to perform an environmental risk assessment of nano-silver. Even if the data quality and quantity about exposure and effect is still sparse, it is possible to make some first conclusions about the risk, using established assessment factors to cope with the uncertainty. It is my opinion, a serious omission is that nothing is said about risk assessment in the whole document, although it is described in Chapter 1 that the CEA-approach is used.

III. SPECIFIC OBSERVATIONS

Figures 2.2 and 2.3 are not necessary – they contain too much detail that is not important for the document.

Chapter 2.3.6. AgCl is not nearly insoluble. Compared to environmental Ag concentrations the solubility limit is far away.

Review By:
Stig I. Olsen, Ph.D.

Peer Review Comments on EPA's Draft Document *Nanomaterial Case Study: Nanoscale Silver in Disinfectant Spray*

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January 12, 2012

I. GENERAL IMPRESSIONS

Overall, the document is a very comprehensive overview of the current knowledge on nano-Ag. I have learnt a lot reading the document, and although I am not an expert in many of the fields based on my knowledge from literature and conferences, I believe that the accuracy of the presented information is high. The clarity of presentation suffers a bit from the comprehensiveness. The wealth of information being served would require more small summaries, e.g., after each subchapter as well as more condensed presentation in the form of tables and figures. I find that the conclusions reflect the information presented in a sound way.

II. RESPONSE TO CHARGE QUESTIONS

1. Chapter 1 provides introductory material regarding the CEA approach used in these case studies along with other background information and a discussion of terminology. Is this information accurately and clearly presented? Please comment on the utility of the chapter in providing background and support for the remainder of the document. In particular, are the figures summarizing the CEA framework and process clear? How might this chapter be improved?

I was not knowledgeable about the CEA framework before. I find it very comprehensive, but nonetheless well described both in the text and in the two figures.

Page 1-5, line 20-21. The list of other efforts (references) is far from exhaustive, so there should be an "e.g." in front of the references.

Bottom of page 1-5 and top of page 1-6. Does raise some doubts as to what is the real purpose of the document. On the one hand it represents the "Compile Information in CEA Framework" step, but apparently only as an example since "it attempts to provide a framework for considering current and future information systematically." But isn't that framework already defined in the CEA? I don't understand why it has not gone through the whole process of compiling information and I don't think the chapter explains the reasoning sufficiently. On page 1-6, line 21 it says that the case study is meant to assist in identifying and prioritizing research – but if the study is not exhaustive how can it be used for that?

2. Chapter 2 presents basic information on conventional silver, including data on usage and historic environmental levels. Information on the physical-chemical properties of nanoscale silver and analytic methods makes up the rest of the chapter. Is this information clear and accurate? How might this chapter be improved?

The chapter does not represent any of my expertise, but I do find that the chapter provides a good overview not just on conventional silver, but when it comes to physiochemical properties very much so on nano-Ag. Since the chapter provides information on both conventional silver and nano-Ag, I think it would be worthwhile to present the data on the use (and amounts) more clearly giving a better overview, e.g., how much additional Ag could be expected in the environment through the use of nano-Ag.

3. Chapter 3 summarizes information on the lifecycle stages of nano-Ag disinfectant spray products, including potential releases to the environment of nano-Ag and by-products. To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-Ag as it might be used in disinfectant spray products? To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

Overall, the chapter includes all relevant life cycle stages in a fairly comprehensive way. The Manufacturing of Nano-Ag seems to be a bit exaggerated in comparison to the other chapters, most likely due to much more available information. Reading through the chapter, it seems that the focus on potential releases is not equal throughout the chapter – the chapters on use, distribution, and disposal have a high focus on this whereas the two preceding chapters do not. I find that the chapter on feedstock is more focused on total quantities produced than on quantities that may enter the environment, but this is a general remark to the chapter. It would be good to summarize what are the main processes for release of which compounds and what are the potential quantities (in line with my comment above, I think it would make sense to provide an overview of the potential releases. I realize it is written several times in different chapters and that there are limited data, but nonetheless this is information, even if estimated, will support planning of future research).

I am wondering about the use stage – all the potential releases mentioned are of course relevant, but will it not be all that is sprayed on that will eventually be released somehow? Or will the surfaces accumulate nano-Ag? I don't think this question has been addressed.

4. Information on the transport, transformation, and fate of nano-Ag in air, water, sediment, and soil is discussed in Chapter 4. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of understanding regarding the known and anticipated behavior of nano-Ag in the environment. To what extent is this information presented in a manner that would inform consideration of likely exposure routes relevant to biota and human health? For each of the environmental media discussed, to what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

This is not my expertise, but from what I have seen in papers and conferences it is an accurate reflection of the current knowledge. The information is well structured and provides a good overview and informative background for planning future research.

5. Chapter 5 provides information on exposure, dose, and translocation of nano-Ag in humans and other biota. Please comment on the extent to which this chapter accurately and

sufficiently characterizes this information and forms a basis for considering the health and ecological impacts of nano-Ag. To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

The chapter is very comprehensive and sums up as far as I can tell the available information. Due to the vast amount of info mixed with the unknowns, it is a bit difficult to read. The summary at the end does provide a good overview.

It is surprising that the potential increase of silver concentration estimated on page 5-5 is so high, given that a large percentage of nano-Ag in waste water should be estimated to be removed in the sludge.

Most of the text (this goes also for many of the other chapters) present in a comprehensive way a good summary of individual studies. It would be worthwhile to sum up/conclude in each subchapter. Also, summing up actual knowledge, e.g., exposure vs. internal dose, which are now referred in the text would be good to present in tables.

I find that there is a considerable amount of information that is repeated several times, reflecting that the structure is perhaps not entirely clear.

6. Chapter 6 characterizes factors that influence ecological and health impacts of nano-Ag and discusses the currently available scientific evidence regarding these impacts. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of the science. To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

The chapter provides a very comprehensive overview of current knowledge of toxic effect and actions. It is good that for ROS the known mechanisms is described. I also appreciate the subchapters summing up and providing the hypothesized mode of action – this is something I missed in the previous chapters. One aspect that has received some attention is the possible resistance development towards antibiotics as cross-resistance developing. This is not at all mentioned here – I am not sure if it is important (again this is not really my expertise), but I do believe that it deserves attention in the text. The chapter is fairly well structured.

7. Chapter 7 summarizes the information and research questions presented in the nano-Ag case study, as well as discusses the role of case studies in the refinement of research strategies and potential future assessment efforts. We would appreciate comment from the peer reviewers on the integration of evidence in this chapter and its usefulness in supporting future development of research strategies and assessments. How might this chapter be improved?

The chapter summarizes the information and integrates the evidence presented in the preceding chapter very well. I don't think the chapter can stand alone, but having gone through the preceding chapters it gives a very good overview for support of research strategies.

I fully agree on the research priorities derived from the workshop and mentioned in the subchapters of each main theme (life cycle stages, exposure, effect), but it lacks a summary of

the overall priority in chapter 7.3.1.

Page 7-1, line 12-13. The “extended cradle-to-grave life cycle approach” is mentioned and I agree that such an approach is necessary to be able to evaluate the potential risks and identify in which life cycle stages the highest risks may occur. This approach has been used in the European Risk Assessment for decades.

8. For the document as a whole, are there ways to improve the structure, scope or presentation of information to better support the identification and prioritization of research needs by diverse stakeholders?

In several comments above I mentioned the organization of the available data in tables or the like in order to get a better overview. Prioritizing and planning future research requires not just the identification of available information, but also the interpretation of current knowledge in terms of where research would be most profitable (and a better overview of the data in terms of tables or figures would improve this). I think this is a bit lacking in the overall document. I do understand that the aim is to provide an objective “state of the art” for current knowledge, but I think it would improve the document if the information was “digested” a little bit more.

9. The case study follows the CEA framework, which combines a product life-cycle perspective with the risk assessment paradigm to support subsequent steps in the CEA process. Please comment on aspects of the CEA framework and process that can be improved in future applications of CEA. We would appreciate input on the overall structure and scope of the framework and process and the extent to which they support the development and refinement of research directions for future CEAs of nano-Ag in particular and nanomaterials in general.

As the name indicates, I think the CEA framework is very comprehensive both in terms of the data compilation and the process. The only improvement I see is, as I mention above, to present the data compilation in a way that provides a better overview and input for prioritization. The scope is very wide, but apparently it can be narrowed a bit down depending on the subject of investigation, as has been done here for nano-Ag. I find that the current document with its comprehensive overview of the current knowledge supports the development of research direction very well.

III. SPECIFIC OBSERVATIONS

Page	Line # or Paragraph	Comment or Question
2-2	5-17	Would be useful to have a table on the distribution of silver usage among different applications.
2-8	11-14	Considering the large use of silver in many other industrial application, I don't find it substantiated that back-ground concentrations will increase in the environment. But background for human exposure probably will.
3-1	25	The study by Johnson et al. is not a life-cycle analysis but a mass flow analysis (MFA).
4-13	16-28	It seems to me that this study does not investigate the uptake of nano-Ag, but the uptake of silver ion from AgNO ₃ -solution. It is however an

Page	Line # or Paragraph	Comment or Question
		interesting finding that silver taken up is stored as nano-particles, so formed by the plant. The mechanism of nature forming silver nanoparticles is seen elsewhere, e.g., http://www.nanowerk.com/news/newsid=21309.php and Loeschner et al. Particle and Fibre Toxicology 2011, 8:18 Nonetheless, it cannot be concluded that plants can be used for sequestering nano-Ag for phytoremediation of nano-Ag!
4-14	3-10	As mentioned previously, I believe that most of the spray-disinfectant in the use stage will eventually end up in water due to washing of surfaces.
4-24	Figure 4-1	The figure seems to miss an arrow from “water/soil” to ecological receptor. The figure also does not reflect the life cycle of a nano-Ag spray disinfectant where both production and disposal are potential releases to ambient air.
6-7	Several lines	Some strange {...} with number inside occurred?
6-13	Table 6.1	While I appreciate seeing a table, I miss an explanation of the toxicity values (or unit if applicable).
7-19	20	I don't understand the term “lifestages” and how it is applied in this context. Is it lifestages of biota and humans?
7-23	7	Would be valuable information to know how many participants in the workshop.

Review By:
James F. Ranville, Ph.D.

Peer Review Comments on EPA's Draft Document *Nanomaterial Case Study: Nanoscale Silver in Disinfectant Spray*

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January 12, 2012

I. GENERAL IMPRESSIONS

As stated frequently, the purpose of the document is to begin the CEA process for nano-Ag in disinfectant sprays. The role of this document is specifically to fulfill the first step in the CEA framework "Compile Information in CEA Framework" (Figure 1-2), and in this respect it achieves its purpose. The document is effective as a non-critical literature review, being a compilation of most of the relevant papers on studies of nano-Ag and "conventional" silver, although I believe there is a body of literature on the environmental health and safety of dissolved ionic Ag that was not included. Given that the document is primarily a literature review, I cannot comment on the accuracy of the information it contains, but assume that results of the studies cited have been accurately presented.

The document also serves as a brief tutorial, both on the relevant processes for nano-Ag, including behavior in the environment (e.g. aggregation, dissolution, etc.), organism exposure, and organism health effects, and on analytical methodology. This aspect of the document is also useful to a reader having minimal background in nanomaterials, but is rather basic for those already practicing in this field.

Perhaps it is an unavoidable consequence of the format of the document, but it suffers from some redundancy, which makes it quite tedious to read the document in its entirety. Many of the concepts and implications seem to be discussed in nearly every chapter. This is especially true of the summary chapter (Chapter 7), which instead of being a succinct, to the point summary, is "watered down" with restatements of things presented in the preceding chapters. Much of Chapter 7 seems to actually be "cut and pasted" from previous sections. I suggest that Chapter 7 should be looked at to see if it could be streamlined. The addition of a 2-3 page executive summary would be very beneficial.

Finally, the document is not designed to draw any conclusions, but is intended to direct efforts at identifying current information gaps, which future research will hopefully close. Therefore, I cannot comment on the soundness of the conclusions as requested.

II. RESPONSE TO CHARGE QUESTIONS

1. Chapter 1 provides introductory material regarding the CEA approach used in these case studies along with other background information and a discussion of terminology. Is this information accurately and clearly presented? Please comment on the utility of the chapter in providing background and support for the remainder of the document. In particular, are the

figures summarizing the CEA framework and process clear? How might this chapter be improved?

I found this chapter to be very useful, perhaps the most so of all the chapters. The figures were very clear and helpful. I don't think this chapter needs any improvement in general. It could be helpful to list other assessments (non-nano) that have successfully used a CEA approach to provide some more background on CEA.

I'm not sure I like the term "cluster", but agree that both "agglomeration" and "aggregation" are used interchangeably and are not well defined. I might argue for "weak-aggregates" and "strong-aggregates" as descriptors that try to convey the same distinction as is attempted with the agglomerate/aggregate terminology. No mention of the term "flocculation" was made, which generally refers to processes involving polymers whereby particles are "bridged" by a single molecule. This could be an important process for constituents such as humic substances in water and high-MW proteins within cells. In the case of the former, the "bridging" effect could overcome the effects of increased surface charge, and in fact lead to a decrease in NP stability in the presence of humic substances.

2. Chapter 2 presents basic information on conventional silver, including data on usage and historic environmental levels. Information on the physical-chemical properties of nanoscale silver and analytic methods makes up the rest of the chapter. Is this information clear and accurate? How might this chapter be improved?

I found this to also be a useful chapter, its contents were clearly presented, and does not need any improvement. I cannot assess its accuracy, but I assume that the data from the studies cited have been accurately presented. The great deal of concern over dissolved ionic silver during the time of heavy use in the photography industry generated many studies on its environmental fate and effects. I feel like there is more data out there on "conventional" silver that was not included. Given that many of the effects of nano-Ag may be a result of the formation of ionic silver, it is paramount to glean as much information as possible from the historical database. Were any authors from these studies or researchers from Kodak involved in the compilation of these data? There are a number of researchers I know that worked extensively on Ag and could be useful sources of data.

In discussing the chemical forms of Ag, there was no mention of silver oxides or hydroxide phases. Given that the common state of bulk silver metal is to have a "tarnish" composed of Ag₂O, I'm surprised there was no discussion of this phase, which could potentially affect nano-Ag behavior. Was Ag₂O eliminated as a result of the investigation of the literature or was it overlooked?

The PEN report is mentioned several times as a listing of nano-Ag containing consumer products. It is stated that no verification is made as to whether the products listed actually contain nano-Ag or some other form of Ag. However, another important point is not made. Once a product appears on the list it is never tracked to see if it remains on the market, so the list continues to grow and is never adjusted downward to reflect the loss of products from the market. As such the current consumer products lists may, or may not, be greatly inflated.

Although Table 2-3 is not intended to be an exhaustive list of methods, it is missing a few key techniques under the category of size distribution. Both nanotracking analysis (NTA) and disc centrifugation (DSC) are important techniques that should be included. Furthermore, we have recently published two papers on single particle ICP-MS, and at least a half dozen other investigators are preparing documents. We feel this approach may prove to be the best approach for detecting and characterizing nano-Ag. Although still relatively new, it would be useful to include this method in Table 2-3.

Pace, H.E., Rogers, N.J., Jarolimek, C., Coleman, V.A., Higgins, C.P., and Ranville, J.F. 2011. Determining transport efficiency for the purpose of counting and sizing nanoparticles via single particle inductively coupled plasma mass spectrometry. Anal. Chem., 83, 9361-9369.

Mitrano, D., Leshner, E., Bednar, A., Higgins, C.P., and Ranville, J.F. 2012. Detecting nanoparticulate silver using single particle inductively coupled plasma mass spectrometry. Environ. Toxicol. Chem. 31, 115-121.

3. Chapter 3 summarizes information on the lifecycle stages of nano-Ag disinfectant spray products, including potential releases to the environment of nano-Ag and by-products. To what extent does this chapter accurately and sufficiently characterize what is known and what is unknown with regard to the various stages of the lifecycle of nano-Ag as it might be used in disinfectant spray products? To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

This chapter is well organized, and presents a good overview, in the abstract sense, of the issues that are likely to be important in the life cycle of nano-Ag in disinfectant sprays. Of course the issue is that no data was presented, and likely does not exist, specifically on spray disinfectants. It is fairly clear from the discussion that almost any research into how nano-Ag behaves after it is sprayed on surfaces would be valuable.

The chapter does not need to be improved for the most part. One suggestion is to try and obtain more information on the other components of the sprays. Do these sprays contain alcohols, surfactants, volatile solvents, etc.? These other, non-metal, components could be problems in themselves.

4. Information on the transport, transformation, and fate of nano-Ag in air, water, sediment, and soil is discussed in Chapter 4. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of understanding regarding the known and anticipated behavior of nano-Ag in the environment. To what extent is this information presented in a manner that would inform consideration of likely exposure routes relevant to biota and human health? For each of the environmental media discussed, to what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

This chapter lays out an extensive list of processes likely to be important for nano-Ag behavior in the environment. All of the possible exposure routes are discussed and as such the chapter is quite complete. It is not quite as clear as to what direction future research should take when looking at nano-Ag transport in all the possible environmental media. Without knowing what process will be most important for the first step in the process, namely the release of nano-Ag

from surfaces that have been sprayed, one cannot be sure what media should be studied in order to prioritize future research. Despite this, I do not see any need for improvement of the chapter. With respect to the discussion of soils (4.3.1), no mention of the role of bioturbation of the upper zone of the soil is made. Earthworms can cause vertical migration of insoluble contaminants, such as uranium and plutonium, despite their very high soil K_d values. This process would also act on nano-Ag that reaches the soil surface.

5. Chapter 5 provides information on exposure, dose, and translocation of nano-Ag in humans and other biota. Please comment on the extent to which this chapter accurately and sufficiently characterizes this information and forms a basis for considering the health and ecological impacts of nano-Ag. To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

The chapter does a good job of presenting all of the available information on exposure issues with respect to nano-Ag in general. Again the problem is that no information specific to nano-Ag containing disinfectant sprays is available. So in this respect, it makes it rather obvious where future research is needed. However, given the extreme breadth of possible exposure routes, it is hard to see what area of exposure research will be most fruitful. The chapter is well organized and needs no improvement.

6. Chapter 6 characterizes factors that influence ecological and health impacts of nano-Ag and discusses the currently available scientific evidence regarding these impacts. Please comment on the extent to which this chapter accurately and sufficiently characterizes the state of the science. To what extent is the material effectively organized and sufficiently informative to support planning for future research? How might this chapter be improved?

My comments for this chapter are the same as for chapter five. The chapter seems sufficient in its current state to direct future research.

One thing that seems lacking is a discussion of photochemical effects. Given that the nano-Ag will be deposited on surfaces, unlike nano-Ag in textiles or other consumer products, it will have direct exposure to light. What is known about the extent to which reactants such as ROS can be formed from interaction of light and nano-Ag?

7. Chapter 7 summarizes the information and research questions presented in the nano-Ag case study, as well as discusses the role of case studies in the refinement of research strategies and potential future assessment efforts. We would appreciate comment from the peer reviewers on the integration of evidence in this chapter and its usefulness in supporting future development of research strategies and assessments. How might this chapter be improved?

As I have discussed previously, I believe chapter 7 could be significantly improved by not restating so much of the material presented in the other preceding chapters. Using a case study approach is a good strategy in general. In this case, the issue is that none of the case studies actually involved the disinfectant sprays under consideration. Although the studies presented are probably all very relevant, they do not directly address the material of concern. This leads to the obvious conclusion that a direct exposure and effects studies of disinfectant sprays will be

helpful.

8. For the document as a whole, are there ways to improve the structure, scope or presentation of information to better support the identification and prioritization of research needs by diverse stakeholders?

The document is extensive and in some respects suffers from this. I do not believe that Chapter 7 works very well as a summary. I suggest a 2-3 page executive summary be added to very succinctly state the most future research directions based on the available information.

9. The case study follows the CEA framework, which combines a product life-cycle perspective with the risk assessment paradigm to support subsequent steps in the CEA process. Please comment on aspects of the CEA framework and process that can be improved in future applications of CEA. We would appreciate input on the overall structure and scope of the framework and process and the extent to which they support the development and refinement of research directions for future CEAs of nano-Ag in particular and nanomaterials in general.

I believe the document makes a strong case for the value of a CEA process in the hazard assessment of nanotechnology. The CEA approach is comprehensive which is its strength and its weakness. In being so comprehensive, the CEA approach identifies all relevant aspects of the problem. The weakness is that it is unlikely that information exists on all these aspects, leaving obvious knowledge gaps. The CEA approach does provide useful guidance for research to fill these gaps.

III. SPECIFIC OBSERVATIONS

Page	Line # or Paragraph	Comment or Question
2-15	Table 2.2	I would argue that hydrocarbons are not surfactants and as such should not be listed in this category
3-5	30	I would suggest changing collide to coalesce
4-16	Table 4-1	Solubility product constants refer to the dissolution of minerals (solids) whereas formation constants describe complexes. Thus, the title is incorrect and should read "Solubility product constants for various silver solids."
7-7	20	It is stated that one analytical limitation is the inability to differentiate natural nano-Ag from engineered nano-Ag. I would argue that it is highly unlikely in most systems there is any appreciable natural nano-Ag. It is likely that the nano-Ag present in a system, especially in wastewater, could be an altered form of engineered nano-Ag or the result of anthropogenically introduced dissolved Ag.