January 26, 2011

In-person meeting participants:

Jaydee Hanson, Policy Director – International Center for Technology Assessment

Colin O'Neil, Regulatory Policy Analyst – Center for Food Safety

Jay Feldman, Executive Director – Beyond Pesticides

Nichelle Harriott, Research Associate – Beyond Pesticides

Jennifer Sass, Senior Scientist – Natural Resources Defense Council

Kathy Dolan, Triclosan Campaign Coordinator – Food & Water Watch

Colleen Cordes – The Loka Institute

Joining by phone:

George Kimbrell, Senior Staff Attorney – International Center for Technology Assessment

Steve Suppan, Senior Policy Analyst – The Institute for Agriculture and Trade Policy

Ian Illuminato, Health and Environment Campaigner – Friends of the Earth US

Michael Hansen, Senior Scientist – Consumers Union

Nano Silver Legal Petition

Jaydee Hanson
Policy Director
International Center for Technology Assessment
Washington DC

300 Nano Silver Products

- Children's products-baby bottles, toys, clothing
- Cutlery, cutting boards, food containers
- Gym wear, bed sheets
- General anti-microbial applications, gels, etc.

Many of the nano-silver infused products are for children (baby bottles, toys, stuffed animals, and clothing) or otherwise create high human exposures (cutlery, food containers, paints, bed sheets and personal care products) despite very little study on nano-silver's potential human health impacts. Studies have questioned whether traditional assumptions about silver's safety are sufficient in light of the unique properties
of nano-scale materials. Potential health risks from nano-silver's widespread use also include increased bacterial and antibiotic resistance and risks created by nanomaterials'
unprecedented mobility in the body.

Many of the nano-silver infused products are for children (baby bottles, toys, stuffed animals, and clothing) or otherwise create high human exposures (cutlery, food containers, paints, bed sheets and personal care products) despite very little study on nano-silver's potential human health impacts. Studies have questioned whether traditional assumptions about silver's safety are sufficient in light of the unique properties
of nano-scale materials. Potential health risks from nano-silver's widespread use also include increased bacterial and antibiotic resistance and risks created by nanomaterials'
unprecedented mobility in the body.

EPA failed to Act

- Despite Concern by Water Utilities and environmental groups that Samsung's new washing machine emitted nano-silver in the waste water, the EPA issued a guidance in 2007 that did not address nano silver as a new pesticide.

Concerns over nano-silver were first raised by national wastewater utilities in early 2006. Their concerns were highlighted by one then-new nano-silver product, Samsung's Silvercare Washer, which releases silver ions into the waste stream with every
wash. In response, the media reported in November 2006 that US EPA would regulate nanosilver
products as pesticides. One year later, EPA published a guidance covering only the Samsung washer and allowed it to remain on the market. EPA denied that this guidance
was "an action to regulate nanotechnology."

Groups petition EPA

- International Center for Technology Assessment
- Center for Food Safety
- Friends of the Earth
- Loka Institute
- Institute for Agriculture and Trade Policy
- Consumers Union
- Beyond Pesticides
- Greenpeace
- Silicon Valley Toxics Coalition
- Center for Environmental Health
- Center for Study of Responsive Law
- Food and Water Watch
- Clean Production Action
- ETC Group

Despite this nano-silver product explosion and its associated environmental and health risks, EPA has yet to take any meaningful regulatory action. The petitioners present both a legal blueprint and impetus to take such needed oversight action.

Nano Silver is a pesticide

- 1. EPA should amend its regulations to clarify that nano-silver is a pesticide

First, EPA called to amend its regulations or otherwise act to clarify that nano-silver is a pesticide and those products incorporating it are pesticide products that must be registered, approved by the agency, and labeled prior to marketing. Nano silver meets the pesticide law's (FIFRA) definition of a pesticide because it is a highly efficient antimicrobial or antibacterial agent and is intended to be used for that purpose.

EPA should clarify that pesticidal intent and public health claims can be both implicit and explicit and that manufacturers cannot avoid pesticide classification simply by stripping their products of labeling, a potential loophole several manufacturers have already exploited.

Nano pesticides new pesticides

- 2. EPA should clarify that nano-pesticides such as nano silver products require new pesticide registrations.

Second, EPA should clarify that nano-pesticides, such as nanosilver products, are new pesticide substances that require new pesticide registrations, with nano-specific toxicity data requirements, testing and risk assessments. Nano-silver must be classified as a separate substance than macro-silver based on the nanomaterial's capacity for fundamentally unique and different properties and because nano-silver many new antimicrobial uses are not previously registered silver uses.

EPA must assess Nano Silver

- 3. EPA must do full assessment of potential human health and environmental risks of nano-silver.

Third, EPA must assess the potential human health and environmental risks of nano-silver. These assessments are required by and must comply with FIFRA, as well as the Food Quality Protection Act (FQPA), the Endangered Species Act (ESA), and the National Environmental Policy Act (NEPA). As part of this assessment, EPA should analyze all existing scientific studies as well as require manufacturers to provide all necessary additional data on nano-silver. Pursuant to FQPA, EPA must assess the potential impacts of nano-silver on children and infants and ensure that no harm will result from aggregate exposures. Additionally, EPA must ensure that its activities regarding nano-silver comply with the ESA and the protection of endangered and threatened species. Finally, EPA must comply with NEPA by ensuring that it assesses the environmental impacts of its actions regarding nano-silver pesticide products.

EPA should stop nano silver sales

- 4. EPA should prohibit sales of nano silver products as illegal pesticides.

Fourth, EPA should take immediate action to prohibit the sale of nano-silver products as illegal pesticide products with unapproved health benefit claims. The nanosilver
consumer products currently on market are in clear violation of FIFRA's mandates. To this end, EPA should issue Stop Sale, Use or Removal Orders or other enforcement penalties or actions to those manufacturers and/or distributors currently selling these unregistered nano-silver pesticide products.

EPA to apply FIFRA to nano

- 5. Amend FIFRA regulations to require submission of nanomaterial specific data for nano silver.

Fifth, should EPA after rigorous assessment approve any nano-silver products as pesticides, the agency must fully apply its pesticide regulations to any registered nanosilver
pesticides. FIFRA's pesticide registration requirement instills with EPA the duty to prohibit, condition, or allow the manufacture and use of nanomaterials in nanopesticides
and prescribe conditions for manufacture or use. These include: requiring nano-specific ingredient and warning labeling; applying conditional registration; applying
requirements for post-registration notification of adverse impacts; applying postregistration
testing and new data development; and requiring the disclosure of all information concerning environmental and health effects, including confidential business
information.

Special Review Needed

- Finally, EPA should use its FIFRA authority to further review the potential impacts of nano-silver and the setting of a Federal Food Drug and Cosmetic Act Tolerance for nano-silver.
-

Finally, EPA should use its FIFRA authority to further review the potential impacts of nano-silver, including: undertaking either a Classification Review or a Special
Review of nano-silver pesticides; amending the FIFRA regulations to require the submission of nanomaterial and/or nano-silver specific data; completing a registration review of existing silver pesticides; regulation of nano-silver pesticide devices; and the setting of a Federal Food Drug and Cosmetic Act Tolerance for nano-silver.

For More Information

Full petition available at:
www.nanoaction.org

Jaydee Hanson
Policy Director
International Center for Technology Assessment
660 Pennsylvania Ave, SE Suite 302
Washington DC 20003
202-547-9359 x24
jhanson@icta.org

For More Information

[illegible]
[illegible]

[illegible]
[illegible]
[illegible]
[illegible]
[illegible]
[illegible]
[illegible]

International Center for Technology Assessment
660 Pennsylvania Ave., S.E., Suite 302, Washington, DC 20003
Phone: (202) 547-9359 Fax: (202) 547-9429

November 18, 2010

The Honorable Stephen A. Owens
Assistant Administrator
Office of Chemical Safety and Pollution Prevention
Ariel Rios Building
Mail Code 7101M
1200 Pennsylvania Avenue, N.W.
Washington, D.C. 20460

Dear Assistant Administrator Owens:

The International Center for Technology Assessment (ICTA) submits this formal request calling for you and your office to investigate a significant issue related to your oversight of nanotechnology and pesticides: nano-copper pesticides.

Introduction

The International Center for Technology Assessment (ICTA) is a non-profit, bi-partisan organization committed to providing the public with full assessments and analyses of technological impacts on society. ICTA is devoted to fully exploring the economic, ethical, social, environmental and political impacts that can result from the applications of technology or technological systems. ICTA seeks to ensure that regulatory agencies adopt accurate, scientific and standardized definitions of nanotechnology and to regulate emerging nanotechnologies as they would other materials whose safety has not been determined.

ICTA has worked on issues of nanotechnology oversight for a number of years and has a specific nanotechnology program, NanoAction. As part of that program, ICTA actively works with the public, policymakers, agencies and other non-profits to further improve awareness and oversight. Most relevant here, as you know, in May 2008, the International Center for Technology Assessment (ICTA) and the Center for Food Safety (CFS) filed a legal petition with the EPA on behalf of a coalition of 14 public interest organizations calling on EPA to regulate nano-silver and other nano-pesticide products pursuant to its authority under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA).[1] The legal petition called on EPA to, *inter alia*: regulate these nanotechnology products as new pesticides; require labeling of all products; assess health and safety data before permitting marketing; analyze the potential human health effects, particularly on children; and analyze the potential environmental impacts on ecosystems and endangered species.

---

[1] A full copy of the petition is available at http://www.icta.org/nanoaction/doc/CTA_nano-silver%20petition__final_5_1_08.pdf

This missive concerns a related issue, nano-copper pesticides.

Summary

Here, ICTA writes specifically regarding the submissions made by Osmose, Inc. (Osmose) to obtain its registrations for the following three pesticide products containing "micronized" copper carbonate:

ORD-X372 (Micro Pro 200), EPA Reg. No. 3008-90 (initial registration 5/12/05)
ORD-X370 (Micro Pro 200C), EPA Reg. No. 3008-92 (initial registration 8/30/05)
ORD-X400 (Micro Pro 200C V3), EPA Reg. No. 3008-99 (initial registration 4/7/08)

In each instance, although the active ingredient copper carbonate was purchased from another registrant, the copper carbonate was subsequently milled intentionally to produce very small particles of copper carbonate, including many particles with at least one dimension measuring less than 100 nanometers (the U.S. Environmental Protection Agency (EPA) Office of Pesticide Programs' (OPP) "working definition" of nanoscale material, however other Agency definitions include particle sizes up to 300nm[2] and 1000nm[3]). Based on a review of publicly available records, it does not appear that Osmose advised EPA when it applied for these three registrations that any of these products included intentionally produced nanoscale material, but, as explained below, it clearly knew this was the case.

It has been the announced policy of OPP since 2008 to "presume that any active or inert ingredient that is or contains nanoscale material is a 'new' ingredient for regulatory purposes under FIFRA."[4] EPA confirmed that it intends to continue this policy in a presentation made to the Pesticide Program Dialogue Committee on April 29, 2010.[5] All registrants were also on notice well before 2008 that OPP wanted any applicant requesting registration of a pesticide product containing a nanoscale active ingredient or inert ingredient to disclose that fact during the application process.

---

2 United States Department of Agriculture National Organic Standards Board Materials Committee, *Guidance Document -- Engineered Nanomaterials in Organic Production, Processing and Packaging* (Sept. 2, 2010) at 156, *available at* http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5086584#nameddest=nanotech

3 Food and Drug Administration Center for Drug Evaluation and Research, *Reporting Format for Nanotechnology-Related Information in CMC Review* (June 3, 2010) at 3, *available at* http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/ManualofPoliciesProcedures/UCM214304.pdf

4 "Nanotechnology and EPA's Office of Pesticide Programs," attachment to e-mail from William Jordan, Senior Policy Advisor, OPP (Nov. 10, 2008) (Exhibit A to this letter).

5 "Nanotechnology and Pesticides," slides of presentation by William Jordan, Senior Policy Advisor, OPP, to Pesticide Program Dialogue Committee (Apr. 29, 2010) (Nanotechnology Slides), at slide 18, *available at* http://www.epa.gov/pesticides/ppdc/2010/april2010/session1-nanotec.pdf.

Osmose appears to have withheld from EPA critical information concerning the presence of nanoscale particles of copper carbonate in the three products ICTA has identified. This has some very significant legal implications. In each instance, it further appears that the company was able to obtain a product registration by claiming the "formulator's exemption." Since EPA would have imposed separate and/or additional data requirements for the "new" active ingredient created when Osmose intentionally modified the structure of the purchased active ingredient to create nanoscale particles, it was clearly improper for Osmose to claim the formulator's exemption for these products. Osmose could not have obtained the registrations in question without generating and submitting data that are different from the data supporting registration of the conventional scale purchased product. Accordingly, ICTA believes that these Osmose nano-copper pesticide registrations should be deemed by EPA to be invalid *ab initio* and subject to immediate cancellation. Moreover, each sale and distribution by Osmose of any version of these three products that contains nanoscale particles should be deemed to be an unlawful act under FIFRA Section 12(a)(1)(C),[6] because the composition of each such product "differs at the time of its distribution or sale from its composition as described in the statement required in connection with its registration under section 136a of this title."

Like the pending petition by ICTA and 13 signatory organizations requesting that EPA further regulate nano-silver pesticide products,[7] ICTA requests that EPA assign a high priority to nanoscale copper compounds in registered pesticides. So have other non-profits that have raised this issue of concern.[8] In fact, EPA itself has recently recognized that, "Nano copper is more acutely toxic than micro copper."[9]

ICTA believes that EPA must act promptly to protect the public from unintended health and environmental hazards resulting from further widespread commercial distribution of Osmose's registered nano-copper wood preservative pesticides. As we will show *infra*, there is substantial scientific evidence that nanoscale copper and copper compounds are highly toxic. And even though EPA has not yet evaluated the safety of the Osmose products containing "micronized" copper carbonate, the company stated over a year ago that: "Over 5 Billion board feet of MicroPro treated wood has been sold since the product

---

6 7 U.S.C. § 136j(a)(1)(C).

7 ICTA, Petition for Rulemaking Requesting EPA Regulate Nano-Silver Products as Pesticides (May 1, 2008), *available at* http://icta.org/nanoaction/doc/CTA_nano-silver%20petition__final_5_1_08.pdf.

8 In a submission to a meeting of the FIFRA Scientific Advisory Panel concerning nanosilver and other nanometal pesticides, the Natural Resources Defense Council (NRDC) also requested that EPA take action concerning micronized copper pesticides, stating that "it appears that EPA has never been provided with any safety data for the nano-scale 'micronized' formulation of this wood treatment biocide. Dr. Jennifer Sass, Comments from the Natural Resources Defense Council for the November 3-6, 2009, FIFRA Scientific Advisory Panel (SAP) Session (October 28, 2009), at 2, Docket No. EPA-OPP-009-0683-0076.1, *available at* http://www.regulations.gov/search/Regs/contentStreamer?objectId=0900006480a4be21&disposition=attachment&contentType=pdf.

9 Nanotechnology Slides, at slide 6.

introduction in 2006."[10] EPA has ample legal basis to cancel the registrations, to determine each is void, and/or to take enforcement action concerning the sale and distribution of these products.

A detailed discussion of the materials and evidence that support these factual and legal conclusions concerning the three specified Osmose products follows.

Composition Information in Osmose Registration Applications

The EPA product chemistry review for ORD-X372 states:

> The applicant has provided a justification for not being required to satisfy the requirements of the following Part A product chemistry data requirements: 830.1620 (Description of Production Process), and 830.1700 (Preliminary Analysis). ORD-X372 is an end-use product that is formulated from registered manufacturing use products by simple mixing.[11]

Thus, EPA's approval of ORD-X372 was based on the premise that the active ingredient in the registered manufacturing use product purchased by Osmose *was not modified* prior to incorporation in the Osmose product. Based on that same premise, Osmose applied for and received the formulator's exemption for ORD-X372. That basic premise is false, because the purchased active ingredient is actually modified by milling before incorporation in the registered end use products.

Mr. Jack Housenger, the Associate Director of the Health Effects Division in OPP, asked personnel in the Antimicrobials Division to review the submissions by Osmose concerning these products. EPA analyst A. Najm Shamin replied to Mr. Housenger's request by stating:

> I looked into the jackets for Reg# 3008-90 and 3008-92 and poured over 300 pages, I could not find any reference about the size of the active product which is called copper carbonate.[12]

Mr. Shamin further stated that he conducted a "quick Google search" and found "that by definition a micronized copper is 500 nm and above."

---

10 Osmose Press Release, "Consumer Safety and Product Performance of Micronized Copper Technology Confirmed" (Feb. 10, 2009) (Osmose Press Release), *available at* http://www.treatedwoodtruth.com/consumer-safety-and-product-performance-of-micronized-copper-technology-confirmed.php.

11 Subject: Product Chemistry Review of ORD-X372, TO: Wallace Powell, EPA Work Assignment Manager, FROM: Joan Cuddleback, CSC/DynCorp Work Assignment Manager (Feb. 21, 2005), at 2 (Exhibit B).

12 Text of e-mail communication from A. Najm Shamin to Jack Housenger (date unknown).

As late as November 21, 2008, Mr. Housenger stated in an e-mail that he thought EPA had determined for the Osmose products that "the particles were greater than 100 nm" and that the particles in question were not "engineered to have special properties." Unfortunately, neither conclusion is correct. ICTA is uncertain whether the incorrect conclusions by EPA staff concerning the composition of these products were based solely on the failures by Osmose to disclose the inclusion of nanoscale material in its registration submissions for ORD-X372 and ORD-X370, or whether Osmose made affirmative representations on which EPA relied in reaching these incorrect conclusions.

ORD-X400 is a newer formulation designed for use with "refractory" wood species. As we will show below, this product contains smaller and more numerous nanoscale particles than ORD-X372 and ORD-X370. ORD-X400 was registered on April 7, 2008, at about the time when EPA was formalizing its policy position that it would treat intentionally created nanoscale particles of existing active and inert ingredients as "new" ingredients. In the letter transmitting its application for ORD-X400, Osmose stated that ORD-X400 is "substantially similar" to ORD-X370, asserting that: "Both products contain the same active ingredient purchased from the same sources," and "The only difference in the two products is the percentage of copper carbonate."[13] In that same letter, Osmose stated that the decreased percentage of copper carbonate in ORD-X400 would "only improve upon the toxicity characteristics," even though the inclusion of smaller and more numerous nanoscale particles in this product raises significant unresolved concerns regarding the hazards associated with its use. ICTA has not determined whether Osmose provided any information to EPA concerning the reductions in particle size in its product chemistry submission for ORD-X400, but the assertions in this letter suggest it did not. In any case, ICTA believes it is improbable that EPA would have registered this product had it been given accurate information on the inclusion of nanoscale particles in its composition.

Nanoscale Composition of Osmose Products

The manufacturing process for Osmose's "micronized" copper carbonate products is described in a U.S. Patent that Osmose applied for on April 9, 2004,[14] shortly before the registration of ORD-X372 and ORD-X370. This Osmose patent defines "micronized" as "a particle size in the range of 0.001 to 25 microns," which is 1 to 25,000 nanometers. The patent claims include wood preservatives that use micronized particles of an insoluble copper compound (such as copper carbonate) with either a soluble organic biocide or micronized particles of an insoluble organic biocide (such as tebuconazole). The patent explains that these small sizes can be attained by "grinding copper compounds using a commercially available grinding mill."

---

13 Letter from Teri Muchow, Manager, Regulatory Administration, Osmose, to Document Processing Desk, OPP, Re: ORD-X400 Application for Product Registration (Dec. 27, 2007), at 2 (Exhibit C).

14 Leach *et al.*, United States Patent No. US 7,674,481 B2, application April 9, 2004, granted March 9, 2010 (Exhibit D). Provisional applications were previously submitted on April 9, 2003, and November 11, 2003.

The first "micronized" copper carbonate product registered by Osmose was ORD-X372, which also contains soluble quaternary ammonium compounds. Osmose subsequently registered ORD-X370, which contains only the "micronized" copper carbonate compound. This second product is labeled for tank mixing with ORD-X300, EPA Reg. No. 3008-97, a tebuconazole product registered by Osmose. Based on the claims in the Osmose patent and the limited solubility of tebuconazole, ICTA believes that it is very likely this product also contains "micronized" particles. Unlike the three "micronized" copper carbonate products, ICTA has not been able to collect sufficient information to confirm that ORD-X300 contains nanoscale particles.

The Osmose patent for "micronized" word preservatives covers a wide range of particle sizes, from sizes that are at the low end of EPA's working definition for nanoscale particles to particles that are much larger than the high end of this definition. Thus, it is critical to determine what particle sizes are actually present in the Osmose "micronized" products.

The first clear evidence that Osmose's "micronized" products contain nanoscale particles of copper carbonate emerged when a group of scientific researchers started evaluating the effects of these products on treated wood. In October 2008, the journal *Nature Nanotechnology* published a letter from several researchers from the Centre for Advanced Wood Processing in Vancouver, Canada, and the Forestry and Forest Products Research Institute in Tsukuba, Japan, describing the "large-scale commercial use of nanoparticles for the biological protection of timber."[15] These researchers described "wood preservatives that consist of copper carbonate particles and an organic co-biocide, both dispersed in water," and referenced the Osmose patent. They also stated unequivocally that, "Nanoparticles, some as small as 20 nm in diameter, are abundant in the aqueous preservative."

This letter followed publication of reports by these same researchers in which they examined wood treated with "micronized" copper carbonate preservatives with a scanning electron microscope and found nanoscale particles of copper carbonate and iron oxide[16] in voids in the structure of the wood.[17] The researchers stated:

> Field Emission Scanning Electron Microscopy (FE-SEM) in combination with x-ray microanalysis (EDX) revealed the presence of nano-sized copper and iron particles in treated

---

15 Evans, P., Matsunaga, H., and Kiguchi, M. (2008), "Large-scale application of nanotechnology for wood protection," *Nature Nanotech.* 3:577 (October 2008) (Exhibit E).

16 ICTA does not know the source of the nanoscale iron oxide particles, or whether iron oxide particles were reported by Osmose as an inert ingredient for any of the "micronized" copper carbonate products. In any case, EPA's policy concerning nanoscale inert ingredients is the same as EPA's policy concerning nanoscale active ingredients.

17 Matsunaga, H., Kiguchi, M., and Evans, P. (2007), "Micro-Distribution of Metals in Wood Treated with a Nano-Copper Wood Preservative," Paper Prepared for the 38th Annual Meeting of the International Research Group on Wood Protection (May 20-24, 2007) (Exhibit F); Matsunaga, H., Kiguchi, M., and Evans, P. (2008), "Microdistribution of copper-carbonate and iron oxide nanoparticles in treated wood," *J. Nanopart. Res.* 11(5):1087-1098 (Exhibit G).

> wood. These particles ranged in size from 10 to 700 nm and were abundantly present in pit chambers and on tertiary wall layers adjacent to the lumens of tracheids and ray parenchyma cells.[18]

The same group of researchers recently presented another paper at a meeting of the International Research Group on Wood Protection. The researchers reported finding even smaller copper carbonate nanoparticles (about 2.5 nm) in the ray parenchyma cell walls of wood treated with a "micronized" copper carbonate product.[19]

At this same meeting of the International Research Group on Wood Protection, Osmose consultant Dr. Craig McIntyre presented a paper that compared particle sizes in several formulations of "micronized" copper carbonate with particles actually deposited in treated wood.[20] In this paper, Dr. McIntyre stated:

> Basically, all of the micronized copper was <1000 nm and roughly the ranges corresponded to:
>
> Formulation 1: mean = 200 to 500 nm
> Formulation 2: mean = 100 to 200 nm
> Formulation 3: mean = 50 to 95 nm[21]

The three formulations described by Dr. McIntyre generally correspond to the Osmose specifications for several formulations containing "micronized" copper carbonate particles that Osmose has marketed pursuant to its FIFRA registrations. Further, testimony given in 2009 by the Osmose Director of Research in a hearing in Federal District Court indicates that the mean particle size specifications for three "micronized" copper carbonate formulations marketed by Osmose are 0.25 to 0.3 microns (250-300 nm), $\leq$ 0.12 microns (120 nm), and $\leq$ 0.08 microns (80 nm).[22] The third Osmose formulation is ORD-X400 (also marketed as Micro Pro 200C V3), a product that is intended for treatment of "refractory" wood species like Douglas fir and hem fir that have an internal structure that resists impregnation with wood preservatives.

---

18 Matsunaga, *et al.* (2007), at 2.

19 Matsunaga, H., Kataoka, Y., Kiguchi, M., and Evans, P. (2010), "Copper nanoparticles in southern pine wood treated with a micronized preservative: Can nanoparticles penetrate the cell walls of tracheids and ray parenchyma?", Paper Prepared for the 41st Annual Meeting of the International Research Group on Wood Protection (May 9-13, 2010) (Exhibit H).

20 McIntyre, C.R. (2010), "Comparison of Micronized Copper Particle Sizes," Paper Prepared for the 41st Annual Meeting of the International Research Group on Wood Protection (May 9-13, 2010) (Exhibit I).

21 McIntyre, C.R. (2010), at 4.

22 Transcript of Preliminary Injunction Hearing, Testimony of Dr. Jun Zhang, Director of Research, Osmose (June 25, 2009), at 288-290, 328-329, Document 200 in *Osmose, Inc. v. Viance, LLC*, No-3:09-CV-23-JTC (N.D.Ga. Nov. 5, 2009), *available at* http://www.pacer.gov/ (Exhibit J).

Although it may initially appear that the first two of the three Osmose formulations have a particle size specification that falls outside of the range specified in OPP's "working definition" for nanoscale material, there is substantial scientific evidence that nanoscale particles of copper carbonate are abundant in all of the Osmose formulations. This is demonstrated both by the data collected in the various studies by Matsunaga, *et al.*, as well as by a study by MVA Scientific Consultants[23] that is included in the public record of a court proceeding. This study shows that the Osmose specifications for particle size are based on a weighted mean that reflects the higher mass of the larger particles, rather than on a mean determined from the numerical abundance of particles of each size in the formulation.

MVA Scientific Consultants is a firm that has conducted many forensic studies of particle size, and thus has considerable expertise in this area. In this study, MVA scientists analyzed a sample of ORD-X372 to determine the particle size distribution by direct visualization using transmission electron microscopy. MVA determined that 188 out of 260 discreet particles (72.3%) in this sample had an equivalent spherical diameter of less than 100 nm.[24] This study demonstrates that a specification based on the mean particle size determined by mass can be misleading because there are actually a much greater number of particles in the range below 100 nm. Because the shape of the visualized particles was irregular, and MVA reported the results by equivalent spherical diameter, the numerical prevalence of particles with at least one dimension smaller than 100 nm would likely be even greater.

The newest Osmose "micronized" copper carbonate formulation for "refractory" wood (ORD-X400) has a mean particle size that is clearly nanoscale under the EPA definition based on Osmose's own specification. In addition, it is clear from the published literature and from the MVA study that each of Osmose's "micronized" copper carbonate products has a composition that meets the EPA definition for a nanoscale active ingredient. Each product contains numerous particles that have been intentionally produced by milling to achieve a particular functionality and that have at least one dimension that measures less than 100 nm. Under EPA policy, the active ingredient in these products is "new." In each instance, it was improper for Osmose to fail to inform EPA that the product contains nanoscale particles, for Osmose to claim the "formulator's exemption" based on its purchase of a conventional sized active ingredient, and for Osmose not to support its application with additional data submissions based on the actual composition of the product.

Potential Risks from Nanoscale Copper Carbonate

Wood products treated with the Osmose "micronized" copper carbonate formulations are used for a variety of consumer applications that may involve direct dermal contact with the treated wood. In addition, copper nanoparticles could be released from the

---

23 Cavaliere, M.R., and Miller, M.A. (2009), MVA Scientific Consultants, "Report of Results: MVA7912, Particle Sizing of Micronized Copper Preservative" (June 19, 2009) , listed as Defense Exhibit 1022 in Appendix A to Defendants and Counter-Plaintiff's Second Revised Amended List of Documents to be Presented at Preliminary Injunction Hearing, Document 157-2 in *Osmose, Inc. v. Viance, LLC*, No-3:09-CV-23-JTC (N.D.Ga. June 30, 2009), *available at* http://www.pacer.gov/ (Exhibit K).

24 Cavaliere and Miller (2009), at 3

treated wood during sawing or machining, during cleaning, through normal wear and tear, or from product decomposition, and then become available for potential inhalation or ingestion. As noted above, Osmose stated in early 2009 that over five billion board feet of wood have been treated with its "micronized" copper products, so the potential for consumer exposure to nanoscale copper particles could be quite large.

Copper is known to be extremely soluble and can leach into the surrounding environment and bind very quickly to both organic and inorganic matter.[25] Copper has detrimental effects on most aquatic species, but especially algae, which in turn can affect entire ecosystems.[26] Studies of the acute toxicity of elemental copper nanoparticles (23.5 nm) in mice found "gravely toxicological effects and heavy injuries on kidney, liver, and spleen."[27] In a study comparing the toxicity of various metal oxide nanoparticles and carbon nanotubes, copper oxide nanoparticles (averaging 43 nm) were the most potent of all the nanoparticles tested at causing cytotoxicity and DNA damage.[28] Although the potential toxicity of nanoscale particles of copper carbonate has not been equally well characterized, the results of the study with copper oxide nanoparticles are of particular concern because both copper oxide and copper carbonate include a bivalent copper ion. Additional, nano-copper particles lead to the accumulation of excessive alkalescent substance and heavy metal ions (copper ions) in mice culminating in metabolic alkalosis and copper ion overload.[29]

To our knowledge, EPA has never evaluated the potential hazards associated with the nanoscale particles of copper carbonate in Osmose products. Yet Osmose issued a press release in 2009 in which it claimed that the "consumer safety" of its products has been "confirmed."[30] In addition to severely misrepresenting the actual degree to which the safety of the Osmose products has been evaluated, this press release clearly violates FIFRA based on applicable EPA policy. EPA construes FIFRA Sections 3(c)(1)(C) and 12(a)(1)(B)[31] to prohibit any claims concerning "safety" of a product in advertising because such claims would not be permissible if they were included in proposed product labeling.[32] EPA

---

25 NOAA (2009) The Use of Treated Wood Products in Aquatic Environments.

26 EPA Office of Pesticide Programs (2008) Copper Facts – Pesticide Reregistration, EPA 738-F-06-014 .

27 Chen, Z., Meng, H., Xing, G., Chen, C., Zhao, Y. Jia, G., Wang, T., Yuan, H., Ye, C., Zhao, F., Chai, Z., Zhu, C., Fang, X., Ma, B. and Wan, L. (2006), "Acute toxicological effects of copper nanoparticles in vivo," *Toxicol. Let.* 163:109-120 (Exhibit L); Meng, H., Chen, Z. Xing., G. Yuan, H., Chen, C., Zhao, F. Zhang, C. Wang, Y., and Zhao, Y. (2007), "Ultrahigh reactivity and grave nanotoxicity of copper nanoparticles," *J. Radioanalyt. Nuc. Chem.* 272:595-598 (Exhibit M).

28 Karlsson, H. L., Cronholm, P., Gustafsson, J., and Möller, L. (2008); "Copper Oxide Nanoparticles are Highly Toxic; A Comparison between Metal Oxide Nanoparticles and Carbon Nanotubes," *Chem Res. Toxicol.* 21:1726-1732 (Exhibit N).

29 "Ultrahigh reactivity provokes nanotoxicity: Explanation of oral toxicity of nano-copper particles" Toxicology Letters Volume 175, Issues 1-3, 10 December 2007, Pages 102-110.

30 Osmose Press Release, note 7 *supra*.

31 7 U.S.C. §§ 136a(c)(1)(C) and 135j(a)(1)(B).

32 *see* EPA, "Pesticide Labeling Questions and Answers," at Section 1 ("Advertising Claims"), *available at* http://www.epa.gov/pesticides/regulating/labels/label_review_faq.htm.

regulations expressly prohibit any labeling that includes "claims as to the safety of the pesticide or its ingredients."[33] Accordingly, it appears that distribution and sale of the three Osmose products following issuance of this Osmose press release was also a violation of FIFRA.

Osmose's MicroPro "ORD-X372" was the first wood preservative to be certified an Environmentally Preferable Product (EPP) by Scientific Certification Systems, a certification based on guidelines developed by EPA. Additionally, Osmose's MicroPro recently earned GREENGUARD Children and Schools Certification from the GREENGUARD Environmental Institute (GEI) and has also earned Green Approved Product Certification from the National Association of Home Builders (NAHB) Research Center under the National Green Building Standard program.

Notwithstanding the evidence that exposure to nanoscale copper carbonate may pose very serious toxicological concerns, Osmose has introduced nanoscale copper carbonate into commerce on a very large scale in wood preservative products registered by EPA. Osmose has not been required to produce any data addressing the potential risks associated with this nanoscale active ingredient, nor has EPA evaluated the risks that may be associated with occupational and consumer exposure to nanoscale copper carbonate resulting from use of these products. EPA may conclude that it was not previously aware of these potential hazards because of the failure of Osmose to disclose information on the composition of these products, but EPA must not neglect this matter now that it is aware of the presence of nanoscale material in these products.

Conclusion and Requests

For all of the above reasons, ICTA requests that EPA immediately investigate the composition of ORD-X372, ORD-X370, and ORD-X400, and take appropriate administrative action. If EPA determines that these products were registered on the basis of an invalid claim of the formulator's exemption, EPA should immediately revoke the registrations for these products. If EPA determines that it must afford the registrant Osmose an opportunity for a hearing prior to cancelling these products, notwithstanding the failure of Osmose to include critical information in its applications, the sole issues in that hearing should be whether Osmose accurately characterized the composition of its products, and whether Osmose was legally eligible to claim the formulator's exemption.

We further request that EPA thoroughly investigate other possible nanoscale copper products, which should include but not be limited to copper-based wood treatment products currently available on the market, as similar actions under FIFRA may be necessary.

If EPA determines that Osmose, or any other manufacturer of copper-based pesticide products, has distributed or sold any product that has a composition that differs from the composition described in the statement Osmose submitted as part of the registration of the product, EPA should take enforcement action under FIFRA Section 12(a)(1)(C).

---

[33] 40 C.F.R. § 156.10(a)(5)(ix).

Finally, EPA should publish its long-awaited industry guidance on nano-scale pesticides (Docket No. EPA-HQ-OPP-2008-0650). A notice on pesticide products containing nanoscale materials was submitted to the US Office of Management and Budget on July 30, 2010; however, no further action has been taken. Industry will have less incentive and ability to violate the law if EPA makes clear its policy regarding nano-pesticides like nano-silver and nano-copper. ICTA assumes that part and parcel of that awaited guidance will be the answer to ICTA's nano-silver petition, discussed *supra*.

Again, clarification and certainty from the agency would lessen the likelihood of future companies failing to divulge new nano-pesticides. The requirement of new data from the prospective registrants would further illuminate the safety and risks of these materials. And programmatic and individual impact assessments, under FIFRA, NEPA and other applicable laws, will further build that needed body of study. Finally, the requirement of labeling any nano-pesticide will provide transparency and causation data for any potential future negative impacts.

ICTA appreciates your prompt consideration of the matters described in this letter and the attached exhibits. Please contact me if you have any questions concerning any matter discussed in this letter.

Sincerely,

Jaydee Hansen
Policy Director

George A. Kimbrell
Staff Attorney

International Center for Technology Assessment

Attachments
cc: James J. Jones, Deputy Assistant Administrator (w/attachments)
Steven P. Bradbury, Ph.D., Director, Office of Pesticide Programs (w/attachments)
Lois Rossi, Director, Registration Division (w/attachments)
Leslye M. Fraser, Esquire, Office of General Counsel (w/attachments)
William Jordan (w/attachments)

International Center for Technology Assessment
660 Pennsylvania Ave., S.E., Suite 302, Washington, DC 20003
Phone: (202) 547-9359 Fax: (202) 547-9429

July 21, 2010

Mr. Steven Bradbury
c/o Jeff Kempter
Acting Director, Office of Pesticide Programs
Regulatory Public Docket (7502P)
U.S. Environmental Protection Agency
1200 Pennsylvania Ave. NW
Washington, DC 20460-0001

CC: submitted to http://www.regulations.gov
Federal Register: May 19 2010 (Volume 75, Number 96)
Docket No. EPA-HQ-OPP-2010-0282

Comments on Draft Guidance for Pesticide Registrants on False or Misleading Pesticide Product Brand Names

EPA Must Address the Hundreds of Nanotech Pesticide Products on the Market

Introduction

The International Center for Technology Assessment (ICTA) is a non-profit, bi-partisan organization committed to providing the public with full assessments and analyses of technological impacts on society. ICTA seeks to force federal regulatory agencies to adopt accurate, scientific and standardized definitions of nanotechnology and to regulate emerging nanotechnologies as they would other materials whose safety has not been determined.

ICTA has been actively working on issues related to nanotechnology for many years, filing separate legal petitions with both the U.S. Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) on behalf of coalitions of public interest organizations, calling for the regulation of nano-enabled products. Additionally, ICTA co-chairs the Nanotechnology Task Force of the Transatlantic Consumers Dialogue, a forum of US and EU consumer organizations, which develops and agrees on joint consumer policy recommendations to the US government and the European Union to promote the consumer interest in EU and US policy making.

The Use of Nanotechnology in Pesticide Products

Nanotechnology ("nano") is a powerful new platform technology for taking apart and reconstructing nature at the atomic and molecular level. Increasingly manufacturers are infusing a large and diverse number of consumer products with nanoparticles, including silver ("nano-silver") and other nano-metals, for their enhanced "germ killing" abilities. Nano-silver is now the most common commercialized nanomaterial with over 250 products containing nano-silver already on market shelves, ranging from household appliances and

cleaners to clothing, cutlery, and children's toys, to personal care products, food packaging, and coated electronics.

In May 2008, the International Center for Technology Assessment (ICTA) and the Center for Food Safety (CFS) filed a legal petition with the EPA on behalf of a coalition of 14 public interest organizations calling on EPA to regulate nano-silver and other nano-pesticide products.[1] The legal petition demands that EPA use its authority under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) to assess the safety of nanomaterials to the public and the environment before permitting commercialization. The petition also calls on the agency to require safety data from manufacturers and require mandatory and approved labeling.

In November 2009, the EPA convened a Scientific Advisory Panel (SAP) to assess and evaluated the hazard and exposure associated with nanosilver and other nanometal pesticide programs.[2] The SAP concluded that data gaps about potential exposure and hazards related to nanoparticles are broad; most existing models are not appropriate for use with nanomaterials and will not accurately predict nanomaterial exposure scenarios; and, existing data on current exposure and toxicity studies vary greatly with respect to metrics, particle size, etc. Ultimately, the SAP concluded that nanoparticles are fundamentally different substances from their larger scale cousins and that nanomaterials can create new and unique health and environmental risks that need new forms of safety testing.

Enforcing Labeling and Marketing Claims Relating to Nano-Pesticide Products

Currently, the largest nano markets are personal care products and antimicrobial products, many of which fall under FIFRA jurisdiction. Numerous nano-pesticides, including nano-silver products, make marketing claims that imply complete or total efficacy, safety, or composition resulting from the use of nanotechnology. As noted in EPA's Draft PR Notice, false or misleading statements of any type are violations of FIFRA and certain regulations promulgated under FIFRA. ICTA's 2008 petition notes that the vast majority of companies marketing their nano-silver products put emphasis on the nano-silver ingredient, touting its antimicrobial and antibacterial qualities, as well as making other sweeping medical claims, including:[3]

- "Antibacterial, Antibiotic effect"
- "eliminates 99.9% of bacteria, fungi and hundreds of other disease causing microorganisms by inhibiting multiplication and growth and preventing transfer"
- "long lasting antibacterial function"
- renders material "permanently anti-microbial and anti-fungal"
- "eliminates the growth of one-celled organisms (such as bacteria and viruses) by deactivating the organism's oxygen metabolism enzymes"

---

[1] A full copy of the petition is available at http://www.icta.org/nanoaction/doc/ICTA_nano-silver%20petition__final_5_1_08.pdf

[2] Environmental Protection Agency FIFRA Scientific Advisory Panel (2009) SAP Meeting Notes 2010-01: "Evaluation of the Hazard and Exposure Associated with Nanosilver and Other Nanometal Pesticide Products," Arlington, VA, Nov. 3-5, 2009.

[3] See Appendix A http://www.icta.org/nanoaction/doc/ICTA%20Petition%20Appendix%20A_nano-silver_product_inventory.pdf

- "antibacterial effect against bacteria, yeasts, mould, and fungi"
- "clinically proven to fight against harmful bacteria"
- "lasting antiseptic that can exterminate bacteria in a short time"
- "can kill and prevent all kinds of disease germs and microorganisms"
- "is proven to kill over 99% of bacteria including MRSA"
- "kills bacteria in vitro in as little as 30 minutes, 2-5 times faster than other forms of silver"
- "kills approximately 650 kinds of harmful germs and viruses with a germ resistance rate of 99.9%"
- "control air free from bacteria, virus, germs, fungus, or even A.I. (Avian Influenza)"
- "can kill and prevent all kinds of disease germs and microorganisms"
- "naturally kills most of bacteria, mold, and germs . . . sterilization benefits for over 650 types of bacteria like "*E. coli, S. aureus, Pneumococcus, Salmonella, Typhus, Vibria. Cholerae*, etc."
- "natural bacteriostat"
- "instant knockdown of bacteria & virus"
- "deactivate enzymes and proteins of bacteria from surviving on the surface of the product
- "when in contact with bacteria and fungus will adversely affect cellular metabolism and inhibit cell growth"
- "works against all types of bacteria and viruses, even killing antibiotic resistant strains as well as all fungal infections . . . remains potent up to 100 washes."
- "sterilizes bacteria of over 650 species."

While the above list is not an exhaustive account of marketing/labeling claims related to nano-silver, EPA should take action to amend its Final Pesticide Registration Notice (PR Notice) to include specific language that would address the hundreds of nano-enabled pesticide products with potentially false or misleading statements appearing on pesticide labeling. Furthermore, EPA should amend its data requirements to include the submission of nano-specific testing data from manufacturers making any claims that relate to the use of nanotechnology.

Mislabeled Nanotech Pesticide Products

Recently, manufacturers of engineered nano products (including pesticide products) have used the terms "micronized", "sub-micron", or "ultra-micronized" to describe their engineered nanotech products in an effort to either distance their products from Federal attention or from any negative association with nanotechnology itself. However, the mislabeling of nano-enabled products is particularly concerning because it prevents the public from making informed decisions when purchasing products as well as preventing employers and workers from taking the necessary precautions against the potential human health and environmental safety hazards of exposure to nanoparticles. EPA should articulate that labeling nano-pesticide products as "micronized" is a clear example of a "false and misleading statement about composition" and therefore represents a violation of FIFRA labeling regulations. EPA should additionally clarify that pesticidal intent and public health

claims can be both implicit and explicit and that manufacturers cannot avoid pesticide classification simply by stripping their products of labeling.

"Micronized Copper" Wood Preservative

Beginning January 1, 2004 EPA began enforcing the voluntary restrictions on the use of chromated copper arsenate (CCA) as a preservative in pressure-treated wood intended for residential purposes.[4] The result has been a shift by wood preservers and pesticide manufacturers to a mixture of copper carbonate particles and an organic co-biocide[5] and more recently from the use of dissolved copper (such as copper azole) to a solution of "micronized" copper particles in a water suspension. However, products advertising "micronized" or "micro" copper can have particle sizes ranging anywhere from 1 nanometer (nm) to 25 microns.[6] Some of the wood preservatives in commercial use in the U.S. have a mean particle size in the 50 to 95 nm range.[7]

A 2008 letter to the editor published in *Nature Nanotechnology* noted that "micronized" copper wood preservatives have captured at least 50% of the North American wood preservative market.[8] A more recent report claims that "micronized" products now account for more than 75% of the wood preservative market. As the market for engineered nano-copper preservatives continues to grow, EPA must clarify its position on the use of the labeling term "micronized" for pesticide products in order to mitigate any future effects on consumers, workers, and manufactures that may result from false or misleading advertising.

Policies for the Oversight of Nanotechnology

Below is an overview of the *Principles for the Oversight of Nanotechnologies and Nanomaterials*, an international declaration agreed upon by a broad coalition of over 80 civil society, public interest, environmental and labor organizations concerned about various aspects of nanotechnology's human health, environmental, social, ethical, and other impacts.

I. A Precautionary Foundation

Government and businesses in the U.S. and EU have invested enormous resources to nanotechnology research and development (R&D), yet both regulation and transatlantic dialogue are woefully lagging behind commercial release. The small size of engineered nanomaterials can imbue them with novel physical, chemical, and biological properties that that are potentially useful; however, the comparatively high reactivity, mobility, and other properties that come with small size are also likely to impart novel toxicity.[9] Existing research on the impacts of nanomaterials on human health and the environment have raised red flags that warrant precautionary action and further study.[9] The EU has begun to

---

4 EPA (2002) "Chromated Copper Arsenate (CCA): Manufacturers to Use New Wood Preservatives, Replacing Most Residential Uses of CCA." Available online at http://www.epa.gov/oppad001/reregistration/cca/cca_transition.htm

5 Evans, et al. (2008) "Letter to the Editor: Large-scale application of nanotechnology for wood protection," *Nature Nanotechnology*. October, Vol. 3, pp. 577.

6 Ibid.

7 McIntyre, Craig R. (2010). "Comparison of Micronized Copper Particles Sizes," Prepared for 41st Annual International Research Group on Wood Protection, McIntyre Associates, Inc., Walls, MS.

8 Op. Cit. 3

9 See, e.g., THE ROYAL SOCIETY AND THE ROYAL ACADEMY OF ENGINEERING, NANOSCIENCE AND NANOTECHNOLOGIES: OPPORTUNITIES AND UNCERTAINTIES (2004);

incorporate the Precautionary Principle into its chemical regulations, while the U.S. regrettably remains in the dark on chemical reform. This disparity will undoubtedly place tremendous trade-relations and regulatory strain on both sides of the Atlantic unless mutually agreed upon policies are underpinned by the Precautionary Principle.[10]

II. Mandatory Nano-specific Regulations

A modified or *sui generis*, nano-specific regulatory regime must be an integral aspect of the development of nanotechnologies. Considering the already advanced and rapidly expanding development and commercialization of nanomaterials, a transatlantic assessment of current oversight mechanisms is urgently needed, taking into account the novel properties exhibited by nanomaterials. Furthermore, regulatory actions should retroactively cover all nanomaterial products already on the market.

Voluntary initiatives are wholly inadequate to oversee nanotechnology. Voluntary programs lack incentives for "bad actors" or those with risky products to participate, thus leaving out the entities most in need of regulation.[11] Under voluntary initiatives, companies may lack motivation to test for long-term or chronic health and environmental effects.[12] Voluntary initiatives often delay or weaken essential regulation, forestall public involvement, and limit public access to vital environmental safety and health data. For these reasons, the public overwhelmingly prefers mandatory regulatory oversight to voluntary initiatives.[13] Additionally, the EU and U.S. should establish mandatory reporting schemes to keep track of the introduction into the marketplace of manufactured nanomaterials and exchange information obtained about products being introduced.

Andre Nel et al., Toxic Potential of Materials at the Nanolevel, 311 SCIENCE 622, 622-23 (2006); Holsapple et al., Research Strategies for Safety Evaluation of Nanomaterials, Part II: Toxicological and Safety Evaluation of Nanomaterials, Current Challenges and Data Needs, 88 TOXICOLOGICAL SCIENCES 12 (2005); Oberdörster et al., Nanotoxicology: an Emerging Discipline from Studies of Ultrafine Particles, 113 ENVIRONMENTAL HEALTH PERSPECTIVES 823 (2005); TRAN et al., INSTITUTE OF OCCUPATIONAL MEDICINE, A SCOPING STUDY TO IDENTIFY HAZARD DATA NEEDS FOR ADDRESSING THE RISKS PRESENTED BY NANOPARTICLES AND NANOTUBES (2005); EUROPEAN COMMISSION'S SCIENTIFIC COMMITTEE ON EMERGING AND NEWLY IDENTIFIED HEALTH RISKS (SCENIHR), *OPINION ON THE APPROPRIATENESS OF EXISTING METHODOLOGIES TO ASSESS THE POTENTIAL RISKS ASSOCIATED WITH ENGINEERED AND ADVENTITIOUS PRODUCTS OF NANOTECHNOLOGIES* 6 (2005); Andrew Maynard, Nanotechnology: The Next Big Thing, or Much Ado about Nothing?, 51 ANNALS OF OCCUPATIONAL HYGIENE 1, 4-7 (2006); J. SASS, NATURAL RESOURCES DEFENSE COUNCIL, NANOTECHNOLOGY'S INVISIBLE THREAT, (2007); FRIENDS OF THE EARTH, NANOMATERIALS, SUNSCREENS AND COSMETICS: SMALL INGREDIENTS, BIG RISKS (2006).

[10] The European Union plans to apply the precautionary principle to issues that may have "potentially dangerous effects on the environment, human, animal or plant health." EUROPEAN COMMISSION, COMMUNICATION FROM THE COMMISSION ON THE PRECAUTIONARY PRINCIPLE (2000).

[11] See, e.g., British Department for Environment, Food, and Rural Affairs, www.defra.gov.uk/environmental/nanotech (voluntary program launched in September 2006, and as of April 2007, has received only six submissions).

[12] J. CLARENCE DAVIES, WOODROW WILSON INTERNATIONAL CENTER FOR SCHOLARS, PROJECT ON EMERGING NANOTECHNOLOGIES, EPA AND NANOTECHNOLOGY: OVERSIGHT FOR THE 21ST CENTURY 18 (2007) ("It is hard to see what will motivate manufacturers to carry out chronic and environmental testing if regulation does not require it.").

[13] JANE MACOUBRIE, WOODROW WILSON INTERNATIONAL CENTER FOR SCHOLARS, PROJECT ON EMERGING NANOTECHNOLOGIES, INFORMED PUBLIC PERCEPTIONS OF NANOTECHNOLOGY AND TRUST IN GOVERNMENT 14 (2005).

III. Health and Safety of the Public and Workers

Adequate and effective nanomaterial oversight requires an immediate emphasis on preventing known and potential exposures to nanomaterials that have not been proven safe. This is essential for both the public and nano-industry workers because some materials present potential hazards and others are largely untested. Free nanoparticles (nanomaterials that are not bound up in other materials) are of particular concern because they appear most likely to enter the body, react with cells, and cause tissue damage.[14] Embedded nanoparticles also pose exposure concerns. Workers may be exposed to such materials throughout the manufacturing process, while disposal and recycling activities may expose the public and the environment.

IV. Environmental Sustainability

A nanomaterial lifecycle[15] assessment – including manufacturing, transport, product use, recycling, and disposal into the waste stream – is necessary to understand how various statutory systems apply and where regulatory gaps exist.[16] Full lifecycle environmental, health and safety effects must be assessed prior to commercialization.

Once loose in nature, manufactured nanomaterials represent an unprecedented class of manufactured pollutants. Potentially damaging environmental impacts can be expected to stem from the novel nature of manufactured nanomaterials, including mobility and persistence in soil, water and air, bioaccumulation, and unanticipated interactions with chemical and biological materials.[17] The limited number of existing studies has raised red flags, such as exposure to high levels of nanoscale aluminum stunting root growth in five commercial crop species,[18] byproducts associated with the manufacture of single-walled carbon nanotubes causing increased mortality and delayed development of a small estuarine

---

[14] See, e.g., THE ROYAL SOCIETY AND THE ROYAL ACADEMY OF ENGINEERING, NANOSCIENCE AND NANOTECHNOLOGIES: OPPORTUNITIES AND UNCERTAINTIES 36, 79-80 (2004); Oberdörster et al., Principles for Characterizing the Potential Human Health Effects From Exposure to Nanomaterials: Elements of a Screening Strategy, 2 PARTICLE AND FIBRE TOXICOLOGY 8, 29 (2005).

[15] A lifecycle assessment is the "systematic analysis of the resources usages (e.g., energy, water, raw materials) and the emissions over the complete supply chain from the cradle of primary resources to the grave of recycling or disposal." THE ROYAL SOCIETY AND THE ROYAL ACADEMY OF ENGINEERING, NANOSCIENCE AND NANOTECHNOLOGIES: OPPORTUNITIES AND UNCERTAINTIES 32 (2004).

[16] See, e.g., THE ROYAL SOCIETY AND THE ROYAL ACADEMY OF ENGINEERING, NANOSCIENCE AND NANOTECHNOLOGIES: OPPORTUNITIES AND UNCERTAINTIES 46 (2004) ("Any widespread use of nanoparticles in products such as medicines (if the particles are excreted from the body rather than biodegraded) and cosmetics (that are washed off) will present a diffuse source of nanoparticles to the environment, for example through the sewage system. Whether this presents a risk to the environment will depend on the toxicity of nanoparticles to organisms, *about which almost nothing is known*, and the quantities that are discharged.") (emphasis added); see, also Wardak et al., The Product Life Cycle and Challenges to Nanotechnology Regulation, 3 NANOTECHNOLOGY LAW & BUSINESS 507 (2006). Scientific experts estimated that it might take until 2012 to have "the ability to evaluate the impact of engineered nanomaterials from cradle to grave." Maynard et al., *Safe Handling of Nanotechnology*, Vol 444 NATURE 267-69 (November 16, 2006).

[17] See, e.g., U.S. ENVIRONMENTAL PROTECTION AGENCY, NANOTECHNOLOGY WHITE PAPER 11 (2006).

[18] Yang L., et al., Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles, 158(2) TOXICOL LETT. 122-32 (2005).

crustacean,[19] and damage to beneficial microorganisms from nanosilver.[20] The U.K. Royal Society has recommended that, "the release of nanoparticles and nanotubes in the environment be avoided as far as possible" and that, "factories and research laboratories treat manufactured nanoparticles and nanotubes as hazardous, and seek to reduce or remove them from waste streams."[21]

V. Transparency

Assessment and oversight of nanomaterials requires mechanisms ensuring transparency, including labeling of consumer products that contain nanomaterials, installing workplace right to know laws and protective measures, and developing a publicly accessible inventory of health and safety information. Polls show that the vast majority of the public lacks even basic information about nanotechnology or the presence of nanomaterials in consumer products.[22]

The public's right to know requires the labeling of all products containing nanomaterial ingredients.[23] Moreover, product labeling facilitates documentation of potential environmental releases, human exposures, and accountability for adverse impacts. On November 20, 2009, The Council of the European Union, the EU's main decision-making body, issued a new regulation requiring cosmetics manufacturers to label any nanoparticles contained in products marketed within the European Union.[24] Transatlantic dialogue on nanotechnology and transparency is urgently needed; the EU has taken positive steps in regards to transparency and now the U.S. must catch up in order to remain competitive in the global marketplace.

Safety testing data must be available for public scrutiny. In light of the poor record of industry in preventing workplace exposures and environmental releases of hazardous chemicals, effective oversight should include strictures on the use of confidentiality shields

---

[19] Templeton R. et al., Life-cycle Effects of Single-walled Carbon Nanotubes (SWNTs) on an Estuarine Meiobenthic Copepod, 40 Environmental Science and Technology 7397-7403 (2006).

[20] R. Senjen, Friends of the Earth Australia, Nanosilver – A Threat to Soil, Water and Human Health?, (2007) available at http://nano.foe.org.au/; J. Sass, Natural Resources Defense Council, Nanotechnology's Invisible Threat (2007).

[21] See, e.g., The Royal Society and the Royal Academy of Engineering, Nanoscience and Nanotechnologies: Opportunities and Uncertainties 46 (2004).

[22] Dan Kahan et al., Woodrow Wilson International Center for Scholars, Project on Emerging Nanotechnologies, Nanotechnology Risk Perceptions 2 (2006) ("Consistent with past surveys (Peter D. Hart Research Associates, 2006), the results suggested that Americans are largely uninformed about nanotechnology: 81% of subjects reported having heard either "nothing at all" (53%) or "just a little" (28%) about nanotechnology prior to being surveyed, and only 5% reported having heard "a lot.").

[23] See, e.g., Paraco Inc v. Dept of Agriculture, 118 Cal. App. 2d 348, 353-54 (1953) (holding that the public "have a right to know what they are buying"); Fredrick H. Degnan, The Food Label and the Right-to-Know, 52 Food & Drug L.J. 49, 50 (1997) (Pursuant to the 'consumer's right to know', "the public has a basic right to know any fact it deems important about food or a commodity before being forced to make a purchasing decision.").

[24] European Council: Regulation on cosmetic products (10 November 2009). Available online at: http://register.consilium.europa.eu/pdf/en/09/st03/st03623.en09.pdf

for nanomaterials. The provisions of international conventions on public access to information should be respected.[25]

VI. Public Participation

The potential of nanotechnologies to transform the global social, economic, and political landscape makes it essential that the public fully participate in the deliberative and decision-making processes.[26] These processes must be *open*, facilitating equal input from all interested and affected parties. Participation must also be *meaningful*: it must proceed and inform policy development and decision-making, rather than be limited to after-the-fact, one-way public 'engagement' in which the government and/or industry 'educates' the public with the goal of quelling debate and smoothing public acceptance.

Finally, *full* public participation requires democratic involvement for the entire range of processes by which nanotechnologies are developed and used and is necessary at each stage of development on a continuing basis to ensure that public concerns, values and preferences inform and guide nanotechnology oversight. Additionally, special efforts must be made to include persons living in poor communities, who have suffered disproportionately from the development of new technologies in the past.

VII. Inclusion of Broader Impacts

Consideration of nanotechnology's wide-ranging effects, including ethical and social impacts, must occur at each stage of the development process. Adequate assessment of both imports and exports containing nanomaterials is essential.

In addition to posing health, safety and environmental risks, nanomaterials present broader socio-economic concerns. For example, as new nanomaterials gain widespread use, they may disrupt markets for existing commodities, with potentially devastating consequences for the economies of commodity-dependent developing countries (i.e., the poorest countries).[27] The adverse impacts of granting patents for fundamental nanomaterials, which may amount to privatizing the building blocks of the natural world, must be considered and addressed. Moreover, the anticipated next generations of

---

[25] United Nations Economic Commission for Europe (UNECE), AARHUS CONVENTION, CONVENTION ON ACCESS TO INFORMATION, PUBLIC PARTICIPATION IN DECISION-MAKING AND ACCESS TO JUSTICE IN ENVIRONMENTAL MATTERS, adopted June 25, 1998.

[26] See, e.g., NATIONAL SCIENCE AND TECHNOLOGY COUNCIL, NATIONAL NANOTECHNOLOGY INITIATIVE, NANOTECHNOLOGY: SHAPING THE WORLD ATOM BY ATOM 4 (1999) (proclaiming nanotechnology as "a likely launch pad to a new technological era because it focuses on perhaps the final engineering scales people have yet to master."); id. at 8 ("If present trends in nanoscience and nanotechnology continue, most aspects of everyday life are subject to change."); id. ("The total societal impact of nanotechnology is expected to be much greater than that of the silicon integrated circuit because it is applicable in many more fields than just electronics."); id. at 1 (stating the nanotechnology revolution will result in "unprecedented control over the material world."); see also ASIA-PACIFIC ECONOMIC COOPERATION INDUSTRIAL SCIENCE AND TECHNOLOGY WORKING GROUP, NANOTECHNOLOGY: THE TECHNOLOGY FOR THE 21ST CENTURY, VOL II: THE FULL REPORT 24 (2002) ("If nanotechnology is going to revolutionize manufacturing, health care, energy supply, communications and probably defense, then it will transform labor and the workplace, the medical system, the transportation and power infrastructures and the military. None of these latter will be changed without significant social disruption.").

[27] See, e.g., THE SOUTH CENTRE, THE POTENTIAL IMPACT OF NANOTECHNOLOGIES ON COMMODITY MARKETS: THE IMPLICATIONS FOR COMMODITY DEPENDENT DEVELOPING COUNTRIES (2005).

nanotechnologies, including the production of more sophisticated nanodevices for manufacturing, military or medical use – including enhancement of human performance – can be expected to pose complex risks as well as social and ethical challenges. Some laboratories are already engineering viruses, yeasts, and bacteria to make nanomaterials. Full public debate on both sides of the Atlantic on all these issues will be crucial.

VIII. Manufacturer Liability

All who market nano-products, including nanomaterial developers, handlers and commercial users, the makers of products containing nanomaterials and retailers who sell nano-containing products to the public must be held accountable for liabilities incurred from their products. While product liability claims are the most likely liability for the nanomaterials industry, other forms of liability, including negligence, derivative liability, nuisance, fraud and misrepresentation are relevant. Incorporating and addressing manufacturer liability would be a key area of focus for any transatlantic dialogue on nanotechnology.

Conclusion

As nanomaterials become increasingly pervasive in the global marketplace, EPA must amend its Final Pesticide Registration Notice (PR Notice) to address the hundreds of nano-enabled pesticide products with potentially false or misleading statements appearing on pesticide labeling. Nanoscale pesticides, moreover, should be labeled as such. Furthermore, EPA should amend its data requirements to include the submission of nano-specific testing data from manufacturers making any claims relating to the use of nanotechnology. Finally, regulatory action should be based on the *Principles for the Oversight of Nanotechnologies and Nanomaterials* and crafted in consultation with civil society, public interest, environmental and labor organizations currently focusing on nanotechnology's human health, environmental, social, ethical, and other impacts.

Respectfully submitted,

Jaydee Hanson
Policy Director
International Center for Technology Assessment
660 Pennsylvania Avenue, SE, Suite 302
Washington, D.C. 20003
Ph: 202-547-9359 Fax: 2025-547-9429

---

[1] See, e.g., Andre Nel et al., Toxic Potential of Materials at the Nanolevel, 311 SCIENCE 622-27, 622, 623 Fig. 1 (2006).

International Center for Technology Assessment

660 Pennsylvania Ave., S.E., Suite 302, Washington, DC 20003
(202) 547-9359 fax (202) 547-9429 www.icta.org

CITIZEN PETITION FOR RULEMAKING TO THE UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

Office of Pesticide Programs
Environmental Protection Agency
1200 Pennsylvania Ave., NW
Washington, D.C. 20460-0001

Office of Pesticide Programs
Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Dr.
Arlington, VA 22202-401

THE INTERNATIONAL CENTER FOR TECHNOLOGY ASSESSMENT,
660 Pennsylvania, Ave., S.E., Suite 302
Washington, DC 20003

et al.,

Petitioners,

Filed With:

STEPHEN L. JOHNSON
in his official capacity as,
Administrator
Environmental Protection Agency
5630 Fishers Lane, Room 1061
Rockville, MD 20852

Docket Number _____

PETITION FOR RULEMAKING REQUESTING EPA REGULATE NANO-SILVER PRODUCTS AS PESTICIDES

Introduction

Nanotechnology and products containing manufactured and engineered nanomaterials have arrived and represent the crest of a product wave spanning many industries. A rapidly expanding universe of products containing nanomaterials is currently widely available, being

sold to the public and disposed of into the environment. These new materials can have fundamentally different properties from their bulk material counterparts–properties that also create unique human health and environmental risks–which create new oversight challenges for the regulatory agencies charged with protecting public health and the environment. A large and increasing percentage of the currently known commercial nanomaterial products are infused with forms of nanoparticle silver ("nano-silver") for its nano-enhanced ability to kill microorganisms and bacteria. While the risks of nano-silver to the environment and human health are not well understood, existing studies have indicated cause for concern, such as harmful impacts on fish and aquatic ecosystems, potential interference with beneficial bacteria in our bodies and the environment, and the potential development of more virulent harmful bacteria.

EPA has recognized that its oversight of materials pursuant to the Federal Insecticide, Fungicide, and Rodenticide Act ("FIFRA") will include the oversight of pesticide products containing nanomaterials ("nano-pesticides"). Despite the explosion of nano-silver products on the market implicating that jurisdiction, the agency has yet to take any meaningful steps pursuant to FIFRA or other applicable statutes to address the human health and environmental impact challenges created by nanomaterials generally or nano-silver products specifically. While not conventional agricultural pesticides, these nano-silver products meet FIFRA's definition of pesticides as substances intended to kill pests such as microorganisms. EPA's Region 9 office recently took action against a manufacturer of a nano-silver product for FIFRA violations, a precedent-setting action that strongly supports the legal arguments outlined in this petition on a broader scale. Petitioners call on EPA to immediately take the steps necessary to properly regulate nano-silver products as pesticides pursuant to FIFRA and other applicable statutes. This legal petition provides both the blueprint and the legal impetus to take such regulatory actions.

Accordingly, pursuant to the Right to Petition Government Clause contained in the First Amendment of the United States Constitution,[1] the Administrative Procedure Act ("APA"),[2] and EPA's FIFRA-implementing regulations,[3] the undersigned submit this citizen petition for rulemaking and collateral relief pursuant to the provisions of the Administrative Procedure Act, 5 U.S.C. §§ 551 et seq., the Federal Insecticide, Fungicide, and Rodenticide Act, 7 U.S.C. § 136w et seq., the Federal Food Drug and Cosmetic Act ("FFDCA"), 21 U.S.C. §§ 301 et seq., the Food Quality Protection Act ("FQPA"), 21 U.S.C. §§ 346 et seq., the Endangered Species Act ("ESA"), 16 U.S.C. §§ 1531 et seq., and the National Environmental Policy Act ("NEPA"), 42 U.S.C. §§ 4321 et seq.

ACTIONS REQUESTED

PETITIONERS REQUEST THAT THE EPA ADMINISTRATOR UNDERTAKE THE FOLLOWING ACTIONS:

I. Classify Nano-silver As a Pesticide and Require the Registration of Nano-silver Products as Pesticides

II. Determine That Nano-silver is a New Pesticide That Requires a New Pesticide Registration

III. Analyze the Potential Human Health and Environmental Risks of Nano-silver

---

[1] U.S. Const., amend. I. ("Congress shall make no law ... abridging ... the right of the people ... to petition Government for a redress of grievances."). The right to petition for redress of grievances is among the most precious of the liberties safeguarded by the Bill of Rights. United Mine Workers of Am., Dist. 12 v. Illinois State Bar Ass'n, 389 U.S. 217, 222 (1967). It shares the "preferred place" accorded in our system of government to the First Amendment freedoms, and has a sanctity and a sanction not permitting dubious intrusions. Thomas v. Collins, 323 U.S. 516, 530 (1945). "Any attempt to restrict those First Amendment liberties must be justified by clear public interest, threatened not doubtful or remotely, but by clear and present danger." Id. The Supreme Court has recognized that the right to petition is logically implicit in, and fundamental to, the very idea of a republican form of government. United States v. Cruikshank, 92 U.S. (2 Otto) 542, 552 (1875).

[2] 5 U.S.C. § 553(e) (2005) ("Each agency shall give an interested person the right to petition for the issuance, amendment, or repeal of a rule.").

[3] See e.g., 40 C.F.R. Chapter I, Subchapter E Pesticide Programs.; 40 C.F.R. § 152.40 (application for new registration of a pesticide product); id. § 154.10 (petition to begin Special Review process); id. Part 158 (pesticide class-specific changes to data requirements); id. § 158.5(data requirements for petition to establish tolerance under FFDCA 408) Part 158 (pesticide class-specific changes to data requirements); 21 U.S.C. § 346a(d) (petition for setting tolerance).

A. *Pursuant to FIFRA, Analyze the Potential Human Health and Environmental Impacts as Part of the Nano-silver Pesticide Registration Process*

B. *Pursuant to the FQPA, Assess the Potential Impacts of Nano-silver Exposures on Infants and Children and Ensure that No Harm Will Result From Aggregate Exposures*

C. *Compliance with the ESA, Including Undertaking Consultation Procedures In Accordance with ESA § 7 for Any EPA Actions, Activities, or Programs Impacting Nano-silver Oversight*

D. *Compliance with NEPA, Including Assessing the Human Health and Environmental Impacts of EPA's Current and Future Actions or Programs Regarding Nano-silver, Including Completing a Programmatic Environmental Impact Statement*

IV. Take Regulatory Actions against the Class of Nano-silver Products Illegally Sold Without EPA FIFRA Approval, Including Issuing Stop Sale, Use or Removal Orders for Illegal and Unlabeled Nano-silver Pesticide Products

V. If any Nano-silver Pesticide Registration is Approved, Apply and/or Amend to Specifically Apply the FIFRA Pesticide Requirements to the Class of Nano-silver Pesticides, Including

1. *Labeling*
2. *Post-Registration Notification of Adverse Effects*
3. *Post-Registration Testing and New Data Development*
4. *Conditional Registration*
5. *Confidential Business Information*

VI. Take Other EPA FIFRA Actions Necessary for Adequate Oversight of Nano-silver Pesticides, Including:

1. *Undertaking a Classification Review of Nano-silver Pesticides*
2. *Undertaking a Special Review of Nano-silver Pesticides*
3. *Requiring the Submission of Nano-specific Data from Nano-silver Registrants*
4. *Amending FIFRA Regulations to Require Nano-Specific Data*
5. *Registration Review of Existing Bulk Silver Pesticide Registration*
6. *Regulate Nano-silver Devices*
7. *Set a Pesticide Tolerance for Nano-silver*

PETITIONERS

Petitioner **The International Center for Technology Assessment** ("CTA") is located at 660 Pennsylvania Ave., S.E., Suite 302, Washington, DC 20003. Formed in 1994, CTA seeks to assist the public and policy makers in better understanding how technology affects society. CTA is a non-profit organization devoted to analyzing the economic, environmental, ethical, political, and social impacts that can result from the application of technology or technological systems. CTA works towards adequate oversight of nanotechnology through its Nanotechnology Project, *NanoAction*.

Petitioner **The Center for Food Safety** ("CFS") is located at 660 Pennsylvania Ave., S.E., Suite 302, Washington, DC 20003 and 2601 Mission Street, Suite 803, San Francisco, CA 94110. CFS is a non-profit public interest and environmental advocacy membership organization established in 1997 by its sister organization, International Center for Technology Assessment, for the purpose of challenging harmful food production technologies and promoting sustainable alternatives.

Petitioner **Beyond Pesticides** is located at 701 E Street, SE, Suite 200, Washington, DC 20003. Founded in 1981, Beyond Pesticides is a non-profit membership organization that serves a nationwide network and works to reduce threats to human health and environmental quality from the use of hazardous pesticides. Beyond Pesticides' primary goal is to educate and advocate for the adoption safe pest management practices and products.

Petitioner **Friends of the Earth** ("FOE") is located at 1717 Massachusetts Avenue, NW, Suite 600, Washington, DC 20036. FOE is a non-profit organization that seeks to create a more healthy, just world. FOE is the U.S. voice of Friends of the Earth International, the world's

largest federation of democratically elected grassroots environmental groups, located in 70 countries.

Petitioner **Greenpeace** is located at 702 H Street, N.W. Suite 300, Washington, D.C. 20001. Greenpeace was founded in 1971 and has 250,000 members in the U.S. and 2.5 million worldwide. Greenpeace is an independent campaigning organization that uses peaceful direct action and creative communication to expose global environmental problems and promote solutions that are essential to a green and peaceful future.

Petitioner **The Action Group on Erosion, Technology and Concentration** ("ETC Group") is an international civil society organization headquartered in Canada, with offices in the USA and Mexico. ETC Group is dedicated to the conservation and sustainable advancement of cultural and ecological diversity and human rights. To this end, ETC Group supports socially responsible developments in technologies useful to the poor and marginalized, and it addresses governance issues affecting the international community. ETC Group also monitors the ownership and control of technologies and the consolidation of corporate power.

Petitioner **Center for Environmental Health** ("CEH") is located at 528 61st Street, Suite A, Oakland, CA 94609. Founded in 1996, CEH is a non-profit organization dedicated to protecting the public from environmental and consumer health hazards. CEH is committed to environmental justice, reducing the use of toxic chemicals and practices, supporting communities in their quest for a safer environment, and corporate accountability.

Petitioner **Silicon Valley Toxics Coalition** ("SVTC") is located at 760 North First Street, San Jose CA, 95112. SVTC is a diverse grassroots coalition that engages in research, advocacy, and organizing around the environmental and human health problems caused by the rapid growth of the high-tech electronics industry. SVTC is interested in incorporating a precautionary

approach and the appropriate regulatory structure to emerging technologies, such as nanotechnology, that have the potential for tremendous good as well as devastating harm to human health and the environment.

Petitioner **Institute for Agriculture and Trade Policy** ("IATP") is headquartered at 2105 First Avenue South, Minneapolis, Minnesota 55404, and has an office in Geneva, Switzerland. IATP is dedicated to policies and practices that support sustainable agriculture and development, healthy and safe food, and fair trade. IATP's interest in the petition concerns hazards to both our rural and urban constituencies posed by the unregulated and unlabeled incorporation of nano-silver materials into a broad array of products, including agricultural chemicals.

Petitioner **Clean Production Action** ("CPA") is a non-profit organization registered in the US. CPA's designs and delivers strategic solutions for the movement to green chemicals, sustainable materials and healthy products. CPA partners with environmental organizations, public health advocates, labor unions, and progressive businesses to develop and build technical and policy support for clean production policies that promote the use of products that are safer and cleaner across their life cycle.

Petitioner **Food & Water Watch** is a national non-profit public interest consumer organization, based in Washington, D.C. that works to ensure safe food and clean water. FWW has worked on many emerging technologies that impact our food supply, by educating consumers, the media, and policymakers about the impact on the food system and public health and by calling for appropriate regulation.

Petitioner **Loka Institute** is located at 736 Bonita Dr., South Pasadena, California 91030. The Loka Institute was founded as a 501(c)3 non-profit organization in 1996 to advocate for

making research, science and technology responsive to democratically-decided social and environmental concerns.

Petitioner **The Center for the Study of Responsive Law** ("CSRL") is located in Washington, DC and contacted at P.O. Box 19367, Washington, DC 20036. CSRL is a non-profit organization that supports and conducts a wide variety of research and educational projects to encourage the political, economic and social institutions of this country to be more aware of the needs of the citizen-consumer. The Center serves to empower citizens, guard the environment, protect consumers and monitor worker health and safety issues.

Petitioner **Consumers Union** is an independent, nonprofit testing, and information organization whose mission is to work for a fair, just, and safe marketplace for all consumers and to empower consumers to protect themselves. To achieve this mission, we test, inform, and protect. To maintain our independence and impartiality, Consumers Union accepts no outside advertising, no free test samples, and has no agenda other than the interests of consumers. Consumers Union supports itself through the sale of our information products and services, individual contributions, and a few noncommercial grants.

FACTUAL BACKGROUND

Nanotechnology

Nanotechnology is a powerful new platform technology for taking apart and reconstructing nature at the atomic and molecular level.[8] The nano-scale is exceedingly tiny: it

[8]The National Nanotechnology Initiative (NNI) defines nanotechnology as

> the understanding and control of matter at dimensions of roughly 1 to 100 nanometers, where unique phenomena enable novel applications. Encompassing nanoscale science, engineering and technology, nanotechnology involves imaging, measuring, modeling, and manipulating matter at this length scale.

is the world of atoms and molecules, involving the manipulation of matter at the nanometer scale (nm), one billionth of a meter.[5] "Nano" means more than just tiny manufacturing. It is well-known that materials engineered or manufactured to the nano-scale exhibit radically different fundamental physical, biological, and chemical properties from bulk materials.[6]

One reason for these fundamentally different properties is that quantum physics comes into play at the nano-scale.[7] Another is that the reduction in size to the nano-scale results in an enormous increase of surface to volume ratio, giving nanoparticles a much greater surface area per unit of mass compared to larger particles.[8] Because growth and catalytic chemical reactions occur at the particle surface, a given mass of nanoparticles will have an increased potential for biological interaction and be much more reactive than the same mass made up of larger particles, thus enhancing intrinsic toxicity.[9] This enormous increase in surface area can change relatively inert substances into highly reactive ones. A material in nano-scale form can then melt faster, absorb more, or simply become more explosive.

Thus, to say that a substance is "nano" does not merely mean that it is tiny, a billionth of a meter in scale; rather, the prefix is best understood to also mean that a substance has the capacity to act in fundamentally different ways. Altered properties can include color, solubility,

---

National Nanotechnology Initiative, Factsheet: What Is Nanotechnology?, http://www.nano.gov/html/facts/whatIsNano.html; 15 U.S.C. 7501-7509; Id. § 7509 (definitions).

[5] For illustration, a hydrogen atom is about .1 nm. A DNA molecule, which carries genetic information in the cell nucleus, is about 2.5 nm long. A human hair is huge by comparison, about 50,000 nm thick; the head of a pin is about 1 million nm across. A sugar molecule, which measures about 1 nm, is about as big in relation to an apple as the apple is in relation to the earth.

[6] National Nanotechnology Initiative, *What is Nanotechnology?*, at http://www.nano.gov/html/facts/whatIsNano.html.

[7] Nanotechnology Now, *Nanotechnology Basics*, at http://www.nanotech-now.com/basics.htm.

[8] See, e.g., Andre Nel et al., *Toxic Potential of Materials at the Nanolevel*, 311 SCIENCE 622 (2006). For example, a gram of nanoparticles has a surface area of a thousand square meters.

[9] See, e.g., European Commission's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), *Opinion on the appropriateness of existing methodologies to assess the potential risks associated with engineered and adventitious products of nanotechnologies*, adopted September 28-29, 2005; Warheit, D.B., *Nanoparticles: Health impacts?*, 7 MATERIALS TODAY 32-35 (2004).

material strength, electric conductivity, and magnetic behavior. For example, a gold wedding ring is yellow in color; but gold nanoparticles appear red. Carbon (like graphite in pencil lead) is relatively soft; but carbon in the form of carbon nanotubes (nano-scale cylinders made of carbon atoms) is a hundred times stronger than steel. An aluminum soda can does not burn; however, aluminum nanoparticles explode when used as rocket fuel catalysts.

The Human Health and Environmental Risks of Nanomaterials

Just as the size and chemical characteristics of engineered nanoparticles can give them unique properties, those same new properties—tiny size, vastly increased surface area to volume ratio, high reactivity— can also create unique and unpredictable human health and environmental risks.[10] Swiss Insurance giant Swiss Re noted that, "Never before have the risks and opportunities of a new technology been as closely linked as they are in nanotechnology. It is precisely those characteristics which make nanoparticles so valuable that give rise to concern regarding hazards to human beings and the environment alike."[11] A growing number of peer-reviewed scientific studies have demonstrated the potential for nanomaterials to present serious toxicity risks for human health and ecosystems.[12] Manufactured nanomaterials move excessively through the environment and have the potential to enter living cells and the environment in ways their larger counterparts do not. For example, the human body absorbs nanomaterials more readily than larger sized particles and nanoparticles cross biological membranes that larger sized particles normally cannot, such as the blood-brain barrier. In addition, research has shown that

---

[10] See, e.g., Andre Nel et al., *Toxic Potential of Materials at the Nanolevel*, 311 SCIENCE 622-27, 622, 623 Fig. 1 (2006); see generally Florini et al., *Nanotechnology: Getting It Right the First Time*, 3 NANOTECHNOLOGY L. & BUS. 38, 41-43 (2006).

[11] Swiss Re, *Nanotechnology-Small Matter, Many Unknowns*, (2004), at 17.

[12] See infra pp. 57-95 and accompanying footnotes.

many types of nanomaterials can be toxic to human tissue and cell cultures, resulting in increased oxidative stress, inflammatory cytokine production, DNA mutation and even cell death.[13]

Once loose in nature, these nanomaterials represent a new class of manufactured non-biodegradable pollutants. Nanomaterials' unique chemical and physical characteristics create foreseeable environmental risks, including potentially toxic interactions or compounds, absorption and/or transportation of pollutants, durability or bioaccumulation, and unprecedented mobility for a manufactured material.[14] Because of their tiny size, nanomaterials may be highly mobile and travel further than larger particles in soil and water. Because nanoparticles tend to be more reactive than larger particles, interactions with substances present in the soil could lead to new and possibly toxic compounds. Environmental impact studies have raised some red flags, including dangers from nano-silver to aquatic life; however, despite rapid nanomaterial commercialization, many potential risks remain dangerously untested due to the government's failure to prioritize and adequately fund environmental impact research.[15] In addition, nanomaterials' unique chemical and physical characteristics create foreseeable, yet unexplored, risks. For example, nanoparticles are the subject of vigorous drug research because of their ability to carry and deliver drugs to specific targets. But this same transport propensity could give nanoparticles the ability to carry toxic chemicals present in the environment.

---

[13] See generally International Ctr. for Technology Assessment, "Petition Requesting FDA Amend its Regulations for Products Composed of Engineered Nanoparticles Generally and Sunscreen Drug Products Composed of Engineered Nanoparticles Specifically," Docket No. 2006P-0210 (filed May 17, 2006), available at http://www.icta.org/doc/Nano%20FDA%20petition%20final.pdf

[14] See generally pp. 86-91 infra and accompanying footnotes.

[15] Woodrow Wilson International Center for Scholars, Project on Emerging Nanotechnologies, Press Release, *Nanotechnology Development Suffers from Lack of Risk Research Plan, Inadequate Funding & Leadership*, September 21, 2006, at http://www.wilsoncenter.org/index.cfm?topic_id=166192&fuseaction=topics.item&news_id=201894

Nanomaterials in Consumer Products: The Future Is Now

Nanotechnology and its material creations are no longer future predictions; they have arrived. Funding is astronomical: global nanotech research and development (R&D) is estimated at around $9 billion, with $1 trillion in U.S. dollars globally estimated by 2015.[16] Investments in federally funded nanotechnology activities coordinated through the National Nanotechnology Initiative (NNI) were approximately $1.3 billion in 2006, and about $2 billion annually of R&D investment is currently being spent by non-federal sectors such as states, academia, and private industry. State governments spent an estimated $400 million on facilities and research aimed at the development of local nanotechnology industries in 2004. Unfortunately, only a paucity of this robust federal funding--4% of the NNI's FY07 budget--was earmarked for environmental health and safety (EHS) research.[17] Other non-governmental estimates put the EHS funding number as actually closer to 1%.[18]

Nanotechnology commercialization is moving forward at lightning speed. Thousands of tons of nanomaterials are already being produced each year.[19] Many materials can be engineered into nanomaterials or nanoparticles with the most common being silver, carbon, zinc, silica, titanium dioxide, gold, and iron.[20] Consumer products containing nanomaterials have been in, and continue to enter, the market at a steady pace. According to Lux Research's 2006

---

[16] See, e.g., Lux Research, *The Nanotech Report, 4th Edition*, 2006, http://luxresearchinc.com/LNR4_TOC.pdf

[17] See, e.g., International Center for Technology Assessment, *Congressional Letter on NNI 2006 Budget*, available at http://www.icta.org/doc/nano%20approps%20letter_Feb_2006.pdf

[18] Woodrow Wilson International Center for Scholars, Project on Emerging Nanotechnologies, Press Release, *Nanotechnology Development Suffers from Lack of Risk Research Plan, Inadequate Funding & Leadership*, September 21, 2006, at http://www.wilsoncenter.org/index.cfm?topic_id=1421&fuseaction=topics.event_summary&event_id=203644

[19] See, e.g., The Royal Society and the Royal Academy of Engineering, *Nanoscience and nanotechnologies: Opportunities and uncertainties*, London, July 2004, pp. 26-27 & Table 4.1, available at http://www.nanotec.org.uk/finalReport.htm (hereafter Royal Society Report).

[20] Lloyd's of London, Risks: Lloyd's Emerging Risks Team Report, *Nanotechnology Recent Developments, Risks and Opportunities*, at 10, 2007.

Nanotechnology Report, more than $32 billion in products incorporating nanotechnology were sold last year, more than double the previous year.[21] Lux predicts that by 2014, $2.6 trillion in manufactured products will be nano-products, 15% of total global manufacturing.

The only publicly available nanomaterial product inventory shows approximately 600 currently available on U.S. market shelves.[22] Since its launch in early 2006 the database shows an addition of about one new product every working day.[23] The nano-products found include: paints, coatings for numerous products, sunscreens, medical devices, sporting goods, cosmetics, stain-resistant clothing, supplements, nanoceuticals, and vitamins, food and food packaging, kitchen and cooking ware, light emitting diodes used in computers, cell phones, and digital cameras, film and photo development products, automotive electronics, automotive exteriors, batteries, fuel additives, and tires, computer accessories, children's toys and pacifiers, laundry detergent and fabric softeners, personal hygiene products, cleaning agents, air conditioning units, pet products, jewelry, bedding and furniture, lubricants and foams, waxes, MP3 players and other electronics.[24] But because there are no labeling requirements for products containing nanomaterials, the total number and range of nano-products is unknown.

Nano-silver Products

Nano-silver has quickly become the most commonly used nanomaterial in consumer products and the fastest growing sector of nanomaterial commercialization. The use of nano-silver as an antimicrobial agent is now widespread, with a wide variety of products now on market shelves. The petitioners discovered no fewer than 260 self-identified nano-silver

---

[21] See, e.g., Lux Research, 2006, http://luxresearchinc.com/TNR4_TOC.pdf

[22] The Woodrow Wilson International Center for Scholars, Project on Emerging Nanotechnologies, *Nanotechnology Consumer Products Inventory*, available at http://www.nanotechproject.org/consumerproducts

[23] March 2006: over 200 products; December 2007: 600 products.

[24] Id.

consumer products, which are listed in Appendix A. These are just the products that are self-identified and many more likely exist since there are currently no labeling requirements. In addition, several of the products were previously marketed as containing nano-silver but have removed advertising or labeling noting that ingredient.[25]

The numerous nano-silver products found include:

- air and water purifiers and their replacement filters
- multipurpose, bathroom, and kitchen cleaning products
- sanitizing sprays
- children's toys, baby bottles and infant products
- laundry detergents and fabric softeners
- food storage containers
- food/produce cleaners and cleaning sprays
- cutlery
- cutting boards
- numerous types of clothing including underwear, socks, shirts, outerwear, gloves and hats
- various fabrics and fibers
- refrigerators
- washing machines
- wet cleaning wipes
- hair care products, brushes, straighteners, and other hair appliances
- personal care products including creams, lotions, masks
- bandages
- razors and shaving accessories, including disposable razor blades
- pet accessories
- soaps
- ingestible "health" drink supplements
- pillows
- humidifiers
- door handles
- computer keyboards and mouses
- printer ink
- shoe inserts
- toothbrushes
- air sanitizers
- showerhead filters
- automobile cleaning and waxing products

[25] See pp. 36-37 infra.

- powdered and liquid nano-silver in bulk form[26]

The nano-silver products' countries of origin include the U.S., U.K, Canada, Korea, Japan, Taiwan, China, New Zealand, and Germany.[27] The vast majority of the companies market their nano-silver products putting emphasis on the nano-silver ingredient, touting its antimicrobial and antibacterial qualities, as well as making other sweeping medical claims, including:

- "Antibacterial, Antibiotic effect"
- "eliminates 99.9% of bacteria, fungi and hundreds of other disease causing microorganisms by inhibiting multiplication and growth and preventing transfer"
- "long lasting antibacterial function"
- renders material "permanently anti-microbial and anti-fungal"
- "eliminates the growth of one-celled organisms (such as bacteria and viruses) by deactivating the organism's oxygen metabolism enzymes"
- "antibacterial effect against bacteria, yeasts, mould, and fungi"
- "clinically proven to fight against harmful bacteria"
- "lasting antiseptic that can exterminate bacteria in a short time"
- "can kill and prevent all kinds of disease germs and microorganisms"
- "is proven to kill over 99% of bacteria including MRSA"
- "kills bacteria in vitro in as little as 30 minutes, 2-5 times faster than other forms of silver"
- "kills approximately 650 kinds of harmful germs and viruses with a germ resistance rate of 99.9%"
- "control air free from bacteria, virus, germs, fungus, or even A.I. (Avian Influenza)"
- "can kill and prevent all kinds of disease germs and microorganisms"
- "naturally kills most of bacteria, mold, and germs . . . sterilization benefits for over 650 types of bacteria like "E. coli, S. Aureus, Pneumococcus, Salmonella, Typhus, Vibria, Cholerae, etc."
- "natural bacteriostat"
- "instant knockdown of bacteria & virus"
- "deactivate enzymes and proteins of bacteria from surviving on the surface of the product
- "when in contact with bacteria and fungus will adversely affect cellular metabolism and inhibit cell growth"
- "works against all types of bacteria and viruses, even killing antibiotic resistant strains as well as all fungal infections . . . remains potent up to 100 washes."
- "sterilizes bacteria of over 650 species."

---

[26] See Appendix A.
[27] Id.

- "sterilize up to 99.9% of harmful bacteria, such as colon bacilli, salmonella, yellow staphylococcus, pseudomonas aeruginosa and salmonella enteritidis."[28]

Nano-silver Risks

Simultaneously with this product explosion, research has mounted to indicate that nano-silver materials pose serious risks to human health and the environment.[29] Even in its bulk form, silver is extremely toxic to fish and other aquatic species.[30] At the nano-scale, nano-silver can be many times more toxic.[31] Because nanoparticles of silver have a greater surface area than larger particles of silver, nano-silver is more chemically reactive and more readily ionized than silver in larger particle form.[32] Nano-silver therefore has greater antibacterial and toxic effects compared to larger silver particles partly because it is more readily converted to silver ions. There is also preliminary evidence that nano-silver can exert effective antibacterial action at a considerably lower concentration than that of silver ions, suggesting that the antibacterial properties and toxicity of nano-silver are not explained only by its chemical composition and by the production of silver ions alone.[33]

While the long-term potential impacts of widespread nano-silver use and disposal are unknown, an increasing number of studies have raised warnings regarding potential toxic effects on human health and the environment.[34] Recent research found that washing nano-silver impregnated clothing caused substantial amounts of nano-silver to leech into the discharge wastewater and eventually into the environment.[35]

---

[28] See Appendix A.
[29] See pp. 58-72, 74-76, 82-84 & 86-91 infra and accompanying footnotes.
[30] See infra pp. 59-60, 82-84 and accompanying footnotes.
[31] See infra pp. 58-59, 60-62, 82-83 and accompanying footnotes.
[32] Id.
[33] See note 29 supra.
[34] See pp. 55-68, 80-86 infra and accompanying footnotes.
[35] See infra pp. 66-67 and accompanying footnotes.

At the nano-scale, silver exhibits remarkably unusual physical, chemical and biological properties.[36] Physical characteristics of nanomaterials, such as shape, size, and surface properties, can exert a toxic effect that goes beyond their chemical composition.[37] Research has demonstrated that nano-silver produce reactive oxygen species (ROS), resulting in oxidative stress toxicity; ROS production is a key mechanism for nanomaterials toxicity. Nano-silver can cause toxicity at a cellular level in mammals and other organisms and has the potential to disrupt key cellular functions.[38] Environmental release and accumulation of nanosilver can also have negative impacts on beneficial bacteria important for soil, plant, and animal health.[39]

Studies have also shown that nanosilver may potentially compromise our ability to control harmful bacteria by creating increased antibiotic resistance which may have an overall negative impact on human health.[40] The powerful antibacterial and toxic effects of nano-silver are of significant concern given that the burgeoning use of nano-silver in disinfectants and other consumer products is likely to result in both human and environmental systems facing greater overall exposures.

PROCEDURAL HISTORY

EPA's Stated Positions on Nanotechnology and Nanomaterials, including Nanosilver

Based on the National Nanotechnology Initiative's ("NNI") definition, EPA has informally defined nanotechnology as

> research and technology development at the atomic, molecular, or macromolecular levels using a length scale of approximately one to one hundred

---

[36] See infra pp. 8-10, 42-46, 49-51, 87-91 and accompanying footnotes.
[37] Id.
[38] See infra pp. 60-73 and accompanying footnotes.
[39] See infra pp. 66-69 and accompanying footnotes.
[40] See infra pp. 64-66 and accompanying footnotes.

> nanometers in any dimension; the creation and use of structures, devices and systems that have novel properties and functions because of their small size; and the ability to control or manipulate matter on an atomic scale.[41]

In its 2007 "White Paper" on nanotechnology, EPA notes that nanomaterials' "special properties" can "cause some nanomaterials to pose hazards to humans and the environment, under specific conditions."[42] EPA believes that "at this point not enough information exists to assess environmental exposure for most engineered nanomaterials"[43] and that "the fundamental properties concerning the environmental fate of nanomaterials are not well understood."[44] There are numerous sources of potential direct and indirect nanomaterial release into the environment, including, *inter alia*, "releases resulting from the use and disposal of consumer products containing nanoscale materials."[45] The "high durability and reactivity of some nanomaterials raise issues of their fate in the environment."[46] Many nanoparticles in current products are non-biodegradable materials (such as metal oxides used in sunscreens) and are not expected to biodegrade.[47] EPA has noted that "the use of nanomaterials in the environment may result in novel by-products or degradates that also may pose risks."[48] EPA has also noted that "nanomaterials may affect aquatic or terrestrial organisms differently than larger particles of the same materials."[49] In general, EPA acknowledges that "there is a significant gap in our

---

[41] Science Policy Council, *U.S. Environmental Protection Agency Nanotechnology White Paper*, U.S. EPA, at 5, (February 2007).
[42] Id. at 13-14.
[43] Id. at 14.
[44] Id. at 33.
[45] Id. at 33.
[46] Id. at 14.
[47] Id. at 36.
[48] Id. at 58.
[49] Id.

knowledge of the environmental, health, and ecological implications associated with nanotechnology."[50]

With regards to "current intentionally produced" nanomaterials, EPA White Paper specifically lists as one category that expressly includes nano-silver:

> (2) Metal-based materials. These nanomaterials include quantum dots, nanogold, **nanosilver** and metal oxides, such as titanium dioxide.[51]

In addition, the EPA White Paper lists examples of products that "use nanotechnology and nanomaterials," that include "wound dressing," "antibacterial socks," "antimicrobial pillows," and "antimicrobial refrigerator,"[52] which are all nano-silver products.[53]

EPA's Stated Position on FIFRA Authority and Pesticide Products Containing Nanomaterials

EPA has recognized that nanotechnology and nanomaterials do and will impact various statutory regimes under its authority, including FIFRA.[54] Specifically with regard to its statutory authority pursuant to FIFRA, EPA has said

> Pesticide products containing nanomaterials will be subject to FIFRA's review and registration requirements. In addition, to the extent that the use of pesticide products containing nanomaterials results in residues in food, the resulting residues require the establishment of a tolerance (maximum allowed residue limit) under the Federal Food, Drug, and Cosmetic Act.[55]

EPA has further stated that in response to the "rapid emergence" of nano-pesticides, the Office of Pesticide Programs (OPP) is currently studying the issue in order to develop policy and evaluating its FIFRA regulatory authority for nano-pesticides:

---

[50] Id. at 52.
[51] Id. at 8 (emphasis added).
[52] Id. at 11 Table 1.
[53] See Appendix A.
[54] EPA, Nanotechnology, at http://es.epa.gov/ncer/nano/ EPA, Science Policy Council, *Nanotechnology White Paper*, February 2007, at http://es.epa.gov/ncer/nano/publications/whitepaper12022005.pdf (hereafter EPA White Paper).
[55] EPA White Paper, supra note 41 at 66.

> [M]embers of the pesticide industry have engaged the Office of Pesticide Programs (OPP) regarding licensing/registration requirements for pesticide products that make use of nanotechnology. In response to the rapid emergence of these products, OPP is forming a largely intra-office workgroup to consider potential exposure and risks to human health and the ecological environment that might be associated with the use of nano-pesticides. Specifically, the workgroup will consider whether or not existing data are sufficient to support additional yet undefined testing. The workgroup will consider the exposure and hazard profiles associated with these new nano-pesticides on a case-by-case basis and ensure consistent review and regulation across the program.[56]

In the interim, voluntary "pre-submission conferences" between companies manufacturing pesticides using nanotechnology and Agency staff are being held.[57] EPA's Office of Pesticide Programs has declined further requests to discuss its ongoing efforts to develop policies for pesticides designed with nanotechnologies.[58]

Concerns Raised over the Samsung Silvercare™ Washing Machine

In early 2006, EPA received letters from both the National Association of Clean Water Agencies (NACWA) and Tri-TAC, a technical advisory group for Publicly Owned Treatment Works in California, expressing concern with the growing number of household products that use pesticides such as nano-silver for general antimicrobial purposes.[59] Both entities pointed out that the silver ions released by the Silver Care washing machine can be highly toxic to aquatic organisms such as plankton,[60] and have the potential to bioaccumulate in some aquatic species.[61]

---

[56] Id. at 20.; see also Pat Phibbs, *Pesticides: Firms Making Nanoengineered Pesticides Urged to Meet with EPA Staff on Data Needs*, DAILY ENVIRONMENT REPORT, May 15, 2006, at A-6.
[57] *Id.*
[58] Pat Phibbs, *Pesticides: Firms Making Nanoengineered Pesticides Urged to Meet with EPA Staff on Data Needs*, DAILY ENVIRONMENT REPORT, May 15, 2006, at A-6.
[59] Letter from Ken Kirk, Executive Director, National Association of Clean Water Agencies, to Stephen Johnson, Administrator, Environmental Protection Agency (February 14, 2006); Letter from Chuck Weir, Chair, Tri-TAC, to James Jones, Director, Office of Pesticide Programs, Environmental Protection Agency (January 27, 2006); Letter from Tobi Jones, Assistant Director, Registration and Health Evaluation Division, Department of Pesticide Regulation, California Environmental Protection Agency, to Chuck Weir, Chair, Tri-TAC (February 22, 2006).
[60] Pat Phibbs and Tripp Baltz, *Pesticides: Examining Use of Nanoscale Silver in Washing Machines as Possible Pesticide*, DAILY ENVIRONMENT REPORT, May 15, 2006, at A-5 - A-6 (quoting Phil Bobel, who works with Tri-TAC).

Widespread use of household products that release silver ions into the sewage system could greatly increase silver concentrations in influents and effluents and adversely affect the nation's waterways.[62] Both entities recommended that EPA require pesticide registration for products using "silver ions" as disinfectants, including washing machines.[63] Both entities also requested that EPA request data regarding wash cycle volumes and silver ion concentrations when registering the Samsung Silver Care Washing Machine.[64]

In its March 10, 2006, response to the letters, EPA stated that the issue was being reevaluated, and it anticipated it would have a decision "within the next few weeks."[65] On May 9, 2006, EPA clarified that it was still examining the question "but does not know when it will make a decision."[66]

EPA November 21, 2006 Announcement

In response to the public concern and calls for action, on November 21, 2006, the media reported that EPA would regulate the nanosilver products used to kill bacteria as a pesticide.[67]

---

[61] Letter from Ken Kirk, Executive Director, National Association of Clean Water Agencies, to Stephen Johnson, Administrator, Environmental Protection Agency (February 14, 2006); Letter from Chuck Weir, Chair, Tri-TAC, to James Jones, Director, Office of Pesticide Programs, Environmental Protection Agency (January 27, 2006).
[62] *Id.*; Pat Phibbs and Tripp Baltz, *Pesticides: Examining Use of Nanoscale Silver in Washing Machines as Possible Pesticide*, DAILY ENVIRONMENT REPORT, May 15, 2006, at A-5 - A-6 (quoting Phil Bobel, who works with Tri-TAC).
[63] Letter from Ken Kirk, Executive Director, National Association of Clean Water Agencies, to Stephen Johnson, Administrator, Environmental Protection Agency (February 14, 2006).
[64] Letter from Ken Kirk, Executive Director, National Association of Clean Water Agencies, to Stephen Johnson, Administrator, Environmental Protection Agency (February 14, 2006); Letter from Chuck Weir, Chair, Tri-TAC, to James Jones, Director, Office of Pesticide Programs, Environmental Protection Agency (January 27, 2006).
[65] Letter from James Jones, Director, Office of Pesticide Programs, Environmental Protection Agency, to Ken Kirk, Executive Director, National Association of Clean Water Agencies (March 10, 2006); Letter from James Jones, Director, Office of Pesticide Programs, Environmental Protection Agency, to Chuck Weir, Chair, Tri-TAC (February 17, 2006).
[66] Pat Phibbs and Tripp Baltz, *Pesticides: Examining Use of Nanoscale Silver in Washing Machines as Possible Pesticide*, DAILY ENVIRONMENT REPORT, May 15, 2006, at A-5 - A-6 (quoting Agency spokeswoman Enesta Jones).
[67] Pat Phibbs, *EPA to Regulate Nanoscale Silver Used in Washing Machines to Kill Bacteria*, Daily Environment, at A-6, BNA, November 21, 2006.

The Washington Post, in a front page article entitled, *EPA to Regulate Nanoproducts Sold as Germ-killing*, explained

> The Environmental Protection Agency has decided to regulate a large class of consumer items made with microscopic 'nanoparticles' of silver, part of a new but increasingly widespread technology that may pose unanticipated risks, a government official said yesterday.[68]

Thus, "companies using nanoscale silver as a pesticide will have to register their product or seek an exemption from federal pesticide rules."[69] As reported, the then-forthcoming EPA action would address the Samsung Washing Machine –reversing its decision to be classified as a "device" and classifying it as a "pesticide" – but would also apply to the broader universe of nano-silver products.[70] EPA spokeswoman Enesta Jones was reported as saying that,

> As for the increasing number of other products that incorporate silver to fight microbes, such as air sanitizers and food-storage containers, Jones said that they will have to be registered or meet a registration exemption if they make pesticide claims.[71]

While the announcement was not limited to the Samsung Washer, it was limited in scope: according to EPA officials, this "large class" of products would be limited only to those nano-silver products advertised as "germ-killing" or the like, and not to those who dropped or did not include such anti-microbial marketing claims.[72]

The Federal Register (FR) notice proposing the new rule was said to be coming "soon."[73]

The EPA September 21, 2007 Federal Register Notice

---

[68] Rick Weiss, *EPA to Regulate Nanoproducts Sold as Germ-killing*, Wash Post, A01, November 23, 2007.
[69] Phibbs, supra note 60.
[70] Id.
[71] Id.
[72] Weiss, supra note 68.
[73] Id.

Nearly a year later, on September 21, 2007 EPA finally issued the long-awaited FR notice, entitled "Pesticide Registration; Clarification for Ion-Generating Equipment."[74] EPA summarized its purpose and scope:

> [The notice] clarifies the Agency's position on the distinction between devices and pesticides with regard to ion-generating equipment and explains why such equipment will now be regulated as a pesticide. The Agency has now determined that these machines will be regulated as pesticides if the machines contain silver or other substances, and if they generate ions of those substances for express pesticidal purposes. [75]

Generally speaking, the FR notice was opaque in its language (i.e., "silver ion generating equipment,") described by one well-known technology reporter as "Washington mumbo jumbo, translated into English, means that Samsung's SilverCare washing machines are covered by pesticide regulations because Samsung claims they kill germs by injecting 100 quadrillion silver ions into each wash load. "[76]

The notice's purpose was stated to: "alert manufacturers of the Agency's determination;" assure that the Agency "will work to identify the information needed to apply to register the machine as a pesticide;" and to "give those products currently out of compliance time to obtain registration."[77] EPA opened a docket, EPA-HQ-OPP-2007-0949, for affected parties to submit information. Producers of the equipment can continue to sell or distribute the equipment as long as they file registration papers by March 23, 2009.[78]

---

[74] See 72 Fed. Reg. 54039 (September 21, 2007).
[75] EPA, Pesticides: Topical & Chemical Fact Sheets, *Pesticide Registration: Clarification for Ion Generating Equipment*, at http://www.epa.gov/oppad001/ion_gen_equip.htm (last visited October 16, 2007).
[76] Barnaby J. Feder, *Samsung's Nanotech Washer Must Follow Bug-Spray Rules*, New York Times *Bits* Blog, September 26, 2007, at http://bits.blogs.nytimes.com/2007/09/26/samsungs-washers-regulated-as-a-pesticide/.
[77] Id.
[78] 72 Fed. Reg. 54039, 54041.

The products covered by the notice are cabined to "ion generators that incorporate a substance (e.g., silver or copper) in the form of an electrode, and pass a current through the electrode to release ions of that substance for the purpose of preventing, destroying, repelling, or mitigating a pest (e.g., bacteria or algae)."[79] Crucially, the notice gave no reference to EPA's oversight of nanotechnology, nanomaterials, or nano-silver ingredients; in fact, it did not contain the prefix "nano" anywhere. Instead, the Agency gave this one-paragraph explanation of that omission on its website:

> While recent press articles have referred to the silver ion generating washing machine as a product of nanotechnology, EPA has not yet received any information that suggests that this product uses nanotechnology. EPA will evaluate any applications to register this type of equipment according to the same regulatory standards as any other pesticide. The notice does not represent an action to regulate nanotechnology.[80]

EPA's statement that it "has not yet received any information" on the nano-aspects of the Samsung Silvercare™ washer defies rationality given that Samsung itself touts its use of nanotechnology on its website, entitled the "Silver Nano Health System" and pictures the washer, among other products.[81]

Finally, in the FR notice no mention is given to the rest of the existing fleet of nano-silver products (besides the "ion generating" equipment) or any proposed action by the agency regarding it, contrary to reports of the quotes from EPA officials in the November 2006 announcement. Nowhere does the notice request information about such products or in any way solicit comment from interested parties or the public on the regulation of nano-silver products.

---

[79] 72 Fed. Reg. 54039, 54040 ("Because these items incorporate a substance or substances that accomplish their pesticidal function, such items are considered pesticides for purposes of FIFRA, and must be registered prior to sale or distribution.").

[80] EPA, Pesticides: Topical & Chemical Fact Sheets, *Pesticide Registration: Clarification for Ion Generating Equipment*, at http://www.epa.gov/oppad001/ion_gen_equip.htm (last visited October 16, 2007).

[81] Samsung, *Silver Nano Health System*, at http://www.samsung.com/ph/silvernano/.

Further communications between petitioners (in an attempt to get further clarification regarding the notice) and an agency official noted that

> The point that was being made was that this notice will not address or represent an action to regulate nanotechnology. It is also pointed out that the Agency at some time in the future may set criteria (in addition to particle size) for determining whether technology would qualify as nanotechnology and until such criteria are established Samsung's claims may or may not be upheld.[82]

However neither the September 21, 2007 FR notice or anything on EPA's website giving further explanation included such notice of any future criteria-setting process.

The February 27, 2008 Consent Agreement Between EPA Region 9 and ATEN Technology, Inc.

On February 27, 2008, EPA's Region 9 office settled an action against a California corporation that manufacturers a nano-silver product for violations of FIFRA.[83] EPA fined the technology company ATEN Technology, Inc., of Irvine, Calif., acting for its subsidiary IOGEAR $208,000 for "nano coating" pesticide claims on its computer peripherals, for selling unregistered pesticides and for making unproven claims about their effectiveness.[84] The IOGEAR products at issue were: wireless laser mouse with nano-silver shield coating, laser travel mouse with nano-silver coating technology, and wireless RF keyboard and mouse combinations. After being contacted by EPA, IOGEAR stopped making claims that their computer peripherals protect against germs.[85] In its complaint EPA alleged that:

1) the IOGEAR electronic equipment with "nano shield coating" was labeled containing pesticidal claims;

---

[82] September 25, 2007 Email from Melba S. Morrow, D.V.M., Special Assistant to the Director, Antimicrobials Division, Office of Pesticide Programs, Environmental Protection Agency to Jaydee Hanson, Policy Analyst, ICTA (on file with author).

[83] In the Matter of: ATEN Technology, Inc. d/b/a IOGEAR, Inc., Docket # FIFRA-09-2008-0003, *Consent Agreement and Final Order Pursuant to Sections 22.13 and 22.18* (February 27, 2008).

[84] Nanowerk News, *EPA fines technology company $208,000 for 'nano coating' pesticide claims on computer peripherals*, March 7, 2008, at http://www.nanowerk.com/news/newsid=4857.php

[85] Id.

2) in the marketing of the products, that IOGEAR had made both "implicit and explicit public health and pesticidal claims," including claims that the nano coating has "mechanisms to deactivate enzymes and proteins to prevent bacteria from surviving on the surface of the product" and "the compound has been tested and proven effective against various bacteria."

3) that "each of the nano products is a 'pesticide' as defined by Section 2(u) of FIFRA, 7 U.S.C. § 136(u). Each of the nano products is not a registered pesticide";

4) and that in 2007 IOGEAR had distributed or sold the nano products on 40 separate occasions, in violation of 7 U.S.C. § 136j(a)(1)(A).[86]

In giving its authority to take this enforcement action EPA explained its relevant FIFRA authority, including, *inter alia*, the definition of a pesticide and that it is unlawful to distribute or sell unregistered pesticides.[87] Thus EPA charged that IOGEAR violated the law by failing to register its products as pesticides prior to distribution and sale as well as making health claims about its products that were unsubstantiated. IOGEAR neither admitted or denied EPA's allegations but consented to the all the conditions of the final order and settlement, waived the right to appeal it, and agreed to pay a fine of $208,000.[88]

As explained in detail in the legal argument section below, the legal bases and analyses by EPA in this IOGEAR enforcement action is precisely the legal argument petitioners herein present regarding the regulatory status of nano-silver products as illegal, unregistered pesticides as well as EPA's FIFRA authority over these products. This precedent-setting enforcement action by EPA strongly supports petitioners' position and highlights the urgency of this matter. Unfortunately press accounts noted that EPA is not making any concerted effort in this area nor does EPA have a new strategy for dealing with these products.[89]

---

[86] In the Matter of: ATEN Technology, Inc. d/b/a IOGEAR, Inc., Docket # FIFRA-09-2008-0003, *Consent Agreement and Final Order Pursuant to Sections 22.13 and 22.18* (February 27, 2008), at p.4.
[87] Id. at 2-3.
[88] Id. at 5-6.
[89] Lacey, *First-Time Fine May Signal New FIFRA Nano Enforcement Effort*, INSIDE EPA, March 14, 2008.

Accordingly, petitioners hereby file this legal petition with EPA in order to, *inter alia*, address the reasonably foreseeable adverse human health and environmental consequences caused by the explosion of nano-silver products on the market that the agency has thus far avoided, and to call on the agency to take the actions required to fulfill its statutory duties of protecting public health and environmental welfare.

EPA ACTIONS REQUESTED REGARGING NANO-SILVER PRODUCTS

Summary of Actions Requested

There are currently at least 260 consumer products in the marketplace that contain nano-silver, which either expressly make pesticidal claims or imply pesticidal effectiveness -- none of which are currently registered with EPA. First, EPA should classify nano-silver as a pesticide and require manufacturers to register nano-silver pesticides pursuant to FIFRA's pesticide regulations. As explained in Section I below, nano-silver products meet the FIFRA definition of a pesticide because nano-silver is a highly efficient antimicrobial or antibacterial agent and is intended to be used for that purpose. Further, EPA should clarify that pesticidal intent and public health claims can be both implicit and explicit and that manufacturers cannot avoid pesticide classification simply by stripping their products of labelling.

Second, EPA should clarify that nano-pesticides, such as nano-silver products, are new pesticide substances that require new pesticide registrations, with nano-specific toxicity testing and risk assessment. As explained in Section II, nano-silver is not covered under previous registrations for bulk silver because nano-silver should be classified as a separate substance than silver based on nanomaterials' capacity for fundamentally unique and different properties and because nano-silver's many new antimicrobial uses are not previously registered silver uses.

Third, EPA must assess the potential human health and environmental risks of nano-silver. As explained in Section III below, these assessments are required by and must comply with the FIFRA, the Food Quality Protection Act (FQPA), the Endangered Species Act (ESA), and the National Environmental Policy Act (NEPA). Pursuant to FIFRA, in order to assess nano-silver pesticides EPA must assess whether nano-silver presents "any unreasonable risk to

man or the environment." As part of this assessment, EPA should analyze all existing scientific studies as well as require manufacturers to provide all necessary additional data on the EHS unknowns of nano-silver. Pursuant to FQPA, EPA must assess the potential impacts of nano-silver on children and infants and ensure that no harm will result from aggregate exposures. Additionally, EPA must ensure that its activities regarding nano-silver comply with the ESA and the protection of endangered and threatened species, including ESA Section 7 Consultation requirements. Finally, EPA must comply with NEPA by ensuring that it assesses the environmental impacts of its actions regarding nano-silver pesticide products, including completing a programmatic environmental impact statement.

Fourth, EPA should take immediate action to prohibit the sale of nano-silver products as illegal pesticide products with unapproved health benefit claims. If a nano-pesticide is unregistered, it may not be distributed or sold in the United States.[90] Similarly, distribution and sale of registered nano-pesticides is prohibited if it is distributed, sold, or used in a manner that departs from the conditions of EPA's approval. This includes: pesticidal claims substantially different from those approved with registration;[91] a composition different than that reviewed in the registration;[92] adulteration;[93] or a use inconsistent with labeling.[94] The nano-silver consumer products currently on market are in clear violation of FIFRA's mandates. To this end, as explained in Section IV below, EPA should issue Stop Sale, Use or Removal Orders ("SSURO") or other enforcement penalties or actions to those manufacturers and/or distributors currently selling these unregistered nano-silver pesticide products.

---

[90] This prohibition is subject to certain exceptions for R&D and exports. 7 U.S.C. §§ 136j(a)(1)(A), 136o(a).
[91] 7 U.S.C. § 136j(a)(1)(B).
[92] 7 U.S.C. § 136j(a)(1)(C).
[93] 7 U.S.C. § 136j(a)(1)(E).
[94] 7 U.S.C. § 136j(a)(2)(G).

Fifth, should EPA, after rigorous assessment, approve any nano-silver products as pesticides, the agency must fully apply its pesticide regulations to any registered nano-silver pesticides. FIFRA's pesticide registration requirement instills EPA with the duty to prohibit, condition, or allow the manufacture and use of nanomaterials in nano-pesticides and prescribe conditions for manufacture or use. As explained in Section V, these include, *inter alia*: requiring nano-specific ingredient and warning labelling; applying conditional registration; applying requirements for post-registration notification of adverse impacts; applying post-registration testing and new data development; and requiring the disclosure of all information concerning environmental and health effects, including "confidential business information."

Finally, as explained in Section VI, EPA should also use its FIFRA authority to further review the potential impacts of nano-silver, including: undertaking either a Classification Review or a Special Review of nano-silver pesticides; amending the FIFRA regulations to require the submission of nanomaterial and/or nano-silver specific data; completing a registration review of existing silver pesticides; regulation of nano-silver pesticide devices; and the setting of a FFDCA Tolerance for nano-silver.

I. Nano-silver and Nano-silver Products Are Pesticides Requiring FIFRA Registration

EPA should clarify that nano-silver and nano-silver products are pesticides requiring registration under FIFRA because nano-silver is a highly efficient pest killer and is incorporated into the products with the intent of using its nano-enhanced antimicrobial properties.

A. *The Federal Insecticide, Fungicide and Rodenticide Act ("FIFRA")*

The Federal Insecticide, Fungicide and Rodenticide Act ("FIFRA")[95] is the federal regulatory scheme for the manufacture, labeling, sale, and application of pesticides.[96] FIFRA controls the manufacture, sale, and use of a broad range of chemicals and biological pest controls, as well as substances to control plant growth.[97] Although first passed in 1947 to ensure product efficacy and accurate labeling,[98] Congress significantly overhauled it in 1972 through the Federal Environmental Pesticide Control Act to shift the regulatory focus to protection of human health and the environment.[99]

Every pesticide chemical to be sold in the United States must be registered with EPA before it can be distributed or sold.[100] If a substance is found to have "unreasonably adverse effects on the environment," it cannot be registered and brought to market.[101] Accordingly, the Agency must conduct a cost-benefit analysis, balancing the risk of allowing a pesticide to be registered and sold in the market with any potentially harmful effects.[102]

---

[95] 7 U.S.C. §§ 136-136y et seq.

[96] The Federal Food, Drug, and Cosmetic Act (FFDCA) also regulates pesticides in a number of ways. In particular the FFDCA requires EPA to establish a "tolerance" for each ingredient of a pesticide used in connection with food or animal feed. 21 U.S.C. § 346a. In addition, various other laws and regulations governing chemical substances such as the Toxic Substances Control Act (TSCA), 15 U.S.C. §§ 2601-2692, Hazardous Materials Transportation Act, 49 U.S.C. §§ 5101-5127, and the Occupational Safety and Health Act Hazard Communication Standard, 29 C.F.R. § 1910.1200, may apply to pesticides.

[97] 7 U.S.C. § 136u. It also includes more limited authority over mechanical pest control devices, including FIFRA labeling and establishment registration requirements. 7 U.S.C. §§ 136(h), 136w(c)(4); 40 C.F.R. § 152.500(a).

[98] Pub. L. No. 80-104, 61 Stat. 163 (1947).

[99] Pub. L. No. 92-516, 86 Stat. 973 (1972); see also Alexandra B. Klass, Bees, Trees, Preemption and Nuisance: A New Path to Resolving Pesticide Land Use Disputes, 32 Ecology L.Q. 763, 771 (2005).

[100] 7 U.S.C. § 136a(a).

[101] No Spray Coalition, Inc. v. City of New York, 351 F.3d 602, 604-05 (2d. Cir. 2003) (citing 7 U.S.C. § 136a(c)(5)(D).

[102] Peter J. Martinez, Damon L. Worden, Luke M. Jones, Jason S. Juceam, Environmental Crimes, 43 Am. Crim. L. Rev. 381, 452 n.540 (2006).

B. Nano-Silver is a Pesticide under the FIFRA Definition of Pesticides

Pursuant to section 2(u) of FIFRA, a pesticide is defined as "any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest."[103] A "pest" is in turn defined as

> **Pest**: (1) any insect, rodent, nematode, fungus, weed, or (2) any other form of terrestrial or aquatic plant or animal life or virus, bacteria, or other micro-organism (except viruses, bacteria, or other micro-organisms on or in living man or other living animals) which the Administrator declares to be a pest pursuant to 7 U.S.C. 136w(c)(1).[104]

The pesticide's "active ingredient" is the ingredient which "will prevent, destroy, repel, or mitigate" pests.[105] Nano-silver is the ingredient in these nanomaterial products infused to fight bacteria, i.e., prevent pests. Therefore, nano-silver meets the definition of a pesticide and/or the active ingredient in a pesticide.[106]

C. The Intent of Nano-Silver Demonstrates that it is a Pesticide

Nano-silver is a pesticide because its intended use is as a pesticide. As noted above, the FIFRA definition of pesticide hinges on the intent: FIFRA defines "pesticide" not in terms of the inherent characteristics of particular substances but rather in terms of the *intent* underlying the use of a substance.[107] EPA's FIFRA-implementing regulations elaborates on intent as the statutory touchstone, providing that a pesticide is "any substance (or mixture of substances)

---

[103] 7 U.S.C. § 136(u)(1); 40 C.F.R. § 152.3.

[104] 7 U.S.C. § 136(t); 40 C.F.R. § 152.5. In addition, the Agency Administrator is authorized, after notice and the opportunity for hearing, to declare as a pest any form of plant or animal life (excluding man and any other bacteria, virus, and micro-organism on or in living man or other animals) that is injurious to human health or the environment. 7 U.S.C. § 136w(c)(1); 40 C.F.R. § 152.5.

[105] 7 U.S.C. § 136(a)(1); 40 C.F.R. § 153.125.

[106] EPA has concluded that one company's nano-silver coated mouses and keyboards were pesticides. See In the Matter of: ATEN Technology, Inc. d/b/a IOGEAR, Inc., Docket # FIFRA-09-2008-0003, *Consent Agreement and Final Order Pursuant to Sections 22.13 and 22.18* (February 27, 2008) at 2-4 (EPA action explaining FIFRA definition of pesticide and concluding that nano-silver coated electronics were pesticides pursuant to 7 U.S.C. § 136(u)).

[107] 7 U.S.C. § 136(u)(1) (emphasis added); Office of Prevention, Pesticides & Toxic Substances, Environmental Protection Agency, Label Review Manual, p. 2-4 (3d ed. 2003).

intended for a pesticidal purpose."[108] The regulations give three factors for determining "intent" i.e., whether "a substance is considered to be intended for a pesticidal purpose, and thus to be a pesticide requiring regulation:"

> A substance is considered to be intended for a pesticidal purpose, and thus to be a pesticide requiring regulation, if:
>
> (a) The person who distributes or sells the substance claims or implies (by labeling or otherwise):
>
> 1) That the substance [] can or should be used as a pesticide; or
> 2) That the substance consists of or contains an active ingredient and that it can be used to manufacture a pesticide; or
>
> (b) The substance contains one or more active ingredient and has no significant commercially valuable use as distributed or sold other than (1) use for pesticidal purpose [], (2) use for manufacture of a pesticide; or
>
> (c) The person distributing or selling the substance has actual or constructive knowledge that the substance will be used, or is intended to be used, for a pesticidal purpose.[109]

Any one of these factors could be sufficient to show intent; in the case of nano-silver products, all of the factors are present. First, the manufacturers of these nano-silver products claim – indeed they proudly tout, by product labeling and/or other advertising -- the highly efficient germ-killing propensities of the nano-silver ingredients in their products. These claims include, *inter alia*, various statements that the nano-silver ingredients have a "*long lasting antibacterial function*;" or renders material "*permanently anti-microbial and anti-fungal*"; or "*kills approximately 650 kinds of harmful germs and viruses with a germ resistance rate of 99.9%.*" See generally supra p. 14-15 and Appendix A infra. According to well-established precedent, labeling or advertising

[108] 40 C.F.R. § 152.15.
[109] 40 C.F.R. § 152.15.

material recommending a product for use against a pest may be clear evidence of that intent.[110]

Second, nano-silver is specifically and solely used for its anti-microbial properties. Research has shown no other "significantly commercially valuable use."

Third, the manufacturers have both actual and constructive knowledge that the nano-silver is infused in said product for a pesticidal purpose. For every nano-silver product listed in the attached appendix and chart,[111] the product description clearly emphasizes its ability to kill, eliminate, curb, prevent or reduce the growth of microorganisms such as fungus and bacteria. These nano-silver product descriptions include: "*can kill and prevent all kinds of disease germs and microorganisms*"; "*natural bacteriostat*"; "*deactivate enzymes and proteins of bacteria from surviving on the surface of the product*"; "*works against all types of bacteria and viruses, even killing antibiotic resistant strains as well as all fungal infections . . . remains potent up to 100 washes*"; "*kills bacteria in vitro in as little as 30 minutes, 2-5 times faster than other forms of silver*"; and so forth. See supra pp. 13-14 and Appendix A infra. These representations and their variants alone are sufficient under the definition of intent provided in the FIFRA-implementing regulations.[112] In addition, these product descriptions make it impossible for manufacturers and distributors to deny they did not have actual or constructive knowledge the substance was to be used, or was intended to be used, for

---

[110] See In re Chemico Indus., Inc., I.F.&R., 1984 WL 50057, *4-5 (EPA Jan. 24, 1984); see also In re Myers, I.F.&R., 1980 WL 19379, *5 (EPA July 31, 1980) ("The intended use of a product may be determined from its label, accompanying labeling, promotional material, advertising and any other relevant sources.") (citing United States v. 216 Bottles, 409 F.2d 734, 739 (2d. Cir. 1969)).

[111] See Appendix A.

[112] 40 C.F.R. § 152.15(a)

pesticidal purposes.[113] The nano-silver product descriptions and the manufacturers' and distributors' actual knowledge that these products would be used as pesticides clearly demonstrate intent as defined in the FIFRA-implementing regulations.

D. Intent Showing Pesticidal Purpose Is Not Limited to Only Product Labeling

EPA should clarify that intent can be shown by means far broader than just labeling. As the factors above illustrate, "a substance is considered to be intended for a pesticidal purpose, and thus to be a pesticide requiring regulation" for reasons including "claims or implies (by labeling *or otherwise*) that the substance can or should be used as a pesticide."[114] In addition, intent can be shown by the active ingredient having "insignificant commercial value as anything else besides a pesticide."[115] Finally, intent can be showing by the "active or constructive knowledge" of the manufacturer that the substance "will be used or is intended to be used for a pesticidal purpose."[116]

At least one Federal Circuit Court of Appeals applies an objective standard to determine intent in the FIFRA context, asking whether the company could expect a reasonable consumer to use the product against pests.[117] "Industry claims and general public knowledge can make a product pesticidal notwithstanding the lack of express pesticidal claims by the producer itself."[118] Accordingly, the general advertising of nano-silver specifically as a germ-killer,[119] creates public knowledge that leads a consumer knowledge and expectation that nano-silver product is an anti-

---

[113] 40 C.F.R. § 152.15(c).
[114] 40 C.F.R. § 152.15(a) (emphasis added).
[115] Id. 152.15(b).
[116] 40 CFR § 152.15(c).
[117] N.Jonas & Co. vs. EPA, 666 F.2d 829, 833 (3d Cir. 1981) ("In determining intent objectively, the inquiry cannot be restricted to a product's label and to the producer's representations. Industry claims and general public knowledge can make a product pesticidal notwithstanding the lack of express pesticidal claims by the producer itself. Labeling, industry representations, advertising materials, effectiveness and the collectivity of all the circumstances are therefore relevant.").
[118] Id.
[119] See Appendix A.

microbial agent, not withstanding any lack of specific germ-killing advertizing on said specific nano-silver product. The appendix includes more than 260 products that contain nano-silver, of which nearly all include some reference to nano-silver's germ-fighting propensity in the manufacturer's advertizing and/or the product's labeling.

Subsequently EPA has incorporated that objective standard into its regulations: "EPA believes that a producer who sells a product with full knowledge of its intended pesticidal use should be held responsible for its regulation."[120] Thus, manufacturers who produce and market products containing nano-silver with "full knowledge" of its intended uses as an anti-microbial – *even if they do not label the material as "nano" and/or "germ killing"*–are still properly subject to FIFRA's pesticide registration requirements.[121]

EPA must clarify that a pesticide classification is not solely based on a product's labeling.[122] This distinction is crucial, as early reports of EPA's planned action on nano-silver products from November 2006 quoted EPA officials erroneously claiming (or erroneously quoted as claiming) that only products marketed or advertised as anti-microbial or germ killing will have to be regulated, providing a huge loophole for companies that drop anti-microbial claims from their nano-silver products.[123] This potential loophole has been exploited: in response to EPA's anticipated proposed action regarding nano-silver, several nano-silver product

---

[120] See Pesticide Registration Procedures, Pesticide Data Requirements, 53 Fed Reg 15952, 15954 (May 4, 1988) (codified at 40 C.F.R. § 152.15(c)); see also Clarification of Treated Articles Exemption, 63 Fed. Reg. 19256, 19257 (April 17, 1998) (discussing 40 C.F.R. § 152.25) ("'The Agency has consistently interpreted and applied this rule to prohibit *implied* or explicit public health claims for unregistered products, and continues to regard any public health claims as not consistent with the provisions of the rule.") (emphasis added).

[121] See, e.g., N.Jonas & Co., 666 F.2d at 833 ("In determining intent objectively, the inquiry cannot be restricted to a product's label and to the producer's representations.").

[122] See In the Matter of: ATEN Technology, Inc. d/b/a IOGEAR, Inc., Docket # FIFRA-09-2008-0003, *Consent Agreement and Final Order Pursuant to Sections 22.13 and 22.18* (February 27, 2008) at 4 (EPA alleging that IOGEAR had made "both implicit and explicit public health claims and pesticidal claims"), 3 (unregistered pesticide products may not be marketed if, *inter alia*, they make any "implied or explicit public health claims").

[123] Weiss, supra note 68.

manufacturers removed their nano-specific labeling. For example, The Sharper Image's *FresherLonger™ Miracle Food Storage* containers were previously marketed with an entire section entitled "*The Silver Nanoparticle Miracle*," noting that the food storage containers were "infused with antibacterial silver nanoparticles" that were "25nm in diameter" and "created by advanced nanotechnology."[124] The nano-silver ingredient was "anti-germ, anti-mold, and anti-fungus" and "compared to regular containers … reduced the growth of microorganisms by over 98 percent."[125] After EPA's November 2006 announcement, Sharper Image stripped its website and all its print and online advertising of any claims to either nano-silver ingredients or that ingredient's biocide activity.[126] Another U.S. company, Pure Plushy, also dropped its claims to be selling toys and stuffed animals made using nanoparticles of 25nm of silver for their antimicrobial effects.[127] Appendix A includes other products previously marketed as nano and/or anti-microbial which are no longer so marketed.[128]

EPA should clarify that manufacturers such as Sharper Image and others cannot purposely evade EPA purview by disclaiming its previous advertising or intentionally misrepresenting its products' ingredients. Manufacturers who produce and market products containing nano-silver with "full knowledge" of its intended uses as an antimicrobial –even if they do not label the material as "nano" or do not label the nano-silver's intended antimicrobial

---

[124] http://web.archive.org/web/20060208021530/http://www.sharperimage.com/us/en/catalog/productdetails/sku__ZN020

[125] Id.

[126] Compare, FresherLonger™ Miracle Food Storage Containers, http://www.sharperimage.com/us/en/catalog/productdetails/sku__ZN020 with FresherLonger™ Miracle Food Storage Containers, http://web.archive.org/web/20060208021530/http://www.sharperimage.com/us/en/catalog/productdetails/sku__ZN020

[127] Andrew Maynard, SafeNano Community Blog, *Benny the Bear and the Case of the Disappearing nanoparticles*, December 15, 2007, at http://community.safenano.org/blogs/andrew_maynard/archive/2007/12/15/benny-the-bear-and-the-case-of-the-disappearing-nanoparticles.aspx

[128] See Appendix A.

effects–are still properly subject to FIFRA's pesticide registration requirements and must be regulated by EPA as such.[129]

E. Nano-silver Products Fit into the Category of Antimicrobial Pesticides

FIFRA also defines one particular subset of pesticides as "antimicrobial pesticides:"

> **Antimicrobial Pesticide**: a pesticide intended to (i) disinfect, sanitize, reduce, or mitigate growth or development of microbiological organisms; or (ii) protect inanimate objects, industrial processes or systems, surfaces, water, or other chemical substances from contamination, fouling, or deterioration caused by bacteria, viruses, fungi, protozoa, algae, or slime.[130]

Thus, an antimicrobial pesticide is one meant either to affect the growth or development of microbiological organisms or to protect inanimate objects, industrial processes, or chemical substances from contamination from such organisms.[131] Common antimicrobial products include disinfectants for medical and household surfaces including floors, walls, linens, and other surfaces, sanitizers for food contact products such as dishes and cooking utensils and non-food contact products such as carpet cleaners and laundry additives.[132] The nano-silver products listed in Appendix A easily fall within this pesticides definition subset, as products include: floor, wall, and other surface cleaners, cutlery and food contact substances, laundry additives and so on, all intended to "reduce, or mitigate growth or development of microbiological organisms" and/or "protect

---

[129] See In the Matter of: ATEN Technology, Inc. d/b/a IOGEAR, Inc., Docket # FIFRA-09-2008-0003, *Consent Agreement and Final Order Pursuant to Sections 22.13 and 22.18* (February 27, 2008) at 4 (EPA alleging that IOGEAR had made "both implicit and explicit public health claims and pesticidal claims").

[130] 7 U.S.C. § 136(mm). Products excluded from this definition include wood preservatives or antifouling paint products, an agricultural fungicide, or an aquatic herbicide. However, the term "antimicrobial pesticide" does include any other chemical sterilant (other than for use with critical devices), disinfectant product, industrial microbiocide product, or preservative product not excluded above. Id.

[131] EPA, Pesticides: Topical & Chemical Fact Sheets, Antimicrobial Pesticide Products, at www.epa.gov/pesticides/factsheets/antimic.htm

[132] Id.

inanimate objects ... or other substances ... from contamination ... caused by bacteria, viruses, fungi...."[133] Accordingly, the logical fit of the nano-silver products in this subset of pesticides further buttresses the conclusion that these products are pesticides and must be regulated as such.

F. Limited FIFRA Pesticide Exemptions Do not Apply to Nano-Silver

Finally, there are several exemptions or exclusions from the FIFRA pesticide definition and accompanying regulations relevant to the nano-silver determination. As discussed below, the nano-silver consumer products do not qualify for these limited regulatory exceptions.

First, there are several classes of substances expressly excluded from regulation by FIFRA for reasons including that they are regulated by other statutes, like those products qualifying as human or animal drug products under FFDCA.[134] The products incorporating nano-silver are consumer products that have come to market already and not new drug products classified and subject to pre-market review by FDA. However any nano-silver drug products approved by FDA pursuant to its drug approval process would be exempt from EPA FIFRA pesticide regulations.

Second, FIFRA also exempts products intended for use only against microorganisms, internal parasites, or nematodes in or on living humans or animals and labeled accordingly.[135] The nano-silver consumer products in Appendix A are not so limited in the scope of their pesticidal intent, nor are they so labeled.

---

[133] See Appendix A.
[134] See 40 C.F.R. §§ 152.6, 152.20.
[135] 40 C.F.R. § 152.8.

Third, some products are exempted from FIFRA regulation because they are not "deemed to be used for a pesticidal effect."[136] This exemption has an explicit lists three types of products exempted which includes, *inter alia*, deodorizers, bleaches, and cleaning agents.[137] This is relevant since several of the nano-silver products currently on market are cleaning agents. See Appendix A. However this exemption expressly does not apply if "a pesticidal claim is made on their labeling or in connection with their sale and distribution."[138] Thus, any nano-silver cleaning agent products would be disqualified by their express labeling and/or advertizing as antibacterial agents. See Appendix A (listing products and advertizing claims).

Finally, some pesticide-treated articles or substances are exempted from FIFRA regulation, if several prerequisites are met.[139] One such class is pesticide "treated" articles. As EPA recognizes

> many products (e.g. cutting boards . . .) are being treated with antimicrobial pesticides. Antimicrobial pesticides are substances or mixtures of substances used to destroy or limit the growth of microorganisms, whether bacteria, viruses or fungi – many of which are harmful – on inanimate objects and surfaces.[140]

"Treated articles" refers to the products treated with an antimicrobial pesticide to protect the article itself.[141] The pesticide is usually added to the treated articles during manufacture or added

---

[136] 40 C.F.R. § 152.10.
[137] 40 C.F.R. § 152.10(a). The complete list of exempted products under this section:

> The following types of products or articles are not considered to be pesticides unless a pesticidal claim is made on their labeling or in connection with their sale and distribution:
> (a) Deodorizers, bleaches, and cleaning agents;
> (b) Products not containing toxicants, intended only to attract pests for survey or detection purposes, and labeled accordingly;
> (c) Products that are intended to exclude pests only by providing a physical barrier against pest access, and which contain no toxicants, such as certain pruning paints to trees.

Id.
[138] 40 C.F.R. § 152.10.
[139] 40 C.F.R. § 152.25.
[140] EPA, Pesticides: Topical & Chemical Fact Sheets, Consumer Products Treated with Pesticides, at www.epa.gov/pesticides/factsheets/treatart.htm
[141] Id.

after manufacture but before use.[142] Such pesticide-treated products can be exempt from FIFRA registration, but only if

1) the pesticide is added only to protect the article itself; and
2) the pesticide added to the treated article "is registered for such use."[143]

Nano-silver consumer products such as those listed in Appendix A do not qualify for this exemption. As to the latter requirement, nano-silver itself is not registered as a pesticide, for these current uses or any other uses for that matter. As to the former, as detailed in the Appendix, the nano-silver products make express claims to protection from bacteria or germs beyond and separate from just the protection of the incorporating product itself. As EPA notes: "Any pesticide-treated product that is not registered by EPA must not make public health claims, such as 'fights germs, provides antibacterial protection, or controls fungus."[144] Many of these nano-silver products do make exactly such beyond product and/or public health claims, including but not limited to "*can kill and prevent all kinds of disease germs and microorganisms*"; provides "*antibacterial effect against bacteria, yeasts, mould, and fungi*"; "*kills approximately 650 kinds of harmful germs and viruses*"; "*fights against cross infection of super bugs such as MRSA*"; "*natural antibiotic that can kill and prevent infections*"; "*kills athlete's foot germs and staphylococcus*", "*nanosilver coated foils have been scientifically proven to reduce redness and irritation*"; and so forth. See Appendix A.

---

[142] Id.

[143] 40 C.F.R. § 152.25(a). Examples include paint treated with a pesticide to protect the paint coating, or wood products treated to protect the wood against insects or fungus infestation. See also EPA, Pesticides: Topical & Chemical Fact Sheets, Consumer Products Treated with Pesticides, at www.epa.gov/pesticides/factsheets/treatart.htm

[144] EPA, Pesticides: Topical & Chemical Fact Sheets, Consumer Products Treated with Pesticides, at www.epa.gov/pesticides/factsheets/treatart.htm

EPA further clarified the interpretation of Section 125.25(a) (the Treated Article Exemption) in Federal Register notice,[145] stating that unregistered products may be marketed only provided that

> (1) no implied or explicit health claims of any kind are made;
>
> (2) the claims concerning the presence of a pesticide in the treated article are limited to protection of the treated article only;
>
> (3) when such claims involve antibacterial properties, (a) the words "antibacterial," "antimicrobial," or "germicidal," or related terms, are not part of the name of the product, and (b) the permissible claims are qualified by statements indicating that the presence of the antibacterial properties does not protect users or others against disease and that users should follow prudent hygienic measures, i.e., cleaning and washing the article;
>
> (4) the pesticide in a treated article is present only as a result of using a pesticide product which is registered under FIFRA and labeled for use in treating the article in question.[146]

As explained above, these conditions are not met and the limited exemption for treated articles does not apply for the nano-silver pesticides.[147]

II. Nano-silver Is a New Pesticide That Requires New Pesticide Registrations

Next, EPA should classify nanomaterial pesticides such as nano-silver pesticides as new pesticides that require new pesticide registrations. The risk assessment for nanomaterials is different from that larger particle substances and must include a nanotoxicology assessment assessing physicochemical characteristics and factors not otherwise assessed. The safety of nanomaterials cannot be reliably predicted or derived from the known toxicity of the bulk

---

[145] Clarification of Treated Article Exemption, 63 Fed. Reg. 19256 (April 17, 1998).
[146] Id. at 19257.
[147] See In the Matter of: ATEN Technology, Inc. d/b/a IOGEAR, Inc., Docket # FIFRA-09-2008-0003, *Consent Agreement and Final Order Pursuant to Sections 22.13 and 22.18* (February 27, 2008) at 3-4 (explaining the Treated Article Exemption before alleging that the IOGEAR nano-silver coated electronics were illegal unregistered pesticides).

material. Further, the claims, composition, and new uses of these nano-silver pesticides are very different from bulk material counterpart pesticides. Finally, the conclusion that nanomaterials—including nano-silver—are distinct and new substances is supported by their patentability, a legal standard which requires, *inter alia*, non-obviousness and novelty.

A. Nano-pesticides Require New Pesticide Registrations

Under FIFRA, a pesticide is considered unregistered if, *inter alia*, 1) its claims differ substantially from the claims made for the registered pesticide, or 2) if its composition differs from the composition of the registered pesticide.[148] In general, claims for nano-pesticides will and do differ from those made for conventional pesticides because nanotechnology allows for many new applications. Nano-silver pesticides and their claims, as discussed infra, are one example. A new registration is required for a pesticide containing an active ingredient that has not been previously registered or used in a registered formulation.[149] Thus, nano-pesticides are not covered by existing registrations of conventional pesticides.

The unique characteristics of nano-pesticides result in different risks and benefits than any macro-scale versions. Product chemistry, toxicology, and other information submitted for macro versions pursuant to 40 C.F.R. Part 158 C & D do not apply to nanomaterials. "Composition" includes the identity of both active and inert ingredients and their ratios. Given the unique characteristics of nanomaterials, nano-pesticides do not have the same composition as bulk material, macro versions.[150] *In short, EPA must employ a different risk assessment based on the actual characteristics of the nano-pesticide.* Any previous analysis/balance of risks and

---

[148] 7 U.S.C. § 136j(a)(1)(B) & (C).
[149] 40 C.F.R. § 152.403 (new chemical registration review).
[150] See, e.g., Reut Snir, *Regulating Risks of Nanotechnologies for Water Treatment*, 38 ENVT'L L. REPORTER 10233, 10244-46 (2008); James Chen et al., ABA-SEER, The Adequacy of FIFRA to Regulate Nanotechnology-Based Pesticides (2006), at 11, available at http://www.abanet.org/environ/nanotech/pdf/FIFRA.pdf

benefits and appropriate control measures for a conventional pesticide containing a macro-ingredient of the same nanomaterial is different, because of the nano-specific properties, the "nano-ness" of the nanomaterial.

Further, under FIFRA § 3(c)(5)(D), registration decisions depend in the main on EPA's determination that a pesticide "will not generally cause unreasonable adverse effects on the environment."[151] To comply with FIFRA, EPA must weigh the *precise* benefits and risks of individual pesticides and determine under what conditions a pesticide may be registered, if any. Key factors in this determination are the claims and composition of the pesticide. Since the balancing of risks and benefits of a nano-pesticide is different from a corresponding conventional pesticide containing a bulk material ingredient of the same substance, EPA must require a new registration for the nano-pesticide.[152] Substitution of a nanoscale ingredient for a macro counterpart constitutes a change in composition *per se* requiring new registration.

"Experts are overwhelmingly of the opinion that the adverse effects of nanoparticles cannot be reliably predicted or derived from the known toxicity of the bulk material."[153] For example, the European Commission's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) concluded: "Experts are of the *unanimous* opinion that the adverse effects of nanoparticles cannot be predicted (or derived) from the known toxicity of material of

---

[151] 7 U.S.C. § 136a(a), (c)(5)(C)-(D).

[152] See also Reut Snir, *Regulating Risks of Nanotechnologies for Water Treatment*, 38 ENVT'L L. REPORTER 10233, 10244-45 (2008); James Chen et al., ABA-SEER, The Adequacy of FIFRA to Regulate Nanotechnology-Based Pesticides (2006), at 11-12, available at http://www.abanet.org/environ/nanotech/pdf/FIFRA.pdf Where a registrant of a conventional pesticide applies for registration of a nano-pesticide, an amended registration may be appropriate. 40 C.F.R. § 152.44, provided it is required to provide additional information specific to the nano-pesticide's risks and benefits.

[153] The Allianz Group and the Organisation for Economic Co-operation and Development (OECD), *Small Sizes that Matter: Opportunities and risks of Nanotechnologies*, (June 3, 2005) at § 6.4, at 30.

macroscopic size, which obey the laws of classical physics."[154] Similarly, the U.K. Royal Society and the Royal Academy of Engineering emphasized: "Free particles in the nanometre size range do raise health, environmental, and safety concerns and their toxicology *cannot be inferred* from that of particles of the same chemical at a larger size."[155] And finally, the British Institute for Occupational Medicine similarly concluded:

> Because of their size and the ways they are used, they [engineered nanomaterials] have specific physical-chemical properties and therefore may behave differently from their parent materials when released and interact differently with living systems. *It is accepted, therefore, that it is not possible to infer the safety of nanomaterials by using information derived from the bulk parent material.*[156]

Toxicology normally correlates health risks with the mass to which an individual is exposed, resulting in an accumulated mass as an internal dose/exposure. However, the biological activity of nanoparticles is likely to depend on physicochemical characteristics that are not routinely considered in toxicity screening studies.[157] There are many more factors affecting the toxicological potential of nanoscale materials, up to at least sixteen in fact, including: size, surface area, surface charge, solubility, shape or physical dimensions, surface coatings, chemical composition, and aggregation potential- a "far cry from the two or three usually measured."[158] Size is one of many factors, but is crucial: The relevance of the nano-size is that unlike larger particles, we cannot predict the toxicity of nanomaterials from the known properties of larger

---

[154] European Commission's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), *Opinion on the appropriateness of existing methodologies to assess the potential risks associated with engineered and adventitious products of nanotechnologies*, at 6 (adopted September 28-29, 2005) (emphasis added); id. at 34.
[155] See, e.g., The Royal Society and the Royal Academy of Engineering, *Nanoscience and nanotechnologies: Opportunities and uncertainties*, London, 2004, supra note 19, at 49 (emphasis added).
[156] Tran et al., *A Scoping Study to Identify Hazard Data Needs For Addressing The Risks Presented By Nanoparticles and Nanotubes*, INSTITUTE OF OCCUPATIONAL MEDICINE Research Report (December 2005), at 34 (emphasis added).
[157] European Commission's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), *Opinion on the appropriateness of existing methodologies to assess the potential risks associated with engineered and adventitious products of nanotechnologies*, at 6 (adopted September 28-29, 2005), at 32; Nuala Moran, *Nanomedicine lacks recognition in Europe*, 24 NATURE BIOTECHNOLOGY, No. 2 (February 2006).
[158] Andrew Maynard, *Nanotechnology: The Next Big Thing, or Much Ado about Nothing?*, at 7 ANNALS OF OCCUPATIONAL HYGIENE, 7 September 2006.

substances. Unless EPA requires a thorough manufacturer testing and investigation of all these variables and then applies a subsequent agency assessment to that submitted data, it cannot properly assess the toxicity of nano-pesticides or assure their safety.

In fact, nanotoxicology is an emerging field in its own right, underscoring the differences of nanomaterial toxicity. In an agenda-setting 2006 article in *Nature*, fourteen international nanotechnology scientists put forth nanotechnology's five "grand challenges," which included the urgent need to develop methods for assessing nano-toxicity.[159] Two recently published articles suggest new paradigms of predictive toxicology for engineered nanoparticle testing.[160] EPA should develop a basic screening framework to guide its testing and data-submission requirements, such as the tiered approach that would start with non-cellular tests to establish particle reactivity, followed by in vitro and in vivo tests for exposure pathways that are relevant to a chemical's anticipated use patterns and lifecycle.[161]

B. Nano-silver Pesticides Require New Chemical Pesticide Registrations Because They are Substances With New Compositions and Claims that Require New, Nano-specific Risk Assessments

Nano-silver exemplifies why nano-pesticides require new pesticide registration. Silver is already registered as a pesticide.[162] It is registered for use in water filter systems as a bacteria inhibitor (90% of use) and in swimming pools as an algicide (3% of use).[163] As of its 1993-94 Re-registration, there were 80 pesticide products registered with silver as an active ingredient.[164] The nano-silver products being used as antimicrobials in consumer applications and appliances

---

[159] Maynard et al., *Safe Handling of Nanotechnology*, NATURE, November 16, 2006.
[160] Andre Nel et al., *Toxic Potential of Materials at the Nanolevel*, 311 SCIENCE 622 (2006); Oberdorster et al., *Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy*, 2 PARTICLE AND FIBRE TOXICOLOGY 8, at 1.0 (2005).
[161] Id.
[162] See EPA, Silver Reregistration Eligibility Document (RED), June 1993.
[163] Id. at p.1.
[164] Id. at p. 2.

differ substantially in both uses and claims from these registered silver pesticides. The nano-silver product explosion has included a broad swath of industries and products including much more than water filtration systems and swimming pools; these new nano-silver products include, but are not limited to, various cleaning and sanitizing products, food storage containers, toiletries, clothing, home appliances, air filters, medical supplies, dietary supplements, and powdered and liquid nano-silver in bulk form. See Appendix A.

These new nano-silver products also differ in the breadth of their product claims, which are much broader than previously-registered silver pesticide products. Silver pesticides are limited to claims as water-based bacteria inhibitors and algicides. In contrast, these new nano-silver products' claims include: "*control air free from bacteria, virus, germs, fungus, or even A.I. (Avian Influenza)*"; "*kills approximately 650 kinds of harmful germs and viruses with a germ resistance rate of 99.9%*"; "*is proven to kill over 99% of bacteria including MRSA*"; "*sterilize up to 99.9% of harmful bacteria, such as colon bacilli, salmonella, yellow staphylococcus, pseudomonas aeruginosa an salmonella enteritidis*"; "*can kill and prevent all kinds of disease germs and microorganisms*"; renders material "*permanently anti-microbial and anti-fungal*"; and so forth. See Appendix A.

Further, the risk assessment needed for nano-silver is wholly different. Exposures are substantially increased and varied. For example, in the 1993 Silver RED, EPA notes that "residential exposure" to silver pesticides was expected at only "very low levels" through the silver drinking water filters and by swimming in treated pools.[165] The Re-registration document lists as "currently registered" uses of silver as only two types: "aquatic non-food residential

[165] EPA, Silver RED, supra note 162 at 3, Appendix A.

(swimming pool systems) and indoor food uses (human drinking water systems).[166] About 90% of the 80 registered silver pesticides are basteriostatic water filters; 7% are media which contain silver for actual filter housing; and 3% are algicides.[167] The sudden appearance of nano-silver consumer products dramatically increases exposure potential and levels as well as the routes of exposure. See Appendix A. These new uses include household cleaners, sprays and wipes, personal care products and soaps, children's toys and bottles, food storage containers and cutlery, clothing and fabrics, and so forth.

Similarly, in the Silver RED, EPA concluded that there were not unreasonable adverse effects to the environment from silver because the exposure from silver pesticides used in swimming pools and drinking water systems would be discharged into municipal water systems and treated.[168] The broad range of new nano-silver products encompasses many environmental discharge and exposure routes, creating a very different environmental risk and exposure assessment. See Appendix A. Moreover, public utility and water treatment experts have already warned EPA of their concerns about nano-silver's potential negative environmental impacts and their inability to adequately treat that substance.[169]

Finally, as discussed, at the nano-scale silver exhibits remarkably unusual physical, chemical and biological properties.[170] Taking into account their unique physicochemical properties, it is likely that nano-silver possesses unique toxicity mechanisms.[171] For example, nano-silver may deplete the antioxidant defense mechanism, which leads to ROS accumulation

---

[166] EPA, Silver RED, supra note 162 at 4.
[167] Id.
[168] EPA, Silver RED, supra note 162 at 4.
[169] Letter from Ken Kirk, Executive Director, National Association of Clean Water Agencies, to Stephen Johnson, Administrator, Environmental Protection Agency (February 14, 2006); Letter from Chuck Weir, Chair, Tri-TAC, to James Jones, Director, Office of Pesticide Programs, Environmental Protection Agency (January 27, 2006).
[170] Chen and Schluesener, *Nanosilver: A nanoproduct in medical application*, 176 TOXICOLOGY LETTERS 1-12 (2008).
[171] Id. at 8.

and can initiate the destruction of mitochondria and eventually cell death.[172] In addition, damage to cell membranes appears to be another part of nano-silver's mechanism of cytotoxicity.[173] There is also preliminary evidence that the nano-silver can exert effective antibacterial action at a considerably lower concentration than silver ions, i.e, is a "far more efficient" conveyer of antibacterial effects.[174] This suggests that the antibacterial properties and toxicity of nano-silver are not explained only by its chemical composition and the production of silver ions alone. As with other nanomaterials, nano-silver will require a nano-specific toxicity assessment.

C. Nano-silver Pesticides Require New Chemical Pesticide Registrations Because Nano-silver is Patented for its Novelty

The U.S. legal patent framework also strongly supports the conclusion that engineered and manufactured nanomaterials generally–and nano-silver specifically–are novel substances, for which manufacturers should be classified and regulated as new products, in this case, new pesticides. As such, nano-silver requires a separate risk assessment and FIFRA registration process. Many of the manufacturers of these nano-silver pesticide products, regulated by EPA, have applied for and received patents for their products and/or the nano-silver in them, a legal and commercial reality that belies any claim that the engineered nanomaterials are not wholly unique substances which must be classified as new substances and new pesticides.

1. Patent Law Requires Novelty

By law, the issuance of a patent requires a determination of novelty and nonobviousness,[175] and claims for novel disclosures are assigned one or more patent classifications. The applicant must demonstrate that the invention is novel, non-obvious, and

---

[172] Id.
[173] Id.
[174] Lok et al., *Proteomoic analysis of the mode of antibacterial action of silver nanoparticles*, 5 J. PROTEAME RES. 916-924 (2007).
[175] 35 U.S.C. §§ 102-103.

useful.[176] It is well-established patent case law that a mere change in size, scale, or dimensions of a known composition are not alone sufficient to establish novelty and nonobviousness and render new material patentable.[177] As early as 1928, the legal principle was well established that a "mere difference in dimension cannot add novelty" to a claimed new product.[178] Courts have since consistently held that the mere scaling of a prior art, capable of being scaled, would not establish patentability in a claim over that prior art.[179] The United States Court of Appeals for the Federal Circuit held that when the only difference between the prior art and its claims was a recitation of relative dimensions of the claimed device, and a device having the claimed relative dimensions would not "exhibit qualitatively different phenomena" from the prior art, the claimed invention was not patentably distinct from the prior art.[180] Thus, whether a nanomaterial is patentable turns on whether the nanomaterial or nanoparticle exhibits "qualitatively different phenomena" than that of its bulk material counterpart.[181]

Nanomaterials meet this threshold because matter behaves uniquely when manufactured or engineered to the nano-scale: nano means more than merely tiny, a billionth of a meter in scale.[182] Rather, it is best understood to mean substances having the capacity to be fundamentally different. "The nano-scale is not just another step toward miniaturization, but a

---

[176] 35 U.S.C. §§ 101-103.
[177] Application of Troiel, 274 F.2d 944, 949 (C.C.P.A. 1960) ("It is well established that the mere change of the relative size of the co-acting members of a known combination will not endow an otherwise unpatentable combination with patentability.").
[178] King Ventilating Co. v. St. James Ventilating Co., 26 F.2d 357, 359 (8th Cir. 1928).
[179] In re Rinehart, 531 F.2d 1048, 1053 (C.C.P.A. 1976); see also U.S. Indus., Inc., v. Norton Co., 210 U.S.P.Q. 94, 104 (N.D.N.Y. 1980) (holding that "mere changes of proportions of a known composition with a resultant increase in strength, size, etc., is generally deemed insufficient to constitute patentability, such changes, though useful, being only of degree rather than kind.").
[180] Gardner v. TEC Sys., Inc., 725 F.2d 1338, 1346 (Fed. Cir. 1984).
[181] See id. at 1345-46 (noting that dimensional limitations do not inherently distinguish the subsequent version from the prior art).
[182] See, e.g., pp. 8-11, 44-46 supra.

qualitatively new scale."[183] Taking advantage of quantum physics, nanotechnology companies have and are continuing to engineer materials that have entirely new properties never before identified in nature, and patenting them in the U.S and other countries.

Recognizing this, in August of 2004, the United States Patent and Trademark Office (USPTO) created an art collection of Nanotechnology, Class 977, in response to the desire to gather in one place all published US Patents and US PreGrant Publications (US PGPUBs) that claim subject matter related to nanotechnology.[184] In December of 2005, the USPTO revised the nanotechnology patent classification, replacing one comprehensive digest with 263 new subclasses for cross-referencing all nano-related patents. Class 977, which establishes the definitions and cross-references for these patents, has a two pronged definition of "nanostructures," a necessary ingredient of all patents for which the class provides disclosures,[185] to be an atomic, molecular, or macromolecular structure that both: 1) "has at least one physical dimension of approximately 1-100 nanometers;" and 2) "*possess[] a special property, provides a*

---

[183] Nat'l Sci. Found., *Societal Implications of Nanoscience and Nanotech.* at 1 (Mihail C. Roco & Sims Bainbridge eds., 2001), http://www.wtec.org/loyola/nano/NSET.Societal.Implications/nanosi.pdf

[184] Patent office Classification Definitions, Class 977, Nanotechnology, (November 2005), available at http://www.uspto.gov/web/patents/classification/uspc977/defs977.htm#C977S000000.

[185] Id. The definition of nanotechnology as a class includes "nanostructures" and their chemical compositions, devices that include at least one nanostructure, mathematical algorithms for modeling confiurations or properties of nanostructures, or specified uses of nanostructure.

Patent Class 977, Nanotechnology, Section I - Class Definition, reads:

> i. Nanostructure and chemical compositions of nanostructure;
> ii. Device that include at least one nanostructure;
> iii. Mathematical algorithms, e.g., computer software, etc., specifically adapted for modeling configurations or properties of nanostructure;
> iv. Methods or apparatus for making, detecting, analyzing, or treating nanostructure; and
> v. Specified particular uses of nanostructure.
>
> As used above, the term "nanostructure" is defined to mean an atomic, molecular, or macromolecular structure that:
> (a) Has at least one physical dimension of approximately 1-100 nanometers; and
> (b) Possesses a special property, provides a special function, or produces a special effect that is uniquely attributable to the structure's nanoscale physical size.

special function, or produces a special effect that is uniquely attributable to the structure's nanoscale physical size."[186] Thus, to be included in USPTO Class 977, a patent must not simply be a reduction in size of an existing element or particle; rather, that new size must alter the original substance creating a unique effect or property that is only possible at the nanoscale. The classification class notes on Class 977 are even more explicit, clarifying that

> Special properties and functionalities should be interpreted broadly, and are defined as those properties and functionalities that are significant, distinctive, non-nominal, noteworthy, or unique as a result of the nanoscale dimension. In general, differences in properties and functionalities that constitute *mere differences of scale are insufficient* to warrant inclusion of the subject matter in Class 977.[187]

2. Nanotechnology Patents Demonstrate the Novelty of Nano-Materials

The President's Council of Advisors on Science and Technology (PCAST) reported in May 2005 that the Patent Office issued over 8,600 "nanotechnology-related" patents in 2003, an increase of 50% from 2000 (compared to about 4% for patents in all technology fields).[188] More discrete surveys have found at least 5,000 nanotechnology patents as of March 2006, with the number of patents growing by over 30% every year since 2000.[189] The "gold rush" for patents on the building blocks of the platform technology continues unabated.[190] Claims include composition of matter claims (claims to nanomaterials themselves, nanotubes, nanowires, and nanoparticles), device, apparatus, or system claims (claims to electrical, mechanical, and optical

---

[186] Id.
[187] U.S. Patent Class 977, Nanotechnology, Classification Definitions, Note (3), available at http://www.uspto.gov/web/patents/classification/uspc977/defs977.htm#C977S000000 (emphasis added);
[188] President's Council of Advisors on Science and Technology (PCAST), *The National Nanotechnology Initiative at Five Years: Assessment and Recommendations of the National Nanotechnology Advisory Panel*, at 15-17 & fig. 4, May 2005. available at http://www.nano.gov/FINAL_PCAST_NANO_REPORT.pdf ; Julie A. Burger et al., *Nanotechnology and the Intellectual Property Landscape*, Chapter 14, p.3, NANOSCALE: ISSUES AND PERSPECTIVES FOR THE NANO CENTURY, ED. NIGEL CAMERON ET AL., (Wiley Pub. 2007).
[189] Nanowerk, *The patent land grab in nanotechnology continues unabated, creating problems down the road*, Marcy 30, 2006. at www.nanowerk.com/spotlight/spotid=386.php
[190] See, e.g., Charles Choi, *NanoWorld: Nano Patents in Conflict*, WASH. TIMES, April 25, 2005.

devices incorporating nanomaterials), and method claims (claims to processes for synthesizing nanomaterials or constructing devices or systems).

3. Nano-silver Patents Demonstrate the Novelty of Nano-Silver Products

Many of these nanotechnology patents are for nano-silver products. An enumerated search of currently-held patents disclosed a number of relevant nano-silver material, formulation, and use patents and patent applications including, *inter alia*,

- U.S. Patent 6,379,712, Yan, et al., April 30, 2002: *Nanosilver-containing antibacterial and antifungal granules and methods for preparing and using the same*

- U.S. Patent 6,979,491, Yan, et al., December 27, 2005: *Antimicrobial yarn having nanosilver particles and methods for manufacturing the same* – "The present invention provides a yarn with antimicrobial effects. The antimicrobial antifungal effect of the yarn is derived from nanosilver particles (diameter between 1 and 100 nm) which are adhered to the yarn."

- U.S. Patent Application 20050287112: *Antibacterial paint containing nano silver particles and coating methods using the same* – December 29, 2005 Kwon, Kyuk-Min; Samsung Electronics – Assignee. An antibacterial paint containing 30 ppm of nano silver particles on a surface. Nano silver particles have a diameter of 5 nm. "Nano technology, as used herein, refers to a technology wherein a material, such as silver, is fabricated into nano-scale particles.... This is based on new phenomena which appear when crystal grain size of a material, such as metal or ceramic, become smaller than 100 nm and which is difficult to explain by conventional theories. It is known in the art that nano silver particles have antibacterial properties."

- U.S. Patent Application 20020051823 (5/2/2002): *Nanosilver-containing antibacterial and antifungal granules and methods for preparing and using the same*

- U.S. Patent Application 20030185889 (10/2/2003): *Colloidal nanosilver solution and method for making same* – "The present invention provides a colloidal nanosilver solution which contains nanosilver particles having diameters between 1 nm and 100 nm."

- U.S. Patent Application 20040135480 (7/15/2004): *Refrigerator with an inner case containing nanosilver particles*

- U.S. Patent Application 20050152992 (7/14/2005): *Antimicrobial surface preparation and method for producing the same* – "The antimicrobial surface preparation of claim 1

wherein said particles of silver have a size between about 5 nanometers and about 100 nanometers on average."

- U.S. Patent Application 20060243675 (11/2/2006): *Novel composite for inhibiting algae growth and use thereof* – "A composite for inhibiting algae growth comprising of a polypore base carrier and a nano-metal mixture coated on a carrier . . . . The composite of claim 4, wherein the nano-metal is nanosilver."

- U.S. Patent Application 20060272542 (12/7/2006): *Nanosilver as a biocide in building materials.*

- U.S. Patent Application 20070256560 (11/8/2007): *Silver nanoparticle-containing polymer film for facilitated olefin transport and method for the fabrication thereof*

The patents and patent claims above belie any argument that manufactured nano-silver particles and materials are not wholly new substances with their novel properties; specifically in the case of nano-silver, that nano-silver pesticides are substantially different from other pesticides made without them. If these substances were the same as their bulk material counterparts (silver pesticides), they would not be patentable, as they would be unable to meet patent law standards for novelty.

D. "New Use" Would Also Require Registration of Nano-silver Pesticides

If a pesticide product to be registered contains an active ingredient that is already registered, but has not previously been used in the manner proposed for the new product, it requires a "new use" registration.[191] For the above reasons, petitioners firmly believe nano-silver is a new active ingredient of a new pesticide that requires its own separate pesticide registration process that accounts for the nano-specific risk assessments, toxicology, and exposures discussed above. However, even if the agency comes to the mistaken conclusion that nano-silver is the equivalent of silver for FIFRA registration purposes, EPA must still act, because nano-silver is a "new use" of previously registered silver pesticides.

---

[191] See generally *Pesticide Regulation Deskbook*, ENVIRONMENTAL LAW REPORTER, 24-25 (2000).

The definition of a "new use" of a pesticide product is:

> New use, when used with respect to a product containing a particular active ingredient, means:
>
> (1) Any proposed use pattern that would require the establishment of, the increase in, or the exemption from the requirement of, a tolerance or food additive regulation under section 408 or 409 of the Federal Food, Drug and Cosmetic Act;
> (2) Any aquatic, terrestrial, outdoor, or forestry use pattern, if no product containing the active ingredient is currently registered for that use pattern; or
> (3) Any additional use pattern that would result in a significant increase in the level of exposure, or a change in the route of exposure, to the active ingredient of man or other organisms.[192]

In this case, nano-silver pesticide products meet all three of the possible ways of creating a new use. First, nano-silver requires the establishment of a tolerance, see Section VI(E) infra, and no tolerance has been set. Second, many unregistered nano-silver products have uses that have the capacity to impact aquatic, terrestrial, and outdoor environments, as discussed infra Section III(A), (C), & (D). Neither nano-silver, nor silver, is registered for such use. And third, nano-silver pesticide products are resulting in new use patterns, with significant increases and new routes of exposure to man and other organisms. See supra & Appendix A.

A pesticide's use is required to be included in the mandatory statement that must accompany the registration.[193] When the use is being changed, or a new use is being added, the registration must be updated if the manufacturer wants to avoid selling an illegal and misbranded product.[194] The registration amendment process[195] is similar to the registration of a new pesticide, requiring a statement, and supporting data, except certain data may be re-used from the initial registration.[196] EPA may also need new data to evaluate the potential effects of the

---

[192] 40 C.F.R. § 152.3.
[193] *See* 7 U.S.C. §136a(c)(1)(C), §136a(c)(1)(E).
[194] 7 U.S.C. §136(q)(1)(F) & 7 U.S.C. §136(q)(2)(B).
[195] 40 C.F.R. §152.44.
[196] *See* 7 U.S.C. §136a(c)(1)(F).

pesticide in the new use application. EPA can conditionally register a pesticide for a new use only if it determines, *inter alia*, that the applicant has submitted "satisfactory data pertaining to the proposed additional use, and (ii) amending the registration in the manner proposed by the applicant would not significantly increase the risk of any unreasonable adverse effect on the environment."[197]

E. Conclusion: Nano-Silver is a New Pesticide

In summary, nanomaterial pesticide products such as nano-silver products are new pesticides. They have new claims and compositions, requiring new risk assessments. Pesticides comprised of engineered or manufactured nano-silver cannot be considered safe and/or to not have an "unreasonable risk to man or the environment,"[198] based on the testing or previous approvals of macro-silver pesticide counterparts. Rather, EPA must require safety information specifically addressing the new dangers presented by these new novel substances. EPA must analyze their nano-specific potential for "unreasonable adverse effects on the environment," as discussed in section III infra. Moreover, consistent legal treatment of nano-pesticides with established patent law necessitates that EPA's pesticide regulatory regime treat nano-pesticides as new pesticides for which manufacturers must complete new and separate pesticide applications. Finally, even if EPA erroneously concludes that silver and nano-silver are the same active ingredient, new use registrations are required for nano-silver pesticides because of the broad swath of new uses of nano-silver pesticide products.

[197] 7 U.S.C. § 136a(c)(7)(B).
[198] 7 U.S.C. § 136(bb).

III. EPA Must Analyze the Potential Environmental and Human Health Risks of Nano-silver Pursuant to EPA's Statutory Obligations under FIFRA, FQPA, ESA, and NEPA

Next, EPA must assess the potential human health and environmental risks of nano-silver. These assessments are required by and must comply with FIFRA, the Food Quality Protection Act (FQPA), the Endangered Species Act (ESA), and the National Environmental Policy Act (NEPA). Pursuant to FIFRA, in order to assess nano-silver pesticides EPA must assess whether nano-silver presents "any unreasonable risk to man or the environment." As part of this assessment, EPA should analyze all existing scientific studies as well as require manufacturers to provide all necessary additional data on the environmental, human health and safety ("EHS") unknowns of nano-silver. Pursuant to FQPA, EPA must assess the potential impacts of nano-silver on children and infants and ensure that no harm will result from aggregate exposures. Additionally, EPA must ensure that its activities regarding nano-silver comply with the ESA and the protection of endangered and threatened species, including ESA Section 7 Consultation requirements. Finally, EPA must comply with NEPA by ensuring that it assesses the environmental impacts of its actions regarding nanomaterial and/or nano-silver pesticide products, including completing a programmatic environmental impact statement.

A. *As Part of the FIFRA Pesticide Registration Process, EPA Must Analyze the Potential Human Health and Environmental Risks of Nano-silver*

1. The FIFRA Pesticide Registration Standard: Unreasonable Adverse Effects on the Environment

EPA can register a pesticide if, in conjunction with any restrictions that it may place on the use of the pesticide, *inter alia*, the expected use of the product will not cause unreasonable environmental harm.[199] FIFRA defines "unreasonable adverse effects on the environment" as

> (1) any unreasonable risk to man or the environment, taking into account the economic, social, and environmental costs and benefits of the use of any pesticide, or (2) a human dietary risk from residues that result from a use of a pesticide in or on any food inconsistent with the standard under The Federal Food, Drug and Cosmetic Act 21 U.S.C. § 346a relating to tolerances and exemptions for pesticide chemical residues.[200]

The "environment" is defined broadly to include the "water, air, land, and all plants and man and other animals living therein, and the interrelationships which exist among these."[201] Thus, as part of the registration process, EPA must assess whether nano-silver specifically creates an unreasonable risk to man or the environment. EPA acknowledges as it must that, "some of the same special properties that make nanomaterials useful are also properties that may cause some nanomaterials to pose hazards to humans and the environment, under specific conditions."[202]

2. *Nano-Silver May Pose Unreasonable Risk to Humans and the Environment*

While the long-term potential impacts of widespread nano-silver use and disposal are unknown, its use as an antimicrobial agent is now widespread including in numerous products such as sprays, liquids, gels, cleaning agents, food containers, clothing, and appliances.[203] These nano-silver products are in direct human contact and direct and/or are indirectly released into the

---

[199] 7 U.S.C. § 136a(c)(5); see also Montana Pole & Treating Plant v. I.F. Laucks & Co., 775 F. Supp. 1339, 1343 (D. Mont. 1991) ("Under FIFRA, the EPA is required to register a pesticide if it determines (1) the pesticide's labeling and other materials comply with FIFRA's requirements; and (2) the pesticide, when used properly, will perform its intended purpose without unreasonable adverse effects on the environment.").
[200] 7 U.S.C. § 136(bb).
[201] 7 U.S.C. § 136(j).
[202] Science Policy Council, *U.S. Environmental Protection Agency Nanotechnology White Paper*, U.S. EPA, at 13, (February 2007)
[203] See Appendix A.

environment. Simultaneously, concerns have been mounting that nano-silver particles pose an unacceptable toxicity risk to human health and the environment.[204] While silver in it's larger forms is already known to be toxic, the dramatically increased surface area of nano-silver enhances that toxic propensity. Existing research has shown that nano-silver is toxic at a cellular level in mammals and other organisms and has the potential to disrupt key cellular functions.[205] Negative impacts can be expected on beneficial bacteria important for soil, plant, and animal health.[206] Studies have also shown that the widespread use of nano-silver may compromise our ability to control harmful bacteria by creating increased antibiotic resistance.[207] The petition summarizes these issues below.

i. Silver Poses Adverse Environmental Impacts

Even in non-nano form silver is extremely toxic to fish,[208] algae, crustaceans, plants, fungi,[209] and bacteria (especially nitrogen fixing heterotrophic and soil forming chemolithotrophic).[210] As noted above, EPA already regulates silver as a pesticide[211] and requires labeling that states silver pesticides are "highly toxic to fish and aquatic invertebrates."[212] Silver also inhibits microbial growth at concentrations far below that of other

---

[204] See, e.g., Lloyd's of London, Risks: Lloyd's Emerging Risks Team Report, *Nanotechnology Recent Developments, Risks and Opportunities*, 2007.
[205] See infra pp. 62-64 and accompanying footnotes.
[206] See infra pp. 66-70 and accompanying footnotes.
[207] See infra pp. 64-66 and accompanying footnotes.
[208] Hogstrand et al., *The toxicity of silver to marine fish*, at 109-112 in Andren, Anders W.; Bober, Thomas W. (ed.) THE 4TH INTERNATIONAL CONFERENCE PROCEEDINGS: TRANSPORT, FATE AND EFFECTS OF SILVER IN THE ENVIRONMENT (1996).
[209] Eisler, R. *A review of silver hazards to plants and animals*, 143-44 in (1996)., pp. 143-144 in Andren, Anders W.; Bober, Thomas W. (ed.) THE 4TH INTERNATIONAL CONFERENCE PROCEEDINGS: TRANSPORT, FATE AND EFFECTS OF SILVER IN THE ENVIRONMENT (1996).
[210] Albright et al., *Sub-lethal effects of several metallic salt-organic compound combinations upon heterotrophic microflora of a natural water*. 8 WATER RES 101-105 (1974).
[211] EPA, R.E.D., supra note 162 at 4.
[212] Brown et al., *Assessing Toxicant Effects in a Complex Estuary: A Case Study of Effects of Silver on Reproduction in the Bivalve, Potamocorbula Amurensis, in San Francisco Bay*, 9 HUMAN AND ECOLOGICAL RISK ASSESSMENT 95, at 117 (2003)

heavy metals.[213] It can also bioaccumulate and persist in water sediment. Silver is toxic to both freshwater and saltwater organisms and is particularly damaging to reproductive systems. In a study of the bivalves, *Potamocorbula amurensis* and *Macoma balthica*, silver presence resulted in a decreased level of reproductive rates. The highest levels of silver were synonymous with the lowest levels of reproductivity.[214] Other studies have shown that silver accumulates in the liver, gills, kidneys and blood plasma of fish causing circulatory failure and ion regulation disruption.[215] Silver can also accumulate in invertebrates and will thus be passed on to different organism when consumed.[216] Silver exposure via direct uptake and trophic transfer can be toxic to zooplankton, a primary food source for developing larvae and fish.[217]

ii. The Nano-Enhanced Toxic Properties And Toxicity Mechanisms of Nano-Silver

In addition to silver's known impacts, nano-scale silver exhibits remarkably unusual physical, chemical and biological properties.[218] The extremely high reactivity and very small mass of nanomaterials means that nanomaterials can be toxic at far lesser weights than bulk materials. Their small size confers greater particle mobility in the environment and in the body. EPA has noted: "Nanoscale materials are typically more reactive than larger particles of the same

---

[213] Braydich-Stolle et al., *In Vitro Cytotoxicity of Nanoparticles in Mammalian Germline Stem Cells* 88 (2) TOXICOLOGICAL SCIENCES 412-19 (2005).
[214] Brown et al., *Assessing Toxicant Effects in a Complex Estuary: A Case Study of Effects of Silver on Reproduction in the Bivalve, Potamocorbula Amurensis, in San Francisco Bay*, 9 HUMAN AND ECOLOGICAL RISK ASSESSMENT 95, at 116 (2003)
[215] Wood et al., *Bioavailability, Physiology and Toxicology of Silver in Freshwater Fish: Implications for Water Quality Criteria*, PROCEEDINGS OF THE 5TH INTERNATIONAL CONFERENCE ON THE TRANSPORT, FATE AND EFFECTS OF SILVER IN THE ENVIRONMENT 205, at 206-207, (1997); Dethloff et al, *Effects of Sodium Chloride on Chronic Silver Toxicity to Early Life Stages of Rainbow Trout (Oncorhynchus Mykiss)*, 26 ENV. TOX. CHEM. 1717, at 1722-1723 (2007).
[216] Fisher et al, *Trophic Transfer of Silver to Marine Herbivores: A Review of Recent Studies*, 17 ENV TOX CHEM 562 (1998).
[217] Hook et al., *Sublethal Effects of Silver in Zooplankton: Importance of Exposure Pathways and Implications for Toxicity Testing*, 20(3) ENVIRON TOXICOL AND CHEMISTRY 568-74 (2000).
[218] Chen and Schluesener, *Nanosilver: A nanoproduct in medical application*, 176 TOXICOLOGY LETTERS 1-12 (2008).

material. *This is true especially for metals and metal oxides.*"[219] The smaller a particle, the greater its surface area to volume ratio and the higher its chemical reactivity and biological activity.[220] The increased chemical reactivity of nanoparticles results in increased production of reactive oxygen species (ROS), including free radicals.[221] ROS production has been found in a diverse range of nanomaterials including carbon fullerenes, carbon nanotubes, and nanoparticle metal oxides.[222] ROS and free radical production is one of the primary mechanisms of nanoparticle toxicity and may result in oxidative stress, inflammation, and consequent damage to proteins, membranes, and DNA.[223] Size is therefore a key factor in determining the potential toxicity of a particle. Other factors influencing toxicity include shape, chemical composition, surface structure, surface charge, aggregation, and solubility.[224]

As with many nanomaterials, the toxicity of nano-silver is greater than that of silver in bulk form; furthermore, nano-silver is considerably more toxic then other metal nanoparticles.[225] At the very small nanometer size the particles' surface area is exponentially large comparative to its volume. The comparatively large surface area of nanoparticles increases their reactivity, which in many instances also increases toxicity. For example, one study showed that the interaction with the HIV-I virus is highly size dependent, with silver nanoparticles in the 1-10nm

---

[219] EPA White Paper, supra note 41 at 38.
[220] Institute of Occupational Medicine for the Health and Safety Executive, *Nanoparticles: An occupational hygiene review* (2004).
[221] Nel A et al., *Toxic potential of materials at the nanolevel* 311 SCIENCE 622-627 (2006).
[222] Oberdörster G et al., *Nanotoxicology: an emerging discipline from studies of ultrafine particles*, 113 (7) ENVIRONMENTAL HEALTH PERSPECTIVES 823-839 (2005).
[223] Nel A et al., *Toxic potential of materials at the nanolevel* 311 SCIENCE 622-627 (2006).
[224] Nel A et al., *Toxic potential of materials at the nanolevel* 311 SCIENCE 622-627 (2006).
[225] Braydich-Stolle, L et al., *In Vitro Cytotoxicity of Nanoparticles in Mammalian Germline Stem Cells*, 88(2): TOXOCOLOGICAL SCIENCES 412–419 (2005).

range exclusively attaching to the virus and consequently inhibiting it from binding to hosts cells.[226]

Moreover, because nano-silver has a greater surface area than larger particles of silver, nano-silver is more chemically reactive and more readily ionized than silver in larger particle form. Nano-silver therefore has greater antibacterial and toxic effects compared to larger silver particles partly because it is more readily converted to silver ions, which are extremely toxic to fish and other aquatic species.[227]

There is also preliminary evidence that the nano-silver can exert effective antibacterial action at a considerably lower concentration than silver ions.[228] This suggests that the antibacterial properties and toxicity of nano-silver are not explained only by its chemical composition and the production of ions alone. Physical characteristics of nanomaterials, such as their size, shape, and surface properties, can exert a toxic effect that goes beyond that associated with their chemical composition.[229] For example one study demonstrated that nano-silver produces reactive oxygen species (ROS) and this can result in oxidative stress-mediated toxicity.[230] Production of ROS, highly reactive molecules which include free radicals, can interfere with cellular metabolism, cause inflammation, and damage proteins, membranes and DNA. ROS production is a key mechanism of nanomaterials' toxicity.[231]

---

[226] Jose Luis Elechiguerra et al., *Interaction of silver nanoparticles with HIV-1*, JOURNAL OF NANOBIOTECHNOLOGY 3:6 (2005), at http://www.jnanobiotechnology.com/content/3/1/6

[227] Hogstrand et al., *The Acute and Chronic Toxicity of Silver to Marine Fish*, Proceedings of the 5th International Conference on the Transport, Fate and Effects of Silver In the Environment, 317-324 (1997).

[228] Lok et al., *Proteomoic analysis of the mode of antibacterial action of silver nanoparticles*, 5 J. PROTEAME RES. 916-924 (2007).

[229] Brunner et al., *In Vitro Cytotoxicity of Oxide Nanoparticles: Comparison to Asbestos, Silica, and the Effect of Particle Solubility*, 40 ENVIRON SCI TECHNOL 4247-81 (2006).

[230] Hussain, S.M. et al., *In vitro toxicity of nanoparticles in BRL 3A rat liver cells*, 19 TOXICOLOGY IN VITRO 975–983 (2005).

[231] See, e.g., Andre Nel et al., *Toxic Potential of Materials at the Nanolevel*, 311 SCIENCE 622-27 (2006).

iii. Studies Show Nano-silver is Toxic to Mammalian Cells and Zebra fish

Numerous studies have shown not only the mobility of nano-silver but also the negative and toxic effects of nano-silver on mammalian cells. *In vitro* (test tube) studies demonstrate that nano-silver is toxic to mammalian liver cells,[232] stem cells[233] and even brain cells.[234] An overwhelming majority of studies reported abnormalities in basic cell functions as a result of nano-silver contact.[235] One study demonstrated the mobility of inhaled nano-silver after it concentrated in the lungs of rats and then followed systematic pathways throughout the body to enter the kidney, brain and heart.[236] In another study, C18-4 germline stem cells from mice exposed to nano-silver underwent dramatic structure changes and apoptosis, a form of cell self-destruction.[237] Silver carbonate had no significant cytotoxic effect on mitochondrial and cell functions while nano-silver caused extreme toxicity and reduced mitochondrial function and cell viability.[238]

Other studies confirmed that cells treated with nano-silver had decreased mitochondrial function and additionally reported that cells shrank and developed irregular shapes.[239]

---

[232]Hussain, S.M. et al., *In vitro toxicity of nanoparticles in BRL 3A rat liver cells*, 19 TOXICOLOGY IN VITRO 975–983 (2005).

[233]Braydich-Stolle, L et al., *In Vitro Cytotoxicity of Nanoparticles in Mammalian Germline Stem Cells*, 88(2): TOXOCOLOGICAL SCIENCES 412–419 (2005).

[234]Hussain, S.M et al., *The Interaction of Manganese Nanoparticles with PC-12 Cells Induces Dopamine Depletion*, 92(2) TOXOCOLOGICAL SCIENCES 456–63 (2006).

[235]Hussain et al. *In Vitro Toxicity of Nanoparticles in BRL 3A Rat Liver Cells*, 19 TOXICOLOGY IN VITRO 977-978, (2005); Stolle et al. *In Vitro Cytotoxicity of Nanoparticles in Mammalian Germline Stem Cells*, 88 TOXICOLOGICAL SCIENCES 412 (2005).

[236] Health & Safety Laboratory, *Health & Safety Executive NanoAlert Service*, http://www.hse.gov.uk/horizons/nanotech/nanoalert001.pdf , December 2006 at p.26.

[237]Stolle et al. *In Vitro Cytotoxicity of Nanoparticles in Mammalian Germline Stem Cells*, 88 TOXICOLOGICAL SCIENCES 412, 414 (2005).

[238]Id.

[239]Hussain et al. *The Interaction of Manganese Nanoparticles with PC-12 Cells Induces Dopamine Depletion*, 92 TOXICOLOGICAL SCIENCES 456, at 460 (2006); Hussain et al. *In Vitro Toxicity of Nanoparticles in BRL 3A Rat Liver Cells*, 19 TOXICOLOGY IN VITRO 977-978, (2005).

Additional research showed that nano-silver agglomerated in cell cytoplasm and fully permeated cell membranes.[240]

Similar studies performed on zebra fish demonstrated that nano-silver could diffuse into developing embryos and affect embryonic development.[241] Zebra fish are commonly used in human drug studies because their protein sequences are similar to humans.[242] Such similarities indicate the potential risks for human embryonic development if exposed to nano-silver. In all studies nano-silver was the most toxic and damaging when tested against several other metal nanoparticles.[243]

Similarly, a study investigating the cytotoxicity of silver nanoparticles in mammalian germline stem cells showed that silver nanoparticles were more toxic than other metal oxides.[244] The authors of the study also pointed out that while silver nanoparticles are proposed to be used as antimicrobial agents in bone cement or other implantable devices, they may in fact be toxic to the bone-lining cells and other tissues.[245] Silver nanoparticles significantly reduced mitochondrial function and interfered with cell metabolism leading to cell leakage. Furthermore, the significant toxicity of silver nanoparticles on mammalian germline stem cells (mice testes) indicates the potential of these particles to interfere in general with the male reproductive system. These findings are of significant practical implications because nano-silver is now available via a

---

[240]Skebo et al. *Assessment of Metal Nanoparticle Agglomeration, Uptake, and Interaction Using High-Illuminating System*,26 INTERNATIONAL JOURNAL OF TOXICOLOGY 135 (2007).
[241]Lee et al. *In Vivo Imaging of Transport and Biocompatibility of Single Silver Nanoparticles in Early Development of Zebrafish Embryos*, 1 ACS NANO 133, 141 (2007).
[242]Id. at 134.
[243]Hussain et al. *The Interaction of Manganese Nanoparticles with PC-12 Cells Induces Dopamine Depletion*, 92 TOXICOLOGICAL SCIENCES 456, at 460 (2006); Hussain et al. *In Vitro Toxicity of Nanoparticles in BRL 3A Rat Liver Cells*, 19 TOXICOLOGY IN VITRO 977-978, (2005); Stolle et al. *In Vitro Cytotoxicity of Nanoparticles in Mammalian Germline Stem Cells*, 88 TOXICOLOGICAL SCIENCES, (2005) at 418; Chen and Schluesener, *Nanosilver: A nanoproduct in medical application*, 176 TOXICOLOGY LETTERS 1-12, 7 (2008).
[244]Braydich-Stolle, L et al., *In Vitro Cytotoxicity of Nanoparticles in Mammalian Germline Stem Cells*, 88(2): TOXOCOLOGICAL SCIENCES 412–419 (2005).
[245]Id.

variety of commercialized products, including contraceptive devices and maternal hygiene items. Fertility problems may occur.[246] These studies establish the risk and toxicity of nano-silver in mammalian animals and denote the possible hazards of nano-silver in humans.

iv. Human Health: Nano-silver Promotes Bacterial and Antibiotic Resistance

Nano-silver poses a unique threat to humans in the form of bacterial and antibiotic resistance. Nano-silver is an antimicrobial biocide that can kill or inhibit the growth of microbes.[247] Certain harmful bacteria may become resistant against nano-silver. In addition, because of the type of resistance mechanism developed, the harmful bacteria could develop resistance to 50% of currently used antibiotics.[248]

Silver resistance genes have been found in some large plasmids (a small ring of genetic material) that also carry several genes that encode for antibiotic resistance. Carrying plasmids is energy intensive so bacteria may lose plasmids that are unnecessary. Yet, with increased silver exposure, bacteria are encouraged to retain plasmids with silver and antibiotic resistant genes, increasing the potential for antibiotic resistance.[249]

"Silver can …constitute a part of selective pressure and may actively contribute to the spread of antibiotic resistance. Silver resistance associated with antibiotic resistance has been observed in isolated bacteria from birds and in salmonella spp."[250] It can also be induced under laboratory conditions, and "is most easily developed in bacteria with already documented resistance mechanisms to antibiotics, such as methicillin-resistant *Staphylococcus aureus*

---

[246] Chen and Schluesener, *Nanosilver: A nanoproduct in medical application*, 176 TOXICOLOGY LETTERS 1-12, 7 (2008).
[247] See generally Sass, Jennifer. *Nanotechnology's Invisible Threat: Small Science, Big Consequences*, NRDC, at 3 (May 2007).
[248] Melhus, A, *Silver threatens the use of antibiotics*, Unpublished manuscript, (on file with author) (2007).
[249] Melhus, A, *Silver threatens the use of antibiotics*, Unpublished manuscript, (on file with author) (2007).
[250] Id.

(MRSA), vancomycin- resistant enterococci (VRE), enterobacteria with production of extended spectrum beta-lactamases (ESBL), multiresistant *Pseudomonas aeruginosa*."

Thomas O'Brien of Harvard Medical School states that, "antimicrobial-resistance genes and their genetic vectors, once evolved in bacteria of any kind anywhere, can spread indirectly through the world's interconnecting commensal, environmental, and pathogenic bacterial populations to other kinds of bacteria anywhere."[251] The widespread introduction of nano-silver into consumer products could thus contribute significantly to the spread of antibiotic resistance throughout the world. Uncertainties about silver and resistance prompted Swedish pharmacies to stop selling band-aids containing silver in April 2006.[252]

v. *Environmental Impacts: Environmental Exposures and Impacts on Beneficial Bacteria*

As a powerful bactericide, when released into the environment nano-silver particles threaten bacteria-dependent processes that underpin ecosystem functions. The release of nano-silver from consumer products into the environment is inevitable after products degrade and/or are thrown away. Exposures will also come from use: a recent study[253] by Arizona State scientists found that socks impregnated with nano-silver released substantial amounts of the nano-silver when washed in both nanoparticle and ionic forms.[254] The study suggested that nano-silver could travel through a wastewater treatment system and enter natural waterways to

---

[251] O'Brien, Thomas F., *Emergence, Spread, and Environmental Effect of Antimicrobial Resistance: How Use of an Antimicrobial Anywhere Can Increase Resistance to Any Antimicrobial Anywhere Else*, 34 (Suppl 3) CID S78, (2002).
[252] Sandquist, Anna, *Swedish Pharmacies Ban Silver Band-Aids*, 3 MILJOAKTUELLT April 2006.
[253] Benn and Westerhoff, *Nanoparticle Silver Released in Water from Commercially Available Sock Fabrics*, Arizona State University, presentation for EMPA nanoECO conference, Ascona, Switzerland, March 3, 2008; forthcoming in ENVIRONMENTAL SCIENCE & TECHNOLOGY.
[254] Rachel Petkewich, *Toxic Socks: Silver nanoparticles intended to control odor release in the wash*, CHEMICAL AND ENGINEERING NEWS, April 7, 2008.

impact aquatic organisms.[255] The study was the first to examine how nanomaterials are released during laundering from commercially available clothing.[256] As discussed infra, nanomaterials can be extremely mobile and can travel large distances in air and water which could have impacts in areas far away from their area of release.[257]

Beneficial bacteria are important for soil, plant and animal health.[258] Once these nanomaterials are released into the environment, their biocidal activity is harmful and potentially deadly to beneficial microbes like bacteria and fungi, and may cause disturbances to critical ecosystems and ecological food webs.[259] Some researchers suggest that nano-silver could damage bacterial cells by destroying the enzymes that transport the cell nutrient and weakening the cell membrane or cell wall.[260] Other researchers believe nano-silver destroys the ability of the bacteria's DNA to replicate. [261]

A recent study provided one example of nano-silver's damage to beneficial bacteria: a 2008 University of Missouri study has found that nano-silver also may destroy benign bacteria that are used to remove ammonia from wastewater treatment systems.[262] The study's authors

---

[255] ScienceDaily, As Nanotechnology Goes Mainstream, 'Toxic Socks' Raise Concerns; Unknown Risks from Nanosilver Cited, April 7, 2008, at http://www.sciencedaily.com/releases/2008/04/080406175050.htm

[256] Id.

[257] See infra pp. 89-90 and accompanying footnotes

[258] For example, bacteria form symbiotic relationships with all animals from insects to humans. Many of these bacteria aid their animal hosts to digest food, others perform more unusual functions. Antibiotic-producing bacteria protect the European beewolf (wasp) from pathogenic fungal infestation. Light- producing bacteria help the Hawaiian squid to camouflage itself from predators.

[259] It is nano-silver particles' increased surface area that is credited with enabling the highly effective destruction of bacteria and other microbes. The actual mechanism by which nano-silver particles interfere with bacteria is as unknown.

[260] In their study of *E. coli* bacteria, Sondi and Salopek-Sondi found that nanosilver damaged and pitted the bacteria's cell walls and accumulated in the cell wall, leading to increased cell permeability and ultimately cell death. Soni, I.and Salopek-Bondi, B, *Silver nanoparticles as antimicrobial agent: a case study on E.coli as a model for Gram negative bacteria*, 275(1) J.COLLOID INTERFACE SCIENCE 1770-82 (2004). *E. coli* is often used as a model for gram negative bacteria, suggesting that these results could be more broadly relevant.

[261] Berger, M. (2007), *Stabilizing antimicrobial nanosilver on a natural porous plant material*, Nanowerk, January 18, 2007, at http://www.nanowerk.com/spotlight/spotid=1276.php.

[262] Choi et al., *The inhibitory effects of silver nanoparticles, silver ions, and silver chloride on microbial growth*, WATER RESEARCH (2008), doi:10.1016/j.watres.2008.02.021

summarized: "that silver nanoparticles are extremely toxic. The nanoparticles destroy the benign species of bacteria that are used for wastewater treatment. It basically halts the reproduction activity of the good bacteria."[263] Further, the study concluded that nano-silver generates more highly reactive oxygen species than do larger forms of silver inhibit bacterial growth. This outcome could impact the use of wastewater treatment "sludge" as land-application fertilizer, which is common practice. If high levels of nano-silver are present in the sludge, soil used to grow food crops may be harmed.[264] The study concluded that "the results of nano-silver toxicity to environmentally sensitive nitrifying microorganisms suggest that stringent regulations of [nano-silver] entering [wastewater] are necessary."[265]

Nano-silver coatings have also been implicated in adverse environmental impacts which, "may result in enhanced interactions with bacteria, algae, and other microorganisms in the environment, and may result in bioaccumulation and possibly biomagnifications up the food chain."[266]

vi. Environmental Impacts: Soil

While limited scientific studies on the microbiological effects of nano-silver in soil systems have been conducted,[267] it is well-established that silver in its bulk form inhibits microbial growth in soils and has the ability to disrupt denitrification processes.[268] Denitrification is a bacteria-driven process, where nitrates are converted to nitrogen gas in some

---

[263] *Too much technology may be killing beneficial bacteria*, Nanowerk, April 29, 2008, at http://www.nanowerk.com/news/newsid=5520.php

[264] Id.

[265] Choi et al., *The inhibitory effects of silver nanoparticles, silver ions, and silver chloride on microbial growth*, WATER RESEARCH (2008), doi:10.1016/j.watres.2008.02.021, at 8.

[266] Mowat et al., *Nanotechnology and the Water Market: Applications and Health Effects*.

[267] Senjen, Rye, *Nanosilver- a threat to water, soil and human health?*, Friends of the Earth Australia, March (2007).

[268] Throback et al., *Silver (Ag+) reduces denitrification and induces enrichment of novel nirK genotypes in soil*, 270 FEMS MICROBIOL LETT 189, (2007); Finnsson, A. et al., *Two Approaches to Prevent Bio Film in Modern Household Washing Machines*, at 10 (June 2006) (on file with author).

soils, wetlands and other wet environments. For example, denitrification bacteria play an important role in removing nitrate from water contaminated by excessive fertilizer use. Denitrification is also important because excess nitrates reduce plant productivity, can result in eutrophication in rivers, lakes and marine ecosystems, and are a drinking water pollutant.

In situ studies have demonstrated that silver, even in larger particle form, inhibits microbial growth at concentrations below that of other heavy metals.[269] It is especially toxic to heterotrophic (ammonifying/ nitrogen fixing) and chemolithotrophic bacteria. Chemolithotropic bacteria belong to the lithotropic family of microbes and consume inorganic material. These organisms liberate many crucial nutrients, and are essential in the formation of soil.[270]

vii. Environmental Impacts: Bioaccumulation

The persistence of nanomaterials and their potential for bioaccumulation is poorly understood, however early studies suggest that microorganisms and plants may be able to produce, modify and concentrate nanoparticles that can then bioaccumulate (or even biomagnify) along the food chain.[271] Once absorbed the nanoparticles may travel up the food chain to larger animals in a similar way to mercury. Mercury is a toxic pollutant that concentrates in marine ecosystems and has the well-known and documented ability to bioaccumulate and biomagnify at all trophic levels in the food web. Mercury is absorbed by micro-organisms which are then consumed by larger organisms. This allows the chemical to continue to be passed along the food chain and in the process increasing in concentration. In large animals, birds and humans mercury concentrations can reach toxic concentrations and may cause birth defects, neurological

---

[269] Murata et al. (2005), *as cited by* Throwback et al., *Silver (Ag(+)) reduces denitrification and induces enrichment of novel nirK genotypes in soil*, FEMS MICROBIOL LETT. (Jan 2007).

[270] http://soils.usda.gov/sqi/concepts/soil_biology/bacteria.html

[271] Tran C, Donaldson K et al., *A scoping study to identify hazard data needs for addressing the risks presented by nanoparticles and nanotubes* Research Report. Institute of Occupational Medicine, Edinburgh (2005).

disorders and death. The deadly effects of mercury were first discovered and publicized in Minimata, Japan, after causing severe disabilities and death among people eating seafood contaminated through industrial mercury discharge, which had accumulated through the food chain.[272] Given how mercury has negatively affected the environment and human health in the past, the potential biological magnification caused through mass manufacturing and disposal of nanomaterials, such as nano-silver, are a definitive possibility that must be investigated and if found to occur addressed. The impact of nanomaterial exposure on plant growth also remains largely uninvestigated; however, high levels of exposure to nanoscale aluminium have been found to stunt root growth in five plant species.[273] No such studies have been performed on silver nanoparticles.

The NACWA and Tri-Tac letters to EPA pointed out that widespread use of household products like the Samsung washing machine will increase the release of nano-silver into sanitary sewer systems.[274] This in turn will greatly increase nano-silver concentrations in treatment-plant discharges, leading to adverse effects, such as bioaccumulation in fish and the killing of aquatic life. It is also possible that nanoparticles, persistent organic pollutants, and other hazardous metals may form associations and spread together, thereby amplifying their toxicity.[275]

---

[272]Booth et al., *Mercury, Food Webs, and Marine Mammals: Implications of Diet and Climate Change for Human Health.* 113(5) ENVIRONMENTAL HEALTH PERSPECTIVES 521–526 (2005) at http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1257541

[273]Yang L. et al., *Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles*, 158 (2) TOXICOL LETT. 122-32 (2005).

[274]Letter from Ken Kirk, Executive Director, National Association of Clean Water Agencies, to Stephen Johnson, Administrator, Environmental Protection Agency (February 14, 2006) (on file with author); Letter from Chuck Weir, Chair, Tri-TAC, to James Jones, Director, Office of Pesticide Programs, Environmental Protection Agency (January 27, 2006) (on file with author).

[275]Tang, H.; Wang, D.; Ge, X., *Environmental nano-pollutans and aquatic micro-interfacial processes*, 50(12), WATER SCI TECHNOL 103-9(2004).

viii. Human Health: Nano-Silver May Adversely Impacts Human through Ingestion and othe Unknown Exposures

Very little attention has been given to the study of nano-silver's potential human health impacts, such as their entry portals into the human body, biodistribution, potential to accumulate in organs as well as their potential interactions with tissues, cells and molecules and their relevant toxicological implications.[276] As discussed above, exposure to nano-silver in the body is becoming increasingly widespread and invasive. Consequently, nano-silver has gained an increasing access to tissues, cells, and biological molecules within the human body.[277] At least one study has noted that the traditional assumptions about silver being only a minimal health risk may not be alone sufficient because "once reaching the nano-scale, certain materials do exhibit significant toxicity to mammalian cells even if they are biochemically inert and biocompatible in bulk size," like carbon.[278]

Ingestion of colloidal silver (a suspension of silver in microparticles and/ or nanoparticles in a gelatinous base) has been linked to neurological problems, kidney damage, stomach upset, headaches, fatigue, and skin irritation. [279] One study demonstrated that silver atoms present in drinking water for purification purposes can accumulate in the cerebellum "which is critical for the motor coordination and functional efficiency of the locomotion system", and oxidative muscle tissue, including the hearts, of rats. The study exposed rats to silver concentrations three times *lower* than the World Health Organization maximum level for drinking water

---

[276] Chen and Schluesener, *Nanosilver: A nanoproduct in medical application*, 176 TOXICOLOGY LETTERS 1-12, 2 (2008).
[277] Chen and Schluesener, *Nanosilver: A nanoproduct in medical application*, 176 TOXICOLOGY LETTERS 1-12, 2 (2008).
[278] Id.
[279] White JM, Powell AM, Brady K, *Severe generalized argyria secondary to ingestion of colloidal silver protein*, 28(3) Clinical and Experimental Dermatology 254-56 (2003); Hori K, Martin TG, Rainey P, *Believe it or not--silver still poisons!*, 44(5) VETERINARY AND HUMAN TOXICOLOGY 291-292 (2002).

disinfection.[280] Considering the growing number of nano-silver water purification systems on the market and the demonstrated ability of silver to pass the blood brain barrier like nanoparticles, this study shows the potential for nano-silver to create similar effects.[281]

One product, a nano-silver coated dressing- Acticoat (Smith & Nephew, Inc.), has generated concern after a previously healthy teenager developed symptoms of hepatotoxicity and argyria symptoms as well as elevated liver enzymes and silver levels in plasma and urine.[282] Six days after treatment the patient developed grayish discoloration with blueish-lips (argyia) and elevated serum aspartate aminotransferase, alanine aminotransferase, and γ-galactosyl transferase without elevation of bilirubin, lactate dehydrogenase, or cholinesterase. The patient had elevated urinary (28 µg/kg) and serum (107 µg/kg) silver levels. Cessation of the nanoscale silver treatment resulted in an immediate decrease of the clinical signs of hepatotoxicity, argyria, and serum and urinary silver; however, serum and urinary levels of silver (42 and 2.3 µg/kg, respectively) were still elevated at 7 weeks.[283]

ix. Additional Research is Needed

One recent study specifically examined the potential of nano-silver coated consumer products to cause environmental damage in freshwater aquatic and terrestrial ecosystems.[284] Noting that there is strong growth potential in the number of nano-silver products in the near future, Blaser et. al conclude that by 2010 nearly 15% of all silver emissions in Europe will be

---

[280] Rungby, J., *An experimental study on silver in the nervous system and on aspects of its general cellular toxicity*. 37DANISH MED. BULL., 442-449 (1990); Pelkonen et al., *Accumulation of silver from drinking water into cerebellum and musculus soleus in mice*, 186 TOXICOLOGY, 151-157 (2003).

[281] Lloyd's of London, Risks: Lloyd's Emerging Risks Team Report, *Nanotechnology Recent Developments, Risks and Opportunities*, at 15, 2007.

[282] Trop et al. *Silver-Coated Dressing Acticoat Caused Raised Liver Enzymes and Argyria-like Symptoms in Burn Patient*, 60 JOURNAL OF TRAUMA-INJURY INFECTION & CRITICAL CARE 648 (2006).

[283] Id.

[284] Blaser et al., *Estimation of cumulative aquatic exposure and risk due to silver: contribution of nano-functionalized plastics and textiles*, 390 SCIENCE OF THE TOTAL ENVIRONMENT 396-409 (2008).

released from biocidal nano-silver products. The study specifically recognizes the prevalence of nano-silver particles imbedded into plastic matrixes and the ability of these plastics to break down in water over time. Additionally, the researchers raise concerns over nano-silver contamination in agricultural fields due to the spreading of sewage sludge and the potential for nano-silver products to decompose in landfills. The study strongly recommends additional research to examine "not only the aquatic exposure to silver from biocidal plastics and textiles…but also the impact on terrestrial ecosystems."[285]

3. Human Health and Environmental Impact Unknowns: EPA Should Require Additional Data from Manufacturers

The approval of a pesticide is contingent on an agency determination that no additional data are necessary to make the determinations required by FIFRA sec. 3(c)(5), including, *inter alia*, the determination that the product will not cause unreasonable adverse effects on the environment.[286] If more data is necessary, EPA should require manufacturers provide it. To perform its statutorily-mandated risk assessment for a pesticide, EPA needs information on the potential risks and benefits of a pesticide. While existing studies show potential risks regarding nanomaterials and nano-silver, there are also many still-unexplored potential human health and environmental impacts that must be "imperatively answered before people rush to indulge in the nano-silver boom."[287]

"If information required generally is not sufficient to evaluate the potential of the product to cause unreasonable adverse effects on man or the environment, additional data

---

[285] Id. at 407.
[286] 40 C.F.R. § 152.112; 7 U.S.C. § 136a(c)(5)(D).
[287] Chen and Schluesener, *Nanosilver: A nanoproduct in medical application*, 176 TOXICOLOGY LETTERS 1-12, 2 (2008).

requirements will be imposed."[288] Therefore, to ensure it has all the data it needs on nano-silver to perform the risk assessments, EPA should require the necessary data from prospective registrants for nano-silver products.[289]

B. PURSUANT TO THE FQPA, EPA MUST ASSESS THE POTENTIAL IMPACTS OF NANO-SILVER ON INFANTS AND CHILDREN AND ENSURE THAT NO HARM WILL RESULT FROM AGGREGATE EXPOSURES

1. EPA Must Apply The Food Quality Protection Act to Nano-Silver Products

Enacted in 1996, the Food Quality Protection Act ("FQPA") amended the regulatory scheme set forth by FIFRA and the Federal Food Drug and Cosmetic Act ("FFDCA") for the movement of pesticides in interstate commerce.[290] The FQPA requires EPA to reevaluate its safety standards for all existing pesticide tolerances using scientific risk factors resulting from "anticipated dietary exposure and all other exposures for which there is reliable information."[291] Pursuant to the FQPA, before granting a tolerance EPA must assess the risks a pesticide poses to infants and children and "ensure that there is a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue."[292]

Among the FQPA requirements for tolerance level reassessment was a mandate for EPA to "apply a presumptive 'tenfold margin of safety in order to take into account potential pre- and post-natal toxicity and completeness of the data with respect to exposure and toxicity to infants and children.'"[293] The EPA Administrator may deviate from the tenfold factor only if, on the basis of reliable scientific data, such deviation is safe to infants and children.[294]

---

[288] 40 C.F.R. § 158.75(a).
[289] 7 U.S.C. § 136a.
[290] New York v. EPA, 350 F. Supp. 2d 429, 432 (S.D.N.Y. 2004); Croplife Am. v. EPA, 329 F.3d 876, 879 (D.C. Cir. 2003) (FQPA "substantially revised" and rewrote most of the FFDCA method for setting tolerances).
[291] Croplife, 329 F.3d at 879 (quoting 21 U.S.C. § 346(b)(2)(A)(ii)).
[292] 21 U.S.C. § 346a(b)(2)(C)(ii)(I).
[293] New York, 350 F. Supp. 2d at 432 (quoting 21 U.S.C. § 346a(b)(2)(C)); Am. Farm Bureau v. EPA, 121 F. Supp. 2d 84, 89 (D.C. Cir. 2000); NRDC v. Johnson, 461 F.3d 164, 168 (2d Cir. 2006) (noting new requirements

2. *EPA Must Assess the Health Risks of Nano-silver on Infants and Children and Set an Exposure Tolerance*

Before setting a tolerance for nano-silver, see Section VI(E) infra, EPA must assess the risks a pesticide poses to infants and children and "ensure that there is a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue.[295] Exposures include both dietary exposures and all other exposures for which there is reliable information."[296] EPA must "apply a presumptive 'tenfold margin of safety in order to take into account potential pre- and post-natal toxicity and completeness of the data with respect to exposure and toxicity to infants and children'" for nano-silver.[297]

The importance of the agency's application of, and compliance with, the FQPA's standards for child safety is underscored by the plethora of nano-silver children and infant products currently on market. As listed in Appendix A, these products include: children's stuffed animals and toys, strollers, baby bottle cleaner, baby textile softener, baby mug, infant teething toy, and baby milk bottle. See Appendix A. In addition, it is foreseeable that many household nano-silver products will also increase exponentially pre-natal, infant, and baby nano-silver exposures. As listed in Appendix A, these nano-silver products include: dietary supplements, bed sheets and pillows, bandages, soaps and personal care products, food storage containers, cutlery and cooking utensils, clothing, filters, washing machines and refrigerators, paints, sprays, cleaners, and bulk and powdered and liquid nano-silver in bulk form. See Appendix A. Healthcare and hygiene spray products containing nano-silver have entered daily

---

pertaining to the safety of several major subgroups of individuals); Physicians Comm. For Responsible Medicine v. EPA, 451 F. Supp. 2d 223, 226 (D.D.C. 2006) ("In other words, the pesticide manufacturer must show that the pesticide is ten times safer than the typical exposure limits for adults").

[294] New York, 350 F. Supp. 2d at 432.

[295] 21 U.S.C. § 346a(b)(2)(C)(ii)(I).

[296] Croplife, 329 F.3d at 879 (quoting 21 U.S.C. § 346(b)(2)(A)(ii)).

[297] See notes 288-89 supra.

use, raising concern of respiratory entry and potential effects.[298] These nano-silver products create dietary and skin exposures to infants and children that must be assessed.

With regard to nanomaterials generally, a growing number of peer-reviewed scientific studies have demonstrated both the potential for nanomaterials to present serious toxicity risks for human health[299] and the capacity for nanomaterials to penetrate the skin in at least some circumstances.[300] Research has shown that many types of nanomaterials can be toxic to human tissue and cell cultures, resulting in increased oxidative stress, inflammatory cytokine production, DNA mutation and even cell death.[301] Nanomaterials' small size confers greater particle mobility both in the environment and in the body.[302] Potential health concerns from nano-silver were addressed above, supra Section III(A)(2), and include *inter alia*, nano-silver toxicity and bacterial and antibiotic resistance concerns, as well as numerous unknowns. These include respiratory impacts from inhalation, as studies have noted the potential for nano-silver, like other nanomaterials, once inside the lungs, to "serve as an efficient facilitator of generation of radicals and ROS" due to their "enormous surface area."[303] Transdermal penetration for some

---

[298] Chen and Schluesener, *Nanosilver: A nanoproduct in medical application*, 176 TOXICOLOGY LETTERS 1-12, 3 (2008).

[299] For overviews of the emerging field of nanotoxicology, see Oberdörster G et al., *Nanotoxicology: an emerging discipline from studies of ultrafine particles*, 113(7) ENVIRONMENTAL HEALTH PERSPECTIVES 823-839 (2005); Oberdörster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, Carter J, Karn B, Kreyling W, Lai D, Olin S, Monteiro-Riviere N, Warheit D, and Yang H, *Principles for characterising the potential human health effects from exposure to nanomaterials: elements of a screening strategy*, 2:8 PARTICLE AND FIBER TOXICOLOGY (2005); Hoet P, Bruske-Holfeld I and Salata O, *Nanomaterials – known and unknown health risks* 2 JOURNAL OF NANOBIOTECHNOLOGY 12 (2004).

[300] See, e.g., Ryman-Rasmussen J, Riviere J, Monteiro-Riviere N, *Penetration of intact skin by quantum dots with diverse physicochemical properties*, 91 TOXICOLOGICAL SCIENCES (1):159-165 (2006); Tinkle S, Antonini J, Roberts J, Salmen R, DePree K, Adkins E, *Skin as a route of exposure and sensitisation in chronic beryllium disease*, 111 ENVIRONMENTAL HEALTH PERSPECTIVES 1202-1208 (2003).

[301] Oberdörster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, Carter J, Karn B, Kreyling W, Lai D, Olin S, Monteiro-Riviere N, Warheit D, and Yang H, *Principles for characterising the potential human health effects from exposure to nanomaterials: elements of a screening strategy*, 2:8 PARTICLE AND FIBER TOXICOLOGY (2005).

[302] Chen and Schluesener, *Nanosilver: A nanoproduct in medical application*, 176 TOXICOLOGY LETTERS 1-12, 3 (2008).

[303] Id.

nanomaterials (titanium dioxide, quantum dots) has been observed in studies, but there is no data for nano-silver.[304] The release of nano-silver from clothing fibers, underwear, socks, lingerie, hospital and lab gowns, under various real life conditions (sweating, laundering, broken skin) remains to be investigated.[305] "Dermal toxicity is still a topic of dispute and concern."[306] Other potential impacts include impacts on the liver, a major accumulation point of circulatory nano-silver[307] and interference with beneficial bacteria in the gut once ingested.[308]

In sum, in setting a nano-silver tolerance EPA must set a 10-fold margin of safety in setting the nano-silver tolerance and ensure that there is a reasonable certainty that no harm will result from aggregate exposure.

C. ANY EPA ACTIVITIES OR PROGRAMS REGARDING NANO-SILVER OVERSIGHT MUST COMPLY WITH ESA, INCLUDING NANO-SILVER PESTICIDE REGISTRATION, REQUIRE ESA SECTION 7 CONSULTATION

1. The Endangered Species Act (ESA)

The ESA obligates federal agencies "to afford first priority to the declared national policy of saving endangered species."[309] To that end, the ESA contains numerous substantive and procedural provisions designed to protect species listed as threatened or endangered under the Act.

One such provision, Section 7, requires federal agencies to "insure that any action authorized, funded, or carried out by such agency . . . is not likely to jeopardize the continued existence of [endangered or threatened species] or result in the destruction or adverse

---

[304] Id. at 5.
[305] Id.
[306] Id
[307] Id at 7.
[308] Lloyd's of London, Risks: Lloyd's Emerging Risks Team Report, *Nanotechnology Recent Developments, Risks and Opportunities*, at 15, 2007.
[309] Tenn. Valley Auth. v. Hill, 437 U.S. 153, 185 (1978).

modification of [critical] habitat."[310] Thus, before engaging in any type of activity that may have direct or indirect effects on endangered species or critical habitat, agencies must "consult" either the Fish and Wildlife Service ("FWS") or the National Marine Fisheries Service (NMFS) in order to evaluate the impact of such agency action.[311] FWS regulations implementing section §7(a)(2) state that such formal or informal consultation must be initiated whenever an agency determines its action may affect a listed species, and that ongoing actions must be re-evaluated when species that may be affect by those actions are listed.[312]

The Act's consultation provision applies to "activities or programs of any kind authorized, funded, or carried out, in whole or in part, by Federal agencies in the United States or upon the high seas."[313] The concept of agency action has been given broad application by the courts and agency regulations, including the promulgation of regulations, the granting of licenses, and actions directly or indirectly causing modifications to land, water, or air.[314] Other examples of activities include the creation of interim management strategies,[315] and ongoing activities and projects.[316] EPA must comply with ESA when acting under FIFRA. "FIFRA does not exempt EPA from complying with ESA requirements when EPA registers pesticides. Indeed, a pesticide registration that runs against the clear mandates of the ESA will most likely cause an unreasonable adverse effect on the environment under FIFRA."[317]

---

[310] 16 U.S.C. § 1536
[311] 16 U.S.C. § 1536(a)(2).
[312] 50 C.F.R. §§ 402.14, 402.16.
[313] 50 C.F.R. § 402.02.
[314] 50 C.F.R. § 402.02.
[315] Lane Cty Audubon Soc'y v. Jamison, 958 F.2d 290 (9th Cir. 1992).
[316] Klamath Water Users Protective Ass'n v. Patterson, 191 F.3d 1115 (9th Cir. 1999).
[317] Defenders of Wildlife v. EPA, 882 F.2d 1294, 1299 (8th Cir. 1989).

FWS regulations under the ESA require agencies to review their action "at the earliest possible time to determine whether any action may affect listed species."[318] The threshold for the requirement to make the determination of whether a particular agency action may affect a listed species is triggered where "an endangered or threatened species may be present in the area of the proposed action."[319]

2. *ESA applies to agency actions taken pursuant to FIFRA and EPA Must Comply with ESA Section 7 With Regard to Nano-Silver*

Any "agency action" EPA takes with regard to nano-silver triggers Section 7 Consultation procedures. This includes oversight programs, and ongoing activities and pesticide projects.[320] EPA should now, "at the earliest possible time" consult with the applicable wildlife agency to determine whether its actions regarding nano-silver may affect listed species.[321]

FIFRA does not exempt EPA from compliance with the ESA's requirements with regard to pesticides.[322] Rather, the statute's mandates apply to agency actions taken pursuant to FIFRA, including pesticide registrations and rescissions. In Washington Toxics Coalition v. EPA, EPA argued that it was bound to follow only the provisions of FIFRA concerning the registration of 54 pesticide active ingredients that plaintiff environmental coalitions argued might harm endangered or threatened salmon in the waters of the Pacific Northwest.[323] EPA argued that the ESA's Section 7 Consultation requirements did not confer independent responsibilities on EPA. The Ninth Circuit disagreed, holding that EPA was not relieved of its obligations to comply with

---

[318] 50 C.F.R. § 402.14(a).
[319] City of Sausalito v. O'Neill, 386 F.3d 1186, 1215 (9th Cir. 2004); Pacific Rivers Council v. Thomas, 30 F.3d 1050, 1055 (9th Cir. 1994) (agency actions 'may affect' the protected salmon where "the plans set forth criteria for harvesting resources within the salmon's habitat").
[320] Klamath Water Users Protective Ass'n v. Patterson, 191 F.3d 1115 (9th Cir. 1999).
[321] 50 C.F.R. § 402.14(a).
[322] Wash. Toxics Coal. v. EPA, 413 F.3d 1024, 1032 (9th Cir. 2005); Defenders of Wildlife v. EPA, 882 F. 2d 1294, 1299 (8th Cir. 1989).
[323] Wash. Toxics Coal, 413 F.3d at 1028; see also Defenders of Wildlife v. EPA, 882 F.2d 1294 (8th Cir. 1989) (EPA's continued registration of strychnine pesticides effected a taking of endangered species).

the ESA by its compliance with FIFRA: "We agree with the Eighth Circuit that even though EPA registers pesticides under FIFRA, it must also comply with the ESA when threatened or endangered species are affected."[324] EPA was required to engage in ESA Section 7 consultation with the National Marine Fisheries Service (NFMS, now NOAA Fisheries) before engaging in pesticide registration. Further, EPA's obligation to comply with the ESA is "continuing" since the agency retains ongoing authority to register pesticides, alter registrations for reasons that include environmental concerns, and cancel registrations.[325]

3. Nano-silver Causes Adverse Environmental Exposures

The proliferation of nano-silver products makes it increasing likely that protected species and their critical habitat may be affected by the increasing release of these materials. The nano-silver products listed in Appendix A create numerous foreseeable direct and indirect environmental exposures. Some nano-silver products will enter the environment directly over the course of the products' use, including: washing machine waste water, laundry detergents and fabric softeners, multipurpose, bathroom, kitchen, and automobile cleaning products, soaps, cleaning and sanitizing sprays and wipes, personal care products, dietary supplements, and powdered and liquid nano-silver in bulk form. See Appendix A. Other nano-silver products will enter the environment at the end of their use during disposal, including brushes, straighteners, and other hair appliances, bandages, food storage containers, pet accessories, various fabrics and fibers, razors and shaving accessories refrigerators, electronics, and other household appliances.

---

[324] Id. at 1032 ("The statutes at issue in this case similarly have different but complementary purposes. FIFRA utilizes a cost-benefit analysis to ensure that there is no unreasonable risk created for people or the environment from a pesticide, taking into account the economic, social, and environmental costs and benefits of a pesticide's use. *Headwaters, Inc.*, 243 F.3d at 532. In contrast, the ESA affords endangered species the "highest of priorities" in assessing risks and benefits. Tennessee Valley Auth. v. Hill, 437 U.S. 153, 174 (1978). The reasoning of our case law therefore leads us to conclude that an agency cannot escape its obligation to comply with the ESA merely because it is bound to comply with another statute that has consistent, complementary objectives.") .

[325] Id. at 1033.

Id. Still other nano-silver products will indirectly leach nano-silver into the environment over the course of their use and cleaning and/or washing including numerous types of clothing such as underwear, socks, shirts, outerwear, gloves and hats, bedding, sheets, and pillows, and air and water purifiers and their replacement filters. Id. A recent study[326] by Arizona State scientists found that socks impregnated with nano-silver released substantial amounts of the nano-silver when washed in both nanoparticle and ionic forms.[327] The study suggested that nano-silver could travel through a wastewater treatment system and enter natural waterways to impact aquatic organisms.[328] The study was the first to examine how nanomaterials are released during laundering from commercially available clothing.[329]

These products will continue to enter the environment through product manufacture, transport, use, and disposal pathways. Because these products are household consumer products available on market shelves across the country, nano-silver environmental disposals and releases will occur nationwide. Many of the nano-silver products are in "free" particle form (such as creams, lotions, sprays), rather than "fixed" in a product matrix, speeding up ecosystem interactions. Even if they are in a product matrix nanomaterials are "highly durable" and will remain in nature long after the disposal of their host products.[330] It is unknown how quickly these materials will leech or dissolve into the environment as the product is washed, broken, or thrown away. These disposals will lead to greater environmental exposures by natural systems

---

[326] Benn and Westerhoff, *Nanoparticle Silver Released in Water from Commercially Available Sock Fabrics*, Arizona State University, presentation for EMPA nanoECO conference, Ascona, Switzerland, March 3, 2008; forthcoming in ENVIRONMENTAL SCIENCE & TECHNOLOGY.

[327] Rachel Petkewich, *Toxic Socks: Silver nanoparticles intended to control odor release in the wash*, CHEMICAL AND ENGINEERING NEWS, April 7, 2008.

[328] ScienceDaily, *As Nanotechnology Goes Mainstream, 'Toxic Socks' Raise Concerns; Unknown Risks from Nanosilver Cited*, April 7, 2008, at http://www.sciencedaily.com/releases/2008/04/080406175050.htm

[329] Id.

[330] Andrew Maynard, *Nanotechnology: A Research Strategy for Addressing Risk*, Woodrow Wilson International Center for Scholars, Project on Emerging Nanotechnologies, at 12 (July 2006).

than those of larger discarded materials since nanoparticles have the ability to reach places that larger particles cannot. Because of their tiny size, nanoparticles move with great speed through aquifers and soils and settle more slowly than larger particles. In addition, because of their large surface area, nanoparticles provide a large and active surface for interacting with and absorbing other materials. The foreseeable result will be a large and quickly increasing aggregate environmental exposure of protected species and their habitat to nano-silver discharges.

4. Nano-silver Causes Environmental Impacts and Potentially Impacts Protected Species

In addition to the potential environmental impacts discussed *infra*, many protected species are potentially impacted by the nano-silver product explosion. For example, it is well-established that silver in larger forms is highly toxic to fish, aquatic invertebrates and estuarine organisms.[331] Products containing silver are not to be applied to marine/estuary environments or oil fields. As explained above, among other nano-specific properties, nano-silver's exponentially increased surface area makes it even more dangerous to these species. Nano-silver therefore has greater antibacterial and toxic effects compared to larger silver particles partly because it is more readily converted to silver ions. There is also preliminary evidence that the nano-silver can exert effective antibacterial action at a considerably lower concentration than silver ions.[332] This suggests that the antibacterial properties and toxicity of nano-silver are not explained only by their chemical composition and the production of ions alone. As EPA has noted, "nanomaterials may affect aquatic or terrestrial organisms differently than larger particles

[331] EPA, Silver RED, supra note 162 at 4.
[332] Lok et al., *Proteomoic analysis of the mode of antibacterial action of silver nanoparticles*, 5 J. PROTEAME RES. 916-924 (2007).

of the same materials" and that "the use of nanomaterials in the environment may result in novel by-products or degradates that also may pose risks."[333]

There are 139 listed species of ESA-protected fish (65 Threatened and 74 Endangered) potentially negatively impacted by widespread nano-silver releases and individual and cumulative exposures.[334] Similarly, there are 70 listed species of protected claims (8 Threatened and 62 Endangered), and 22 listed species of protected crustaceans (3 Threatened and 19 Endangered) also potentially negatively impacted by nano-silver releases and exposures.[335] Finally, there are at least 10 water-based protected reptiles (6 Threatened and 4 Endangered) and 15 water-based mammals (4 Threatened and 11 Endangered) potentially negatively impacted by nano-silver releases and exposures.[336]

Unfortunately, despite rapid nanomaterial commercialization, many potential environmental risks of nanomaterials such as nano-silver remain dangerously untested due to the failure to prioritize relevant research and paucity of funding for environmental impact research. However some extrapolations from the known risks of silver are helpful to show potential risks to species. It is well-known that silver is among the most toxic metals for aquatic organisms.[337] The highly toxic levels generally have been considered to result from the presence of the free silver ion in water.[338] Because nano-silver has a greater surface area than larger particles of silver, nano-silver is more chemically reactive and more readily ionized than silver in larger particle form. Nano-silver therefore has greater antibacterial and toxic effects compared to larger

---

[333] EPA White Paper, supra note 41 at 58.
[334] See http://www.fws.gov/endangered/wildlife.html#Species
[335] Id.
[336] See http://www.fws.gov/endangered/wildlife.html#Species
[337] Fisher et al., *Trophic Transfer of Silver to Marine Herbivores: A Review of Recent Studies*, 17 ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY 562-571 (1998).
[338] Call et al., Toxicity *of Silver in Water and Sediment To the Freshwater Amphipod Hyallella Azteca*, 25 ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY 1802-08 (2006).

silver particles, partly because it is more readily converted to silver ions. Thus, 139 federally protected species of fish, as well as other protected aquatic species, are potentially at risk from widespread and cumulative nano-silver releases.

Free silver ions are extremely toxic to fish.[339] For example, studies have shown the severe toxicity of silver to juvenile rainbow trout,[340] a fish closely related to several endangered members of the *Salmonidae* family (trout and salmon). The *Salmonidae* family includes numerous distinct population segments of pacific salmon (Chinook, Sockeye, Chum, and Coho), atlantic salmon, and trout (steelhead, bull, gila, cutthroat and others), collectively representing at least 40 different federally protected fish species,[341] with critical habitats from coast to coast.

Silver is also toxic to aquatic invertebrates[342] such as sea urchins[343] and amphipods.[344] Studies have shown that the young life stages of numerous marine and estuarine life forms such as mollusks (e.g, clams, snails) and crustacean (e.g., lobsters) are highly susceptible to silver toxicity.[345] There are 75 federally protected members of the snail species,[346] 70 different protected clam species,[347] five members of the amphipod family,[348] and four members of the crayfish family.[349]

---

[339]Hogstrand et al., *The Acute and Chronic Toxicity of Silver to Marine Fish*, Proceedings of the 5th International Conference on the Transport, Fate and Effects of Silver In the Environment, 317-324 (1997).
[340] Naddy et al., *Effects of Sodium Chloride on Chronic Silver Toxicity to Early Life Stages of Rainbow Trout*, 26 ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY 1717-25 (2007).
[341] http://ecos.fws.gov/tess_public/SpeciesReport.do?groups=F&listingType=L&mapstatus=1
[342]Naddy et al., *Chronic Toxicity of silver nitrate to Ceriodaphnia dubia and Daphnia magna, and potential mitigating factors*, 84 AQUATIC TOXICOLOGY 1-10 (2007).
[343]Ward et al., *Chronic Toxicity of Silver to the Sea Urchin*, 25 ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY 1568-73 (2006).
[344] Call et al., Toxicity *of Silver in Water and Sediment To the Freshwater Amphipod Hyallella Azteca*, 25 ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY 1802-08 (2006).
[345] Luoma et al., *Fate, Bioavailability, and Toxicity of Silver in Estuarine Environments*, 31 MARINE POLLUTION BULLETIN 44-54, Table 1 (1995).
[346] http://ecos.fws.gov/tess_public/SpeciesReport.do?groups=G&listingType=L&mapstatus=1
[347] Idhttp://ecos.fws.gov/tess_public/SpeciesReport.do?groups=F&listingType=L&mapstatus=1
[348] http://ecos.fws.gov/tess_public/SpeciesReport.do?groups=K&listingType=L&mapstatus=1
[349] http://ecos.fws.gov/tess_public/SpeciesReport.do?groups=K&listingType=L&mapstatus=1

Additionally, low levels of silver when ingested can be toxic to both marine and freshwater zooplankton.[350] These are important components of marine and freshwater food webs since they are the primary grazers in many ecosystems and are often the major food source for developing larvae and fish.[351] Contaminant impacts on these animals are important because they can affect food web structures by altering the grazing on phytoplankton communities and affecting the food supply of predators and/or impact the critical habitat of protected species.

4. *Conclusions Made for Bulk Silver Are Not Sufficient To Protect Species from Nano-Silver Releases*

Conclusions of potential species' safety and/or the lack of a need for ESA consultation with regard to bulk silver are inadequate for nano-silver releases. First, as explained above, nanomaterials such as nano-silver require a specific nanotoxicology analysis; a bulk materials toxicity assessment is not alone sufficient.[352] In addition, the nano-silver product explosion is creating a vastly increased aggregate environmental exposure than previous releases of bulk silver. One reason that EPA concluded, in the 1993 Silver Re-registration Eligibility Document, that it did not expect "unreasonable adverse effects" on aquatic organisms from silver was because only "*little exposure* to fish and aquatic invertebrates is expected from these uses" and that "the agency does not expect unreasonable adverse effects *from these uses*."[353] In contrast, nano-silver products are creating many more opportunities for exposure from increased and different uses/products, as listed above and in Appendix A. Thus, EPA's 1993 conclusion of no unreasonable adverse effects is inadequate for a plethora of 2008 products of nano-silver.

---

[350] Fisher et al., *Silver Accumulation and Toxicity in Marine and Freshwater Zooplankton*, PROCEEDINGS OF THE 5TH ANNUAL CONFERENCE ON THE TRANSPORT, FATE, AND EFFECTS OF SILVER IN THE ENVIRONMENT, pp. 265-274 (1999).

[351] Hook et al., *Sublethal Effects of Silver in Zooplankton: Importance of Exposure Pathways and Implications for Toxicity Testing*, 20 ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY 568-574 (2001).

[352] See pp. 9-11, 43-46 supra and accompanying footnotes.

[353] EPA, Silver RED, supra note 162 at 17 (emphases added).

Moreover, because nano-silver has a greater surface area than larger particles of silver, nano-silver is more chemically reactive and more readily ionized than silver in larger particle form. Nano-silver therefore has greater antibacterial and toxic effects compared to larger silver particles partly because it is more readily converted to silver ions, which are extremely toxic to varied protected species.

There is also preliminary evidence that nano-silver can exert effective antibacterial action at a considerably lower concentration than silver ions.[354] This suggests that the antibacterial properties and toxicity of nano-silver are not explained by its chemical composition and the production of ions alone. Physical characteristics of nanomaterials, such as their size, shape, and surface properties, can exert a toxic effect that goes beyond that associated with their chemical composition.[355] For example one study demonstrated that nano-silver produces reactive oxygen species (ROS) and this can result in oxidative stress-mediated toxicity.[356] Production of ROS, highly reactive molecules which include free radicals, can interfere with cellular metabolism, cause inflammation, and damage proteins, membranes and DNA. ROS production is a key mechanism for nanomaterials' toxicity.[357]

5. EPA Must Comply with ESA Requirements

Accordingly, EPA must act as soon as possible to protect endangered and threatened species by complying with the ESA, including *inter alia* by consulting with the appropriate wildlife agency about the impacts on protected species of EPA's oversight actions, including *inter alia* any pesticide registration or classification decisions, for nano-silver.

---

[354] Lok et al., *Proteomoic analysis of the mode of antibacterial action of silver nanoparticles*, 5 J. PROTEAME RES. 916-924 (2007).
[355] Brunner et al., *In Vitro Cytotoxicity of Oxide Nanoparticles: Comparison to Asbestos, Silica, and the Effect of Particle Solubility*, 40 ENVIRON SCI TECHNOL 4247-81 (2006).
[356] Hussain, S.M. et al., *In vitro toxicity of nanoparticles in BRL 3A rat liver cells*, 19 TOXICOLOGY IN VITRO 975–983 (2005).
[357] See, e.g., Andre Nel et al., *Toxic Potential of Materials at the Nanolevel*, 311 SCIENCE 622-27 (2006).

D. EPA MUST COMPLY WITH THE NATIONAL ENVIRONMENTAL POLICY ACT (NEPA) TO ASSESS THE ENVIRONMENTAL IMPACTS OF EPA'S DECISIONS REGARDING NANO-PESTICIDES AND/OR NANO-SILVER PESTICIDE PRODUCTS, INCLUDING COMPLETING A PROGRAMMATIC ENVIRONMENTAL IMPACT STATEMENT (PEIS)

1. The National Environmental Policy Act

The National Environmental Policy Act ("NEPA") is the "basic national charter for protection for the environment."[358] NEPA is intended to "promote efforts which will prevent or eliminate damage to the environment and biosphere and stimulate the health and welfare of man."[359] Agency NEPA duties are not "inherently flexible."[360] Recognizing the effects of new technologies on the environment, Congress explicitly states in NEPA that "new and expanding technological advances" are activities that could threaten the environment.[361] Thus, in order to understand and control the effects of new technologies like nanotechnology, Congress requires federal agencies to consider the environmental effects of new technology by complying with the requirements of NEPA.

2. The Potential Environmental Impacts of Nanomaterials, Including Nano-Silver

This Section hereby incorporates the above Sections' discussions of the potential environmental impacts of nano-silver pesticides. See supra pp. 57-73, 79-85 and accompanying footnotes. In addition, summarized below is more general information on the potential environmental impacts of nanomaterials. Engineered and manufactured nanomaterials are entering the natural environment throughout their lifecycle: via manufacturing, transportation,

---

[358] 40 C.F.R. § 1500.1.
[359] 42 U.S.C. § 4321.
[360] Calvert Cliffs Coordinating Comm. Inc. v. U.S. Atomic Energy Comm'n, 449 F.2d 1109, 115 (D.C. Cir. 1971). In fact, "[c]onsideration of administrative difficulty, delay or economic cost will not suffice to strip the section of its fundamental importance." Id.
[361] 42 U.S.C. § 4331(a). In the legislative history, Congress expressed its concern with "[a] growing technological power * * * far outstripping man's capacity to understand and ability to control its impact on the environment." Found. on Economic Trends v. Heckler, 756 F.2d 143, 147 (D.C. Cir. 1985) quoting S. Rep. No. 91-296 (1969).

use, disposal, and/or intentional introduction.[362] All of these lifecycle stages present possible environmental impacts and are potential foci of a comprehensive NEPA impacts assessment.[363]

Nanomaterials' unique chemical and physical properties can create reasonably foreseeable environmental risks. Nanomaterials' potential health and ecological impacts could occur as a result of direct and/or new routes of exposure; the toxicity of the materials themselves; and alterations or byproducts from interactions with other compounds and the environment over time.[364] Cumulative exposures with other manufactured nanomaterials as well as bulk-scale pollutants could also create impacts.[365] Once loose in nature manufactured nanomaterials represent a new class of non-biodegradable pollutants.

Toxicity: Studies assessing the role of size on toxicity have generally found that nanoparticles are more toxic than larger particles of the same substance.[366] Other studies have shown that some nanoparticles are toxic in ways that cannot be attributed to particle size alone.[367] Scientists have yet to determine what physicochemical properties will be most important in determining ecological and toxicological properties of nanomaterials.[368]

There is an emerging literature on the ecotoxicity of nanomaterials. Given all the unknowns about nanomaterials, researchers have focused on the traits that make nanomaterials

---

[362] The Royal Society and the Royal Academy of Engineering, *Nanoscience and nanotechnologies: Opportunities and uncertainties*, London, July 2004, pp. 37 Fig. 5.1, 46, available at http://www.nanotec.org.uk/finalReport.htm.

[363] See, e.g., Environmental Protection Agency, *Draft Nanomaterials Research Strategy (NRS)*, January 24, 2008, at 22, available at http://cs.epa.gov/ncer/nano/publications/nano_strategy_012408.pdf ; J. Michael Davis, *How to Assess the Risks of Nanotechnology: Learning from Past Experience*, 7 JOURNAL OF NANOSCI AND NANOTECHNOLOGY, 402, 406-07 (2007).

[364] See, e.g., Environmental Protection Agency, *Draft Nanomaterials Research Strategy (NRS)*, January 24, 2008, at 2, 38, available at http://cs.epa.gov/ncer/nano/publications/nano_strategy_012408.pdf

[365] Id.

[366] Science Policy Council, *U.S. Environmental Protection Agency Nanotechnology White Paper*, U.S. EPA, supra note 41, at 54 (February 2007).

[367] Id.

[368] See, e.g., Maynard et al., *Safe Handling of Nanotechnology*, Vol 444 NATURE 267-69 (November 16, 2006); Oberdorster et al., *Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles*, 113 ENVIRON HEALTH PERSPECT 823-839 (2005).

attractive for applications in industry and medicine—their ability to enter cells and carry other materials as well as a slew of other behaviors that make nanomaterials potentially damaging for humans and the environment. A number of studies have shown respiratory toxicity of various types of nanoparticles in small mammals.[369] These mammalian studies raise concerns that some nanomaterials may also be toxic to wildlife. EPA noted that "nanomaterials may affect aquatic or terrestrial organisms differently than larger particles of the same materials."[370] Several studies on the effects of various nanomaterials on fish and aquatic species have shown potentially negative impacts.[371] Significant lipid peroxidation was found in the brains of fish (largemouth bass) after exposure to carbon fullerenes, demonstrating the toxic effects of these nanoparticles on aquatic and possibly other organisms.[372] This is especially important given that this fish species is seen as a model for defining ecotoxicological effects. Studies on fullerenes have shown other potential impacts on aquatic ecosystems.[373] Similarly studies on various nanomaterials currently in use commercially have shown potential negative impacts on fish and aquatic organisms, e.g. carbon nanotubes,[374] copper nanoparticles,[375] titanium dioxide nanoparticles,[376] and silver nanoparticles.[377]

---

[369] Handy et al., *Toxic effects of nanoparticles and nanomaterials: implications for public health, risk assessment, and the public perception of nanotechnology*, 9 HEALTH, RISK AND SOCIETY 125-144 (2007).

[370] Science Policy Council, *U.S. Environmental Protection Agency Nanotechnology White Paper*, U.S. EPA, at 58, (February 2007).

[371] Handy et al., *Ecotoxicity of nanomaterials to fish: Challenges for ecotoxicity testing*, 3 INTEGRATED ENVIRONMENTAL ASSESSMENT AND MANAGEMENT 458-60 (2007).

[372] Oberdorster et al., *Manufactured Nanomaterials (Fullerenes, C60) Induce Oxidative Stress in the Brain of Juvenile Largemouth Bass*, 112 ENVIRONMENTAL HEALTH PERSPECTIVES 10 (2004).

[373] Fortner et al., *C60 in water: Nanocrystal formation and microbial response*, 39 ENVIRON SCI & TECH 4307-16 (2005); Rick Weiss, *Nanoparticles Toxic in Aquatic Habitat, Study Says*, WASH. POST (March 29, 2004) at A2; Press Release Rice University's Center for Biological and Environmental Nanotechnology, *CBEN: Buckyball aggregates are soluble, antibacterial*, (June 22, 2005), available at http://www.eurekalert.org/pub_releases/2005-06/ru-cba062205.php; Geoff Brumfiel, *A Little Knowledge . . .*, Vol 424 NATURE 246 (July 17, 2003); Sayes C. et al., *The differential cytotoxicity of water-soluble fullerenes*, 4 NANOTECHNOLOGY LETTERS 1881-87 (2004).

[374] Smith et al., *Toxicity of single walled carbon nanotubes to rainbow trout: respiratory toxicity, organ pathologies, and other physiological effects*, 82 AQUAT. TOXICOL. 94-109 (2007);

There is little research thus far on impacts of nanomaterials on plants, for instance in terms of bioaccumulation. One study found that engineered nanoparticles of aluminum oxide slowed the growth of roots in at least five species of plants.[378] Nanoparticles also can be "taken up" by bacteria, creating a means of potential bioaccumulation up the food chain.[379]

Mobility and Durability: Because of their tiny size nanomaterials may be highly mobile and travel further than larger particles in soil and water, which could foreseeably create environmental impacts.[380] Initial studies on potential remediation uses indicate that nanoparticles of iron can travel with groundwater over a distance of twenty meters and remain reactive for up to two months.[381] Early studies on the effects of nanomaterial exposure to biological systems have shown a high mobility in organisms or cells.[382] The translocatory potential of nanomaterials that makes them commercially attractive for drug delivery could cause unintended consequences as nanomaterials are released into natural systems.

---

[375] Griffitt et al., *Exposure to Copper Nanoparticles Causes Gill Injury and Acute Lethality in Zebrafish (Danio rerio)*, 41 ENVIRON. SCI. TECHNOL., 8178–8186 (2007).

[376] Federici, *Toxicity of titanium dioxide nanoparticles to rainbow trout (Oncorhynchus mykiss): Gill injury, oxidative stress, and other physiological effects.*, AQUAT TOXICOL. 2007 Jul 25; : 17727975 (P,S,E,B,D); Zhang et al., *Enhanced bioaccumulation of cadmium in carp in the presence of titanium dioxide nanoparticles*, 67 CHEMOSPHERE 160-67 (2007).

[377] Lee et al, *In Vivo Imaging of Transport and Biocompatibility of Single Silver Nanoparticles in Early Development of Zebrafish Embryos*, ACS Nano, 1(2), 133–143 (2007).

[378] Watts, D., *Particle Surface Characteristics May Play an Important Role in Phytotoxicity of Alumina Nanoparticles*, 158 TOXICOLOGY LETTERS 122-132 (2005); *Study Shows Nanoparticles Could Damage Plant Life*, SCIENCEDAILY (November 22, 2005), available at http://www.sciencedaily.com/releases/2005/11/051122210910.htm.

[379] Science Policy Council, *U.S. Environmental Protection Agency Nanotechnology White Paper*, U.S. EPA, at 36, (February 2007).

[380] Science Policy Council, *U.S. Environmental Protection Agency Nanotechnology White Paper*, U.S. EPA, at 34, (February 2007).

[381] Zhang et al., *Nanoscale Iron Particles for environmental remediation: An overview*, 5 JOURNAL OF NANOPARTICLE RESEARCH 323-332 (2003).

[382] See, e.g., Limbach et al., *Oxide nanoparticle uptake in human lung fibroblasts: Effects of particle size, agglomeration, and diffusion at low concentrations*, 39 ENVIRON. SCI. TECHNOL. 9370-9376 (2005); Rothen-Rutishauser et al., *Interaction of fine particles and nanoparticles with red blood cells visualized with advanced microscopic techniques*, 40 ENVIRON. SCI. TECHNOL. 4353-4359 (2006); Geiser, et al., *Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells*, 113 ENVIRON. HEALTH PERSPECT. 1555-1560 (2005).

Little is known about the potential of biodegradation of nanoparticles and mechanisms will depend on the nature of the material. The "high durability and reactivity of some nanomaterials raise issues of their fate in the environment."[383] Many nanoparticles in current products are non-biodegradable materials (such as metal oxides used in sunscreens).[384]

Interactions and Transport of Pollutants: Possible interactions between nanoparticles and harmful environmental chemicals may lead to unique exposures and impacts. Because nanoparticles tend to be more reactive than larger particles, interactions with substances present in the soil could lead to new and possibly toxic compounds. EPA has noted that "the use of nanomaterials in the environment may result in novel by-products or degradates that also may pose risks."[385] Many nanomaterial products (such as cosmetics and sunscreens) consist of "free" nanoparticles not fixed in a product matrix which will speed up their interaction in the environment.

Nanoparticles are the subject of vigorous drug research because of their ability to carry and deliver drugs to specific targets.[386] This same transport propensity could give nanoparticles the ability to carry toxic chemicals present in the environment. Natural and accidentally-created ultrafine particles can similarly carry toxic chemicals such as hydrocarbons and metals which can then damage natural systems.[387] The large surface area, crystalline structure and reactivity of

---

[383] Science Policy Council, *U.S. Environmental Protection Agency Nanotechnology White Paper*, U.S. EPA, at 14, (February 2007).
[384] Science Policy Council, *U.S. Environmental Protection Agency Nanotechnology White Paper*, U.S. EPA, at 36, (February 2007).
[385] Science Policy Council, *U.S. Environmental Protection Agency Nanotechnology White Paper*, U.S. EPA, at 58, (February 2007).
[386] See, e.g., Chavanpatil et al., *Nanoparticles for cellular drug delivery: mechanisms and factors influencing delivery*, 6 J. Nanosci. NANOTECHNOL 2651-2663 (2006).
[387] See, e.g., Penn et al., *Combustion-derived ultrafine particles transport organic toxicants to target respiratory cells*, 113 ENVIRON HEALTH PERSPECTIVES 956-79 (2005); Gutierrez-Castillo et al., *Effect of chemical composition on the induction of DNA damage by urban airborne particulate matter*, 47 ENVIRON MOL MUTAGEN 199-211 (2006); Schwarze et al., *Particulate matter properties and health effects: consistency of epidemiological and toxicological studies*, 25 HUM EXP TOXICOL 559-79 (2006).

some nanoparticles may facilitate transport of toxic pollutants in the environment.[388] Moreover, recent research has discovered a possible "trojan horse"-like toxicity mechanism of nanoparticles, which could carry harmful metals into cells.[389] Once inside the cell, the metal ions can leach from the nanoparticle and create oxidative stress.[390]

3. EPA's NEPA responsibilities

To accomplish NEPA's purposes, all federal agencies are required to prepare a "detailed statement"–known as an Environmental Impact Statement (EIS)– regarding all "major federal actions significantly affecting the quality of the human environment . . ."[391] To determine whether an EIS is required, federal agencies must prepare an Environmental Assessment (EA), that provides sufficient evidence and analysis to support the agency's determination on whether a proposed action will significantly affect the environment.[392] In addition to environmental concerns, the proposed action's possible direct, indirect, and cumulative impacts on public health must be reviewed if they are linked to its environmental impacts.[393]

Beyond just assessing the impacts of particular project-related actions, EPA is also required to assess the broader impacts of its programmatic actions and to consider alternative program approaches. A programmatic EIS (PEIS) is called for under the CEQ NEPA regulations, which define a "Federal action" broadly to include, in pertinent part, when there is:

---

[388] Zhang et al., *Environmental Technologies at the nanoscales*, 37 ENVIRON SCI. TECHNOL. 102A-108A (2003).
[389] Limbach et al., *Exposure of Engineered Nanoparticles to Human Lung Epithelial Cells: Influence of Chemical Composition and Catalytic Activity on Oxidative Stress*, 41 ENVIRON. SCI. TECHNOL. 4158-4163 (2007).
[390] Id.
[391] 42 U.S.C. § 4332(c). The EIS must describe (1) the "environmental impact of the proposed action," (2) any "adverse environmental effects which cannot be avoided should the proposal be implemented," (3) "alternatives to the proposed action," (4) "the relationship between local short-term uses of man's environment and the maintenance and enhancement of long-term productivity," and (5) any "irreversible or irretrievable commitment of resources which would be involved in the proposed action should it be implemented." Id.
[392] 40 C.F.R. §§ 1501.4(b), 1508.9.
[393] 40 C.F.R. § 1508.8; Baltimore Gas & Elec. Co. v. NRDC, 462 U.S. 87, 106 (1983)(explaining that "NEPA requires an EIS to disclose the significant health, socioeconomic, and cumulative consequences of the environmental impact of a proposed action").

> Adoption of programs, such as a group of concerted actions to implement a specific policy or plan; systematic or connected agency decisions allocating agency resources to implement a specific statutory program or executive directive.[394]

If EPA grants this petition and enacts new regulations, or amends existing regulations with an aim at regulating nano-silver products, or adopts an official policy in another form, such programmatic regulatory action would necessitate a PEIS if the action "significantly affects the quality of the human environment."[395] Moreover, an agency "program" or "proposal" that exists in fact, but is not necessarily expressly declared by the agency, also requires a PEIS.[396] Accordingly, if EPA declines to enact or amend its regulations, but instead continues acting pursuant to a "de facto" nano-silver regulatory policy, such concerted action would also necessitate a PEIS.

At least one Court has said that EPA does not need to prepare an EIS before it can register a pesticide.[397] That said, the registration and labeling of a pesticide under FIFRA does not exempt an agency from its general NEPA obligations.[398] A pesticide registration under

---

[394] 40 C.F.R. § 1508.18(b)(3) (defining "Federal action"). CEQ's "Question 24a" is instructive here as it addresses programmatic compliance on the topic of: "When are EISs required on policies, plans or programs?" It provides:

> An EIS mst be prepared if an agency proposes to implement a specific policy, to adopt a plan for a group of related actions, or to implement a specific statutory program or executive directive. In addition, the adoption of official policy in the form of rules, regulations, and interpretations pursuant to . . . formal documents establishing governmental or agency policy which will substantially alter agency programs, could require an EIS . . . . It should be noted that a proposal may exist in fact as well as by agency declaration that one exists.

46 Fed. Reg. 18026, 18033 (Forty Most Asked Questions Concerning CEQ's NEPA Regulations) (Question and Answer 24(a)).

[395] 21 C.F.R. § 25.22(b).

[396] See 40 C.F.R. § 1508.23 (Defining "Proposal" to include that a "proposal may exist in fact as well as by agency declaration that one exists").

[397] Merrill v. Thomas, 807 F.2d 776 (9th Cir. 1986).

[398] 42 U.S.C. § 4332; Oregon Envtl. Council v. Kunzman, 714 F.2d 901, 905 (9th Cir. 1983); Save Our Ecosystems v. Clark, 747 F.2d 1240, 1248 (9th Cir. 1984).

FIFRA does not require the same examination of environmental concerns that an agency is required to make under NEPA.[399]

5. *EPA regulatory action or program regarding nano-silver and nanotechnology is "significant" and requires a PEIS*

CEQ's implementing regulations list factors to determine whether a Federal action, such as EPA's pesticide regulatory approach to nanotechnology and nanomaterials, is "significant," which include:

> -- The degree to which the proposed action affects public health or safety
>
> -- The degree to which the effects on the quality of the human environment are likely to be highly controversial
>
> -- The degree to which the possible effects on the human environment are highly uncertain or involve unique or unknown risks
>
> -- [t]he degree to which the action may establish a precedent for future actions with significant effects or represents a decision in principle about a future consideration.[400]

In this case, all the above factors are present. First, given the unprecedented environmental and human health risks of nanomaterials, EPA regulatory actions or programs (or inaction) for nano-silver will greatly affect public health and safety. The petition discusses the significant risks nano-silver poses to public health and safety and the environment. These nano-silver pesticide products being released into the environment are under EPA's FIRFA jurisdiction, and represent the highest percentage of known nanomaterial consumer products currently on markets and being disposed into the environment.[401]

---

[399] Save Our Ecosystems, 747 F.2d at 1248; Washington Toxics Coal. v. EPA, 413 F.3d 1024, 1032 (9th Cir. 2005).
[400] 40 C.F.R. § 1508.27(b)(2),(4),(5),(6) & (9). The Supreme Court has held that CEQ's NEPA implementing regulations are entitled to substantial deference by the courts. Andrus v. Sierra Club, 442 U.S. 347, 358 (1979); Marsh v. Oregon Natural Resources Council, 490 U.S. 360, 372 (1989). FDA has expressly adopted CEQ's "significantly" definition in its own NEPA regulations. 21 C.F.R. § 25.5(a)(19).
[401] The Woodrow Wilson International Center for Scholars, Project on Emerging Nanotechnologies, *Nanotechnology Consumer Products Inventory*, available at http://www.nanotechproject.org/consumerproducts

Second, EPA's current general stance is that it has "no information" regarding nano-silver pesticide products.[402] Yet this petition includes an appendix with over 260 such products, over 100 pages and 400 footnotes providing information publicly available. Further, EPA's Region IX has taken an enforcement action against one nano-silver product manufacturer for violating FIFRA, using the same statutory provisions and statutory authority outlined in this petition. Still, EPA has also limited any proposed action to the "ions" of the Samsung Washing Machine, without even mentioning nanotechnology or nanomaterials. This is at odds with the scientific studies on nanomaterials regarding their fundamentally unique properties and risks. Thus, the agency's regulatory stance, if not corrected, is highly controversial at best and grossly negligent at worst.

Third, due to the paucity of research funding on the environmental and health impacts of nanomaterials, the possible effects on the human environment are highly uncertain;[403] given the fundamental differences of engineered nanoparticles from bulk materials, those risks are also quite unique.[404] The nano-ness created capacity for fundamentally different properties and the associated unknowns about potential adverse environmental and health impacts of nanotechnology apply to both nanomaterial writ large as well as nano-silver specifically.

Finally, no U.S. regulatory agency has enacted regulations governing the release and marketing of nanomaterials. However, EPA has acknowledged that products containing nanomaterials such as nano-silver are currently available to consumers and fall under its pesticide

---

[402] See notes 75-82 and accompanying text supra.
[403] EPA White Paper, supra note 41, at 35 ("The fundamental properties concerning the environmental fate of nanomaterials are not well understood [], as their are few available studies on the environmental fate of nanomaterials.) (footnote omitted).
[404] See id. at 35-44 (discussing, *inter alia*, the different behavior of nanoparticles in water and soil, the inability to meaningfully predict the biodegradation, bioavailability, or bioaccumulation of nanomaterials, and the inability of existing methods to detect or track nanomaterials in the environment).

regulation. Accordingly, EPA's pesticide regulatory and/or policy stance on nanopesticides and nano-silver regulation is significant and precedential.

The "presence of one or more of these factors should result in an agency decision to prepare an EIS."[405] In this case, at least four factors are present.[406] Accordingly, NEPA requires EPA to conduct a PEIS before enacting, adopting, or amending its regulations to create a regulatory program for nano-silver pesticide regulation, and before continuing to act under its regulatory program on nano-silver pesticide regulation.[407]

IV. EPA Must Take Immediate Action to Prohibit the Sale of the Class of Illegal Nano-silver Pesticide Products with Unapproved Health Claims

A. Both Nano-silver as an Active Ingredient and Nano-silver Products are Illegal Pesticide Products

Under the above statutory and regulatory framework, the nano-silver infused consumer and household products are illegal pesticides that require registration.[408] The products easily meet the FIFRA definition of pesticides, even a specific subset of antimicrobial pesticides.[409] The products are intended for such use. Their labeling illegally connotes a germ-killing propensity without registration.[410] Even if unlabeled or if such labeling is stripped, the nano-silver products are pesticides because manufacturers have actual knowledge of the nano-silver's germ killing powers and advertising has created a reasonable expectation of that use from industry-wide ads on other nano-silver products.[411] The nano-silver pesticide used to treat many consumer items is not registered for use in the items or use (or registered at all for any use).

---

[405] Public Service Co. of Colo. v. Andrus, 825 F. Supp. 1483, 1495 (D. Idaho 1993); See Friends of the Earth, Inc. v. U.S. Army Corp of Eng'rs, 109 F. Supp. 2d 30, 43 (D. D.C. 2000).
[406] See 40 C.F.R. § 1508.27(b)(2),(4),(5),(6) & (9).
[407] Id. §§ 1502.4(c)(3), 1508.18(b)(1).
[408] See supra pp. 30-42.
[409] See pp. 30-38 supra and accompanying footnotes.
[410] See supra pp. 14-15, 32-34.
[411] Id. at 34-37.

EPA itself lists several types of common "illegal pesticides," including antimicrobial products used in households:

> Many common household products, ranging from cleansers to cutting boards, claim to protect against bacteria. Such claims are illegal unless the product is registered with EPA or the claim only applies to protecting the item itself from damage by microorganisms, not to provide any additional health benefits. In addition, the pesticide used to treat the item must be registered for use in or on the treated item.[412]

In this case, the nano-silver pesticide products are not registered, and the widespread claims made include various other additional health benefits besides protecting the product itself. These claims include claims like "*sterilization benefits for over 650 types of bacteria like "E. coli, S. Aureus, Pneumococcus, Salmonella, Typhus, Vibria, Cholerae, etc.*"; "*kills bacteria in vitro in as little as 30 minutes, 2-5 times faster than other forms of silver*"; "*works against all types of bacteria and viruses, even killing antibiotic resistant strains as well as all fungal infections . . . remains potent up to 100 washes*"; and "*sterilize up to 99.9% of harmful bacteria, such as colon bacilli, salmonella, yellow staphylococcus, pseudomonas aeruginosa an salmonella enteritidis.*" See Appendix A. Further, nano-silver itself is not registered for use on the items or any items for that matter. Thus, the claims and products are clearly illegal.

B. EPA Must Act to Stop the Sale of Illegal Nano-silver Pesticides by All Means Possible, Including the Issuance of Stop Sale, Use or Removal Orders

With express limited exemptions, no pesticide products may be distributed or sold if not registered.[413] EPA's statement on "illegal pesticides" notes:

> EPA is concerned about these claims because, in addition to being unlawful, they are also potentially harmful to the public (e.g., if people believe that a product has a self-sanitizing quality, they may become lax in their hygiene practices). Practicing standard hygiene practices has been proven to prevent the transmission

[412]EPA, Illegal Pesticide Products, at www.epa.gov/pesticides/health/illegalproducts/index.htm
[413]40 C.F.R. §§ 152.15, 152.42 (application for new registration must be approved before product may be legally distributed or sold).

> of harmful microorganisms and, therefore, reduce the possibility of public health risk.
>
> In response to the marketing of unregistered pesticide-treated products with illegal, unsubstantiated public health claims, EPA has acted quickly and decisively to prohibit sales of such products. It will continue to be the Agency's policy to take action against companies that make such illegal claims.[414]

In accordance with the mandates of FIFRA and EPA's own regulations and policies, petitioners call on EPA to act "quickly and decisively" to prohibit the sale of these nano-silver products and take further actions it deems necessary against the companies making these illegal claims.

To that end, EPA should issue Stop Sale, Use or Removal Orders ("SSURO") to those manufacturers and/or distributors currently selling these unregistered nano-silver pesticide products. EPA may issue a stop sale, use or removal order (SSURO) under FIFRA § 13(a) to any person who owns, controls, or has custody of a pesticide or device that EPA has reason to believe, *inter alia*, is in violation of any FIFRA provision or has been or is intended to be distributed or sold in violation of FIFRA.[415] EPA may issue such orders based on only a reasonable belief of a FIFRA violation. According to the EPA's FIFRA Enforcement Response Policy, a SSURO must be issued for a number of instances, including

> a pesticide for which there is reason to believe that there is a potential hazard to man or the environment because: (1) *they are not registered* or are so over-formulated, under-formulated or adulterated as to present a serious health hazard . . .[416]

---

[414]EPA, Pesticides: Topical & Chemical Fact Sheets, Consumer Products Treated with Pesticides, at www.epa.gov/pesticides/factsheets/treatart.htm

[415]7 U.S.C. § 136k(a).

[416]U.S. EPA, Enforcement Response Policy for the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (July 2, 1990) (FIFRA ERP) at 6 (emphasis added).

EPA should issue stop sale orders to the manufacturers of nano-silver products not properly registered as pesticides. Appendix A lists many of these illegal pesticide products and their manufacturers.

Finally, as discussed above, there is already precedent for such actions: EPA's recent consent agreement with ATEN Technology fining that company for unlawfully marketing and selling unregistered nano-silver pesticide products.[417]

V. If Any Nano-silver Pesticide Registration is Approved, EPA Must Apply the EPA Pesticide Requirements To Nano-silver Pesticides, Including Requiring Labeling and Post-Registration Notification Requirements

If approved, EPA must insure that nano-silver pesticides abide by all pesticide requirements. The pesticide registration requirements provide EPA authority to require the generation of data necessary for risk assessment on nano-pesticides; to prohibit the use of a nano-pesticide that is determined to present unreasonable adverse effects to human health or the environment; and to condition the use of nano-pesticides to ensure that it does not present the threat of unreasonable adverse effects. Accordingly, when making registration decisions, EPA should impose appropriate restrictions on the registration of a nano-silver pesticide in order to prevent it from causing unreasonable adverse effects. These restrictions include but are not limited to: Registration for general use or restricted use under FIFRA Section 3(d) and 40 C.F.R. Part 152, Subpart I; Labeling restrictions under FIFRA Section 3(c)(5)(B) and 40 C.F.R. Part 156. (including the use of personal protective equipment, disposal restrictions, use restrictions, etc.); Tolerances under the FFDCA Section 408 and 40 C.F.R. Part 180; Worker protection standards under FIFRA Section 25(a) and 40 C.F.R. Part 170; and Packaging standards under

[417] See pp. 25-26 supra and accompanying footnotes.

FIFRA Section 25(c)(3) and 40 C.F.R. Part 157. Further, the pesticide registration requirement is supported by strong enforcement powers that can be exercised over unregistered pesticides under FIFRA §§ 12, 13, 14, & 19. Finally, in addition to information required to be submitted under § 3(c)(2)(B), registrants are under a continuing obligation under FIFRA § 6(a)(2) to submit factual information regarding unreasonable adverse effects on the environment of the pesticide whenever the registrant has such information. 7 U.S.C. § 136d(a)(2).; 40 C.F.R. § 152.125.

A. EPA Must Require Labeling of Nano-Silver Products

Registered pesticides must have EPA-approved labels, including a proper ingredient statement, directions for use, classification for restricted use, and hazard and precautionary statements.[418] In addition, all other written, printed, or graphic matter accompanying the pesticide or any other such matter to which the label or literature accompanying the pesticide refers must conform to EPA requirements.[419] Warnings and precautionary statements include statements for environmental risks,[420] such as those to non-target organisms.[421] For example, silver pesticides must carry a label stating:

> the pesticide [silver] is toxic to fish and aquatic invertebrates.
>
> Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans or other waters unless in accordance with the requirements of a National Pollution Discharge Elimination System (NPDES) permit....[422]

Current nano-silver pesticide products are in violation of FIFRA for their commercial sale without proper labeling. EPA must require a unique identifier to be commonly understood to designate a nano-formulation; these products need to be labeled as containing nano-silver,

---

[418] 40 C.F.R. § 156.10.
[419] 7 U.S.C. § 136(p)(1-2).
[420] 40 C.F.R. § 156.80.
[421] 40 C.F.R. § 156.85.
[422] EPA, Silver RED, supra note 162 at 5.

including any nano-specific environmental precautionary statements; and any other limitations the agency saw appropriate to mandate.

B. EPA Must Require Post-Registration Notification of Adverse Effects

Registration of nano-silver pesticides places upon registrants a continuing obligation to report to EPA any new factual information the registrant learns about unreasonable adverse effects on the environment from the pesticide.[423] This includes information from scientific studies, including toxicological, ecological, and human epidemiological and exposure studies.[424] Any study that suggests a pesticide may present greater risks than previously known is reportable. In addition, registrants must provide information they know or should know that EPA might regard as raising concerns about the continued registration of the pesticide or about the terms or conditions of the registration.[425]

This post-registration notification requirement is especially crucial for emerging technologies and materials such as nanotechnologies, with rapid commercialization happening ahead of EHS research. Significant health, safety and environmental impact information on nanomaterials and nano-silver will continue to appear. EPA must require nano-silver pesticide registrants to timely provide all information related to unreasonable adverse effects on the environment from nano-silver.

Further, this post-registration reporting obligation includes information related to a class of pesticides, not just individual pesticides.[426] EPA has previously tailored post-registration reporting requirements for certain types or classes of pesticides, such as plant-incorporated

---

[423] 7 U.S.C. § 136d(a)(2); 40 C.F.R. § 159.152(a); 40 C.F.R. Part 159 (specifying the kinds of information required to be submitted).
[424] 40 C.F.R. §§ 159.155(a)(1), (3); 159.165; 159.170.
[425] 40 C.F.R. § 159.195(a).
[426] See PR Notice 98-3, "Guidance on Final FIFRA Section 6(A)(2) Regulations for Pesticide Product Registrants" (Apr. 3, 1998), § X, available at http://www.epa.gov/opppmsd1/PR_Notices/pr98-3.pdf.

protectants[427] and specifically singled out genetically-engineered microbial pesticides on their duty to report adverse effects.[428] EPA should undertake similar actions for nanomaterial and/or nano-silver pesticides as well, to ensure the agency timely receives all pertinent data on the impacts of these new materials in order to best inform its oversight actions.

C. EPA Must Require Post-Registration Testing and New Data Development

EPA should also require nano-silver registrants to develop new data post-registration. EPA can require post-registration testing of nanopesticides under FIFRA § 3(c)(2)(B) and 4. EPA has the authority to require registrants conduct new studies whenever EPA determines such data is "required to maintain in effect an existing registration of a pesticide."[429] As new scientific data on nano-silver emerges EPA should use its authority to ensure FIFRA's standards are maintained. In addition, EPA should require, as part of reregistration, submission of missing or inadequate data.[430]

D. Conditional Registration

When EPA does not have enough data to make an unconditional registration decision it may conditionally register a pesticide.[431] Most new pesticide registrations are conditional.[432] EPA can conditionally register a pesticide for a time period sufficient to allow the generation and submission of additional data.[433] Because of the many unknowns about nanomaterials and nano-silver specifically, EPA should use its conditional registration authority.

---

[427] 40 C.F.R. § 174.71
[428] 51 Fed. Reg. 23313, 23320 (June 26, 1986).
[429] 7 U.S.C. § 136a(c)(2)(B).
[430] 7 U.S.C. §136a-1(d)(3).
[431] 7 U.S.C. § 136a(c)(7).
[432] 40 C.F.R. § 152.111
[433] 136a(c)(7)(C).

E. Disclosure of Confidential Business Information is in the Public Interest

All information concerning the environmental or health effects of a registered pesticide or its ingredients is available for public disclosure.[434] Data submitted with registrations must be made part of the public record and be available for public inspection.[435] In addition, EPA may disclose confidential business information (CBI) concerning production, distribution, sale, or inventories of a pesticide in connection with a public proceeding to determine whether the pesticide causes unreasonable adverse effects on health or the environment, if EPA finds such disclosure is necessary in the public interest.[436] In the case of nanotechnology, nanomaterials, and nano-silver products, disclosure of claimed CBI is in the public interest because of the dearth of information on the risks of nanotechnology. The public interest is benefited from a transparent and open dialog on the risks of any new and emerging technology such as nanotechnology. Here, nano-silver product information would substantially enhance and inform the public interest and EPA should require the disclosure of such information with regard to nano-silver pesticide products.

VI. Other EPA Actions Requested for Adequate Assessment and Oversight of Nano-silver Pursuant to FIFRA

FIFRA grants EPA general authority to prescribe regulations to carry out the provisions of the Act,[437] and separate sections of FIFRA include more specific grants of rulemaking authority.[438] EPA thus has broad powers under FIFRA to make regulatory changes as it sees necessary to protect public heath and the environment from the potential dangers of nano-

---

[434] 7 U.S.C. § 136h(d)(1).
[435] 40 C.F.R. §152.119
[436] 7 U.S.C. § 136h(d)(2).
[437] 7 U.S.C. § 136w(a)(1).
[438] See, e.g., 7 U.S.C. § 136a(c)(2)(A)(registration data guidelines shall be revised from time to time); § 136f(a) (regulations for recordkeeping requirements necessary for effective enforcement).

pesticides, including nano-silver products. Accordingly, in addition to the above delineated agency actions, EPA should also use all other relevant FIFRA oversight mechanisms to adequately address the potential environmental and human health impacts of nano-silver and determine whether nano-silver presents an unreasonable risk to man or the environment.

A. EPA should Undertake a Classification Review of Nano-silver Pesticides

EPA should undertake a classification review of nano-silver pesticides. Pursuant to its classification procedure regulations, EPA may, by regulation, prescribe classification restrictions relating, *inter alia*, to a pesticide product's composition, labeling, packaging, uses, or distribution and sale.[439] EPA may identify "a group of products having common characteristics or uses and may classify for restricted use same or all of the products or uses included in that group."[440] Such a group can be comprised of products that:

> (1) Contain the same active ingredients.
> (2) Contain the same active ingredients in a particular concentration range, formulation type, or combination of concentration range and formulation type.
> (3) Have uses in common.
> (4) Have other characteristics, such as toxicity, flammability, or physical properties, in common.[441]

Thus, EPA can conduct a classification review of such a group of products with the same active ingredient, same usage, or same characteristics in common if it deems such review necessary to avoid unreasonable adverse affects on the environment.[442] All of the nano-silver products have the same active ingredient (nano-silver), in the same concentration range

---

[439] 40 C.F.R. § 152.160.
[440] 40 C.F.R. § 152.164.
[441] Id. § 152.164(a).
[442] 40 C.F.R. § 152.164(a)-(b).

(nanoscale), for the same or similar use (antimicrobial effects). In this case, the environmental impacts of nano-silver and existing unknowns warrant such a classification review.[443]

Classification reviews are often conducted as part of a review of an application for a new registration of a product containing an active ingredient not contained in any currently registered product.[444] Nano-silver is not registered for use and is a new active ingredient. If the EPA determines that a product or one or more of its uses should be classified for restricted use, it can do so by regulation.[445]

B. EPA Should Undertake a Special Review of Nano-silver Pesticides

Alternatively, EPA should undertake the Special Review process for nano-silver pesticide products.[446] The purpose of Special Reviews is for the agency to determine whether to initiate procedures to cancel, deny, or reclassify registration of a pesticide product because that product may cause unreasonable adverse effects on the environment under FIFRA sections 3(c)(6) and 6.[447] The Special Review procedures expressly note that even though EPA is taking review action, the burden of persuasion that a pesticide is entitled to registration remains on the pesticide product manufacturer/applicant.[448]

The EPA Administrator may conduct a Special Review of a pesticide use for a broad array of reasons, including, *inter alia*:

> (3) May result in residues in the environment of nontarget organisms at levels which equal or exceed concentrations acutely or chronically toxic to such organisms, or at levels which produce adverse reproductive effects in such organisms, as determined from tests conducted on representative species or from other appropriate data.

---

[443] See supra pp. 58-91.
[444] 40 C.F.R. § 152.164(b)(1).
[445] 40 C.F.R. § 152.164(c)(1).
[446] 40 C.F.R. §§ 154.1-154.35
[447] 40 C.F.R. § 154.1
[448] 40 C.F.R. § 154.5.

> (4) May pose a risk to the continued existence of any endangered or threatened species designated by the Secretary of the Interior or the Secretary of Commerce under the Endangered Species Act of 1973, as amended.
>
> (5) May result in the destruction or other adverse modification of any habitat designated by the Secretary of the Interior or the Secretary of Commerce under the Endangered Species Act as a critical habitat for any endangered or threatened species.
>
> [and the catch-all provision]
>
> (6) May otherwise pose a risk to humans or to the environment which is of sufficient magnitude to merit a determination whether the use of the pesticide product offers offsetting social, economic, and environmental benefits that justify initial or continued registration.[449]

Nano-silver poses environmental risks pertaining to one or more of these types of risks sufficient to conduct a Special Review.[450] Nano-silver aimed at killing bacteria and microorganisms in or on consumer products, homes, and other goods, when released into the environment pose dangers to non-target species such as fish and other aquatic species. These residues may exceed levels toxic to such organisms. In addition, many of these fish and aquatic species may be federally protected as endangered or listed species. See Section III(C) infra. The current Fish and Wildlife Service (FWS) protected species listing counts at least 258 protected relevant fish or other aquatic species, including 139 threatened or endangered fish, 70 threatened or endangered clams, and 22 threatened or endangered crustaceans, and 25 reptiles or mammals.[451] Given the widespread usage and potential disposal routes, nano-silver releases could also result in the destruction or adverse modification of these species' habitat. Finally, nano-silver releases may pose other risks to humans or the environment, see supra, of sufficient magnitude to merit a determination.

---

[449] 40 C.F.R. § 154.7(a).
[450] See supra pp. 58-91 and accompanying footnotes
[451] See Appendix C; http://www.fws.gov/endangered/wildlife.html#Species

As part of the Special Review of nano-silver the Administrator should, among other duties, open a public docket for comments,[452] request a Scientific Advisory Panel hold a public meeting to review the scientific issues related to the Special Review,[453] hold hearings,[454] and meetings with interested parties.[455]

C. *EPA should Require the Submission of Nano-specific Data from Prospective Nano-Silver Registrants*

EPA should require the necessary data from prospective registrants for nano-silver products. EPA must ensure it has all the data it needs on nano-silver necessary to perform its risk assessments. Where data does not exist, EPA must require its development.[456] The data requirements for registration are intended to generate data and information necessary to address concerns pertaining to the identity, composition, potential adverse effects and environmental fate of each pesticide.[457] Data needs include, *inter alia*, data on physical and chemical characteristics of a pesticide active ingredient, wildlife and aquatic organism data, environmental fate data, mobility studies, accumulation studies, and hazards to nontarget organisms.[458] To perform its statutorily-mandated risk assessment for a pesticide, EPA needs information on the potential risks and benefits of a pesticide. There are many unknowns currently about potential the human health and environmental impact of nanomaterials, including nano-silver. "If information required generally is not sufficient to evaluate the potential of the product to cause unreasonable adverse effects on man or the environment, additional data requirements will be imposed."[459]

---

[452] 40 C.F.R. § 154.15, 154.26.
[453] 40 C.F.R. § 154.25(d).
[454] 40 C.F.R. § 154.29.
[455] 40 C.F.R. § 154.27.
[456] 40 C.F.R. § 152.111
[457] 40 C.F.R. § 158.130
[458] Id.
[459] 40 C.F.R. § 158.75(a).

D. *EPA should Amend FIFRA Regulations to Require Nanomaterial and/or Nano-silver Specific Data*

To account for the unique challenges of nanomaterials and nano-pesticides, including nano-silver products, EPA should amend its regulations to require nano-specific data for nano-pesticides.[460] The data requirements are intended to generate the data necessary to address concerns. FIFRA section 25(a) instructs EPA to "take into account the difference in concept and usage between various classes of pesticides [] and differences in environmental risk and the appropriate data for evaluating such risk between agricultural, non-agricultural, and public health pesticides."[461] Accordingly, FIFRA gives EPA the ability to make regulatory data requirements for specific types of pesticide products.[462]

Current data requirements for product composition, certified limits, and physical and chemical characteristics do not address information regarding some of the key unique properties of nanomaterials.[463] For example the regulations do not require either identifying or testing the surface area, shape, or aggregation of particles, all of which can modify cellular uptake, protein binding, translocation, and the potential for injury. Further the regulations define threshold limits by mass concentration rather than surface area.[464]

There is well established precedent for actions amending data requirements for specific types of pesticide products. For example, EPA has promulgated regulations that apply specifically to testing of genetically modified microbial pesticides.[465] The data requirements for this category of pesticides differ from those typically required for other types of pesticides.

---

[460] 40 C.F.R. Part 158 (Data Requirements).
[461] 7 U.S.C. § 136w(a).
[462] 40 C.F.R. § 158.1.
[463] See 7 U.S.C. § 136a(c)(2)(A); 40 C.F.R. Part 158.
[464] 40 C.F.R. § 158.175(b).
[465] 40 C.F.R. §§ 172.43-.59

E. EPA should Undertake Registration Review of Existing Bulk Silver Pesticide Registration

A registration review decision is "the Agency's determination whether a pesticide meets, or does not meet the standard for registration under FIFRA."[466] "Registration review is intended to ensure that each pesticide's registration is based on current scientific and other knowledge regarding the pesticide, including its effects on human health and the environment."[467] Silver last re-registered in 1993.[468] Since then, nanotechnology has come of age and a fleet of nano-silver products have come to market and thus entered the natural environment.[469] "At any time, the Agency may undertake any other review of a pesticide under FIFRA, irrespective of the pesticide's past, ongoing scheduled, or not yet scheduled registration review."[470]

EPA should undertake a registration review for its existing pesticide registrations for the active ingredient silver, in order to take in account and properly analyze the new scientific issues of nanotechnology and nano-silver. This review is needed not only because of the new scientific challenges and risks created by nanotechnology and nanomaterials but also the new nanomaterial uses and nanomaterial products, and nanomaterial created routes of exposure for humans and the environment.

As part of the silver registration review EPA should issue a data call-in notice under FIFRA Section 3(c)(2)(B) to gather the nano-specific health and safety and exposure data necessary to conduct the registration review.[471] Additionally, as part of the registration review process, EPA should: open a public docket;[472] "assess changes since the pesticide's last review;"

---

[466] 40 C.F.R. § 155.57
[467] 40 C.F.R. § 155.40(a)(1).
[468] Silver Re-registration Eligibility Document (RED), 1993.
[469] See, e.g., supra pp. 11-14, 66-67, 89-90
[470] 40 C.F.R. § 1555.40(c)(1).
[471] 40 C.F.R. § 155.48, 155.53(b)(1); 7 U.S.C. 136a(c)(2)(B)
[472] 40 C.F.R. § 155.50.

"consider whether any new data or information on the pesticide … warrant conducting new risk assessment or a new risk/benefit assessment;" and "conduct new assessments as needed."[473] Any proposed findings, revised or new risk assessments, risk mitigation measures, and/or labeling changes must be subject to public notice and comment.[474]

F. EPA should Ensure that Nano-silver Pesticide Devices Comply with FIFRA

Some nano-silver products may qualify as a pesticide device in addition to (or instead of) classification as a pesticide. A pesticide device is defined as

> **Pesticide Device**: any instrument or contrivance (other than a firearm) which is intended for trapping, destroying, repelling, or mitigating any pest or any other form of plant or animal life (other than man and other than bacteria, virus, or other microorganism on or in living man or other living animals).[475]

In general, an article is a device if it uses physical or mechanical means (as opposed to chemical or biological agent) to control a pest. Some of the nano-silver products in Appendix A contain not only nano-silver intended to prevent and destroy pests, but also a mechanism such as a filter, coating, or other process where the product itself is intended to trap or mitigate pests.[476] The possible "co-packs" not only contain nano-silver, but also are items capable of trapping or repelling the microorganisms that come into contact with them.

Nano-silver products properly classified as devices are still subject to FIFRA regulation. Devices are subject to FIFRA labeling requirements.[477] They are also subject to establishment registration requirements, record requirements, inspection requirements,

---

[473] 40 C.F.R. § 155.53(a)-(b).
[474] 40 C.F.R. §§ 155.53(c), 155.58.
[475] 7 U.S.C. § 136(h); 40 C.F.R. Part 152, Subpart Z (Devices).
[476] 7 U.S.C. § 136(h).
[477] 7 U.S.C. § 136w(c)(4); 40 C.F.R. § 152.500(b)(1), Part 156 (labeling requirements); 7 U.S.C. § 136(q)(1) (misbranded definition).

import and export requirements, and child-resistant packaging requirements.[478] Devices are subject to FIFRA's violation, enforcement and penalty provisions.[479]

Accordingly, if EPA determines that one or more of the nanosilver products are properly classified as pesticide devices rather than pesticides, the agency should ensure each complies with FIFRA's pesticide device requirements, including accurate labeling.

G. EPA should Set a FFDCA Tolerance for Nano-silver

1. Pesticide Tolerances and Exemptions

In addition to direct oversight and regulation of pesticides, EPA regulates pesticide residues in food and animal feed. EPA cannot register a pesticide under FIFRA until the applicant has obtained the necessary tolerance or exemption under the FFDCA. Under § 301, FFDCA prohibits the shipment in interstate commerce of "adulterated food."[480] Under FFDCA § 402(a)(2)(B), a food is considered adulterated if "it bears or contains a pesticide chemical residue that is unsafe" within the meaning of § 408(a).[481] Section 408(a) provides that a pesticide is "unsafe" (and the food containing it adulterated) unless EPA has established a tolerance for the pesticide and the pesticide residue is within that tolerance; or EPA has exempted the pesticide from the requirement for a tolerance.[482] No food containing any pesticide residue can be introduced into commerce unless the amount of the pesticide residue is within the prescribed tolerance.[483]

---

[478] 40 C.F.R. §§ 152.500(b); 7 U.S.C. §§ 136e (registration and reporting of establishments), 136f (books and records), 136g (inspection of establishments), 136o (imports and exports), 136w(c)(3) (child-resistant packaging).
[479] 40 C.F.R. §§ 152.500(b); 7 U.S.C. §§ 136j (unlawful acts), 136k (stop sale, use, removal, and seizure), 136l (penalties).
[480] 21 U.S.C. § 331.
[481] 21 U.S.C. § 342(a)(2)(B).
[482] 21 U.S.C. § 346a(a)(1).
[483] 21 U.S.C. § 331(a)-(c).

A "tolerance" is the maximum level of a pesticide residue that may be present in food or animal feed;[484] it is established by substantial testing demonstrating that it meets statutory standards for safety.[485] The statutory standard of "Safe" is defined as a "reasonable certainty that no harm will result from the aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures and all other exposure for which there is reliable information."[486] Section 408 of the FFDCA and its regulations layout the procedures for the establishment of a tolerance and factors to be considered by the agency, which can be begun with the filing of a petition to establish a tolerance.[487]

Alternatively to a tolerance, EPA can register a pesticide if an applicant obtains an "exemption" from the tolerance requirement if EPA determines that there is a "reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue."[488] However such an exemption would be arbitrary and capricious given the information provided in this petition.

2. In Order to Register Nano-silver Pesticides EPA Must Set a Nano-silver Tolerance

EPA establishes tolerances and exemptions for specific chemicals not products.[489] Silver is not registered for use on food or feed crops or for use on processed commodities.[490] There is no tolerance for silver or exemptions from the requirements of a tolerance. In the Silver RED, EPA concluded that "Silver is a natural element and trace amounts are normally present in the human diet." EPA further concluded that only "minimal dietary exposure may result from the

---

[484] 21 U.S.C. § 346a.
[485] Id. 346a(b)(2)(A).
[486] Id. § 346a(b)(2)(A)(ii).
[487] Id. § 346a(d)(1).
[488] Id. § 346a(c)(2)(A).
[489] 40 C.F.R. § 152.112(g).
[490] EPA, Silver RED, supra note 161 at 3.

use of silver in human drinking water systems. EPA does not anticipate that dietary exposure to these low levels of silver will be associated with any significant degree of risk."[491]

In sharp contrast, the recent explosion of nanosilver consumer products presents much higher human exposures. See Appendix A. These exposures are dietary through colloidal silver "health" drinks. Pesticides can reach food or feed several different ways, including by the migration of pesticidal chemicals from containers or processing equipment. Nano-silver is being used in a number of food-related products, including storage containers, cutting boards, cutlery, baby bottles, refrigerators, food and produce spray cleaners, toothbrushes, and dietary supplements. See Appendix A. While the nano-silver is in a "fixed" matrix in some products, it is unknown how and if they will migrate to food. Given their close proximity to food by many different products it seems likely that they the nano-silver particles will cause aggregate contamination and ingestion by the public, creating an internal build-up of the nanomaterial within the body before the toxicological effects of the nanomaterial are fully known. For example, the effect of organs storing nano-silver over a long period of time is unknown. Nano-silver could also interfere with beneficial bacteria in the gut.

Moreover, these nano-silver exposures are also occurring as skin-contact exposures. The statutory standard of "Safe" is defined as a "reasonable certainty that no harm will result from the aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures and all other exposure for which there is reliable information."[492] Many nano-silver products will create direct and indirect skin exposures. These nano-silver products include personal care products, hair products, soaps, various cleaning products, detergents and softeners, clothing, pillows, bandages, and shaving accessories. See Appendix A. Nano-silver clothing in

[491] Id.
[492] 21 U.S.C. § 346a(b)(2)(A)(ii).

particular will be in direct contact with skin over prolonged periods of time. EPA must assess the safety of these materials with regard to these exposures as well when setting a tolerance for nano-silver.

CONCLUSION

EPA has said that "in response to the marketing of unregistered pesticide-treated products with illegal, unsubstantiated public health claims, EPA has acted quickly and decisively to prohibit sales of such products. It will continue to be the Agency's policy to take action against companies that make such illegal claims."[493] Yet with one recent exception EPA has not acted to prohibit the widespread sale of illegal nano-silver pesticide products, including products with false and misleading claims. Instead, EPA has taken action only with regard to a limited category of these substances ("ion machines") while still permitting them to remain on market and expressly denied that its action in any way was related to nanotechnology or nanomaterials.

Petitioners urge EPA to act to remedy these failings in a timely fashion. EPA has jurisdiction over and a continuing statutory obligation to regulate nano-silver pesticide products. EPA has set precedent already for this with its action and consent agreement with IOGEAR Inc. Yet EPA has thus far denied its actions are even nanotech-related, or that oversight measures are needed to account for nanomaterials' regulatory and testing challenges, including those of nano-silver pesticide products. In general, there is currently a vacuum of regulation in the field of nanotechnology and nanomaterials. Industry has no guidance regarding the classification of these nano-silver products.[494] This legal petition provides both the blueprint for EPA's needed regulatory actions with regard to nano-silver and the legal impetus to take those actions. In

---

[493] www.epa.gov/pesticides/factsheets/treatart.htm

[494] See, e.g., Feder, *New Device for Germophobes Runs Into Old Law*, NEW YORK TIMES, March 6, 2008.

addition, FIFRA grants EPA general authority to prescribe regulations to carry out the provisions of the Act,[495] and separate sections of FIFRA include more specific grants of rulemaking authority.[496] EPA thus has broad powers under FIFRA to amend its regulations as it sees necessary to protect public heath and the environment from the potential dangers of nano-silver.

Specifically, petitioners requests EPA take the following actions with regard to nano-silver pesticides:

PETITIONERS REQUEST THAT THE EPA ADMINISTRATOR UNDERTAKE THE FOLLOWING ACTIONS:

I. Classify Nano-silver As a Pesticide and Require the Registration of Nano-silver Products as Pesticides

II. Determine That Nano-silver is a New Pesticide That Requires a New Pesticide Registration

III. Analyze the Potential Human Health and Environmental Risks of Nano-silver

A. *Pursuant to FIFRA, Analyze the Potential Human Health and Environmental Impacts as Part of the Nano-silver Pesticide Registration Process*

B. *Pursuant to the FQPA, Assess the Potential Impacts of Nano-silver Exposures on Infants and Children and Ensure that No Harm Will Result From Aggregate Exposures*

C. *Compliance with the ESA, Including Undertaking Consultation Procedures In Accordance with ESA § 7 for Any EPA Actions, Activities, or Programs Impacting Nano-silver Oversight*

D. *Compliance with NEPA, Including Assessing the Human Health and Environmental Impacts of EPA's Current and Future Actions or Programs Regarding Nano-silver, Including Completing a Programmatic Environmental Impact Statement*

IV. Take Regulatory Actions against the Class of Nano-silver Products Illegally Sold Without EPA FIFRA Approval, Including Issuing Stop Sale, Use or Removal Orders for Illegal and Unlabeled Nano-silver Pesticide Products

---

[495] 7 U.S.C. § 136w(a)(1).

[496] See, e.g., 7 U.S.C. § 136a(c)(2)(A)(registration data guidelines shall be revised from time to time); § 136f(a) (regulations for recordkeeping requirements necessary for effective enforcement).

V. If any Nano-silver Pesticide Registration is Approved, Apply and/or Amend to Specifically Apply the FIFRA Pesticide Requirements to the Class of Nano-silver Pesticides, Including

1. *Labeling*
2. *Post-Registration Notification of Adverse Effects*
3. *Post-Registration Testing and New Data Development*
4. *Conditional Registration*
5. *Confidential Business Information*

VI. Take Other EPA FIFRA Actions Necessary for Adequate Oversight of Nano-silver Pesticides, Including:

1. *Undertaking a Classification Review of Nano-silver Pesticides*
2. *Undertaking a Special Review of Nano-silver Pesticides*
3. *Requiring the Submission of Nano-specific Data from Nano-silver Registrants*
4. *Amending FIFRA Regulations to Require Nano-Specific Data*
5. *Registration Review of Existing Bulk Silver Pesticide Registration*
6. *Regulate Nano-silver Devices*
7. *Set a Pesticide Tolerance for Nano-silver*

In accordance with the APA, petitioners request that EPA provide an answer to this petition within a reasonable time.[497]

Respectfully submitted,

_____

George A. Kimbrell
Staff Attorney
International Center for Technology Assessment

_____

Joseph Mendelson III
Legal Director
International Center for Technology Assessment

---

[497] 5 U.S.C. § 555(b) ("[W]ithin a reasonable time, each agency shall proceed to conclude a matter presented to it.") id. § 706(1) (The reviewing court shall … compel agency action unlawfully withheld or unreasonably delayed."); id. § 555(e) ("Prompt notice shall be given of the denial in whole or in part of a written application, petition, or other request of an interested person made in connection with any agency proceeding.").

International Center for Technology Assessment

660 Pennsylvania Ave., S.E., Suite 302, Washington, DC 20003
(202) 547-9359 fax (202) 547-9429 www.icta.org

EXECUTIVE SUMMARY

Legal Petition Challenges EPA's Failure to Regulate Environmental and Health Threats from Nano-Silver

On May 1, 2008, the International Center for Technology Assessment (CTA) and a coalition of consumer, health, and environmental groups filed a legal petition with the Environmental Protection Agency (EPA), demanding the agency use its pesticide regulation authority to regulate numerous consumer products now using nano-sized versions of silver. The petition is the first legal challenge to EPA's failure to regulate nanomaterials. Nano-silver is the most common commercialized nanomaterial.

Nanotechnology and Nano-silver Products have arrived

Nanotechnology takes apart and reconstructs nature at the atomic and molecular level. Nanotechnology and products containing manufactured nanomaterials have arrived and represent the crest of a product wave spanning many industries. Hundreds of consumer products composed of manufactured and engineered nanomaterials are now widely available. The largest percentage of the currently known commercial nanomaterial products are infused with forms of nanoparticle silver ("nano-silver") for its nano-enhanced ability to kill microorganisms and bacteria.

The Products

The petitioners discovered no fewer than 260 self-identified nano-silver consumer products being sold in the U.S. The products listed in the petition's appendix include: air and water purifiers and filters; bathroom, kitchen and multipurpose cleaning sprays and wipes, children's toys, baby bottles and infant products; laundry detergents and fabric softeners; food storage containers, cutlery, and cutting boards; numerous types of clothing including underwear, socks, shirts, outerwear, gloves and hats; various fabrics and fibers; soaps, personal care and hair products; pet accessories; refrigerators and washing machines; computer hardware; ingestible "health" drink supplements; automobile products; and powdered and liquid nano-silver in bulk form. The products come from the U.S., the U.K., Canada, Korea, Japan, Taiwan, China, New Zealand, and Germany.

The nano-silver products make broad claims about the power of their nano-silver ingredients, such as: "eliminates 99% of bacteria"; renders material "permanently anti-

microbial and anti-fungal"; "kills approximately 650 kinds of harmful germs and viruses" and "kills bacteria in as little as 30 minutes, 2-5 times faster than other forms of silver."

The Environmental and Human Health Risks of Nano-silver

The same property that makes these nanomaterials attractive to manufacturers—their highly enhanced antimicrobial action—can be highly destructive to the environment and raise serious human health concerns. Even in bulk form, silver is toxic to fish, aquatic species and microorganisms and a 2005 study found that nano-silver is approximately 45 times more toxic than standard silver. In addition, nanomaterials such as nano-silver exhibit remarkably unusual physical, chemical and biological properties, such as the ability to be harmful in new ways. Impacts are occurring through use and disposal: A 2008 study showed that washing nano-silver socks releases substantial amounts of the nano-silver into the laundry discharge water, which will ultimately reach natural waterways and ecosystems and potentially poison fish and other aquatic organisms. Another 2008 study found that releases of nano-silver destroy benign bacteria used in wastewater treatment.

Many of the nano-silver infused products are for children (baby bottles, toys, stuffed animals, and clothing) or otherwise create high human exposures (cutlery, food containers, paints, bed sheets and personal care products) despite very little study on nano-silver's potential human health impacts. Studies have questioned whether traditional assumptions about silver's safety are sufficient in light of the unique properties of nano-scale materials. Potential health risks from nano-silver's widespread use also include increased bacterial and antibiotic resistance and risks created by nanomaterials' unprecedented mobility in the body.

EPA's Failure to Act

Concerns over nano-silver were first raised by national wastewater utilities in early 2006. Their concerns were highlighted by one then-new nano-silver product, Samsung's Silvercare Washer, which releases silver ions into the waste stream with every wash. In response, the media reported in November 2006 that EPA would regulate nano-silver products as pesticides. One year later, EPA published a guidance covering only the Samsung washer and allowed it to remain on the market. EPA denied that this guidance was "an action to regulate nanotechnology."

The Petition

Despite this nano-silver product explosion and its associated environmental and health risks, EPA has yet to take any meaningful regulatory action. The petitioners present both a legal blueprint and impetus to take such needed oversight action.

First, the petition calls on EPA to amend its regulations or otherwise act to clarify that nano-silver is a pesticide and those products incorporating it are pesticide products that must be registered, approved by the agency, and labeled prior to marketing. Nano-

silver meets the pesticide law's (FIFRA) definition of a pesticide because it is a highly efficient antimicrobial or antibacterial agent and is intended to be used for that purpose. EPA should clarify that pesticidal intent and public health claims can be both implicit and explicit and that manufacturers cannot avoid pesticide classification simply by stripping their products of labeling, a potential loophole several manufacturers have already exploited.

Second, the petition calls on EPA to clarify that nano-pesticides, such as nano-silver products, are new pesticide substances that require new pesticide registrations, with nano-specific toxicity data requirements, testing and risk assessments. Nano-silver must be classified as a separate substance than macro-silver based on the nanomaterial's capacity for fundamentally unique and different properties and because nano-silver many new antimicrobial uses are not previously registered silver uses.

Third, EPA must assess the potential human health and environmental risks of nano-silver. These assessments are required by and must comply with FIFRA, as well as the Food Quality Protection Act (FQPA), the Endangered Species Act (ESA), and the National Environmental Policy Act (NEPA). As part of this assessment, EPA should analyze all existing scientific studies as well as require manufacturers to provide all necessary additional data on nano-silver. Pursuant to FQPA, EPA must assess the potential impacts of nano-silver on children and infants and ensure that no harm will result from aggregate exposures. Additionally, EPA must ensure that its activities regarding nano-silver comply with the ESA and the protection of endangered and threatened species. Finally, EPA must comply with NEPA by ensuring that it assesses the environmental impacts of its actions regarding nano-silver pesticide products.

Fourth, EPA should take immediate action to prohibit the sale of nano-silver products as illegal pesticide products with unapproved health benefit claims. The nano-silver consumer products currently on market are in clear violation of FIFRA's mandates. To this end, EPA should issue Stop Sale, Use or Removal Orders or other enforcement penalties or actions to those manufacturers and/or distributors currently selling these unregistered nano-silver pesticide products.

Fifth, should EPA after rigorous assessment approve any nano-silver products as pesticides, the agency must fully apply its pesticide regulations to any registered nano-silver pesticides. FIFRA's pesticide registration requirement instills with EPA the duty to prohibit, condition, or allow the manufacture and use of nanomaterials in nano-pesticides and prescribe conditions for manufacture or use. These include: requiring nano-specific ingredient and warning labeling; applying conditional registration; applying requirements for post-registration notification of adverse impacts; applying post-registration testing and new data development; and requiring the disclosure of all information concerning environmental and health effects, including confidential business information.

Finally, EPA should use its FIFRA authority to further review the potential impacts of nano-silver, including: undertaking either a Classification Review or a Special

Review of nano-silver pesticides; amending the FIFRA regulations to require the submission of nanomaterial and/or nano-silver specific data; completing a registration review of existing silver pesticides; regulation of nano-silver pesticide devices; and the setting of a Federal Food Drug and Cosmetic Act Tolerance for nano-silver.

The full petition is available at www.icta.org and www.nanoaction.org

Relief Requested

Should EPA grant the petition, the result would be that nano-silver is classified as a new substance and nano-silver products regulated as new pesticides. That would require current and future nano-silver products to undergo mandatory EPA pre-market approval. Current products would have to be removed until and unless they received EPA approval. Approval would only occur if the agency found the products did not create an unreasonable risk to the environment. EPA would also have to assess nano-silver's potential impacts on human health, particularly on children and infants, and on the environment, particularly on endangered species and their habitat. EPA would require manufacturers to submit any needed data about the nanomaterials and current EHS unknowns to conduct its assessments. If any of the nano-silver products were approved and registered as pesticides, their use would be conditioned as necessary to protect the environment and human health, including the use of warning labeling. EPA would also amend its regulations to require nano-specific data, testing, and risk assessments for nanomaterial pesticide products.

The Petitioners

Joining the CTA petition are: the Center for Food Safety, Beyond Pesticides, Friends of the Earth, Greenpeace, ETC Group, Center for Environmental Health, Silicon Valley Toxics Coalition, Institute for Agriculture and Trade Policy, Clean Production Action, Food and Water Watch, the Loka Institute, the Center for Study of Responsive Law, and Consumers Union.

CTA

CTA is a non-profit, non-partisan organization committed to providing the public with full assessments and analyses of technological impacts on society. CTA works towards adequate oversight of nanotechnology through its Nanotechnology Project, *NanoAction*, www.nanoaction.org

CTA's uses a variety of legal and policy tools to fulfill its mission, including administrative law petitions. This is the second legal action CTA has filed on the health and environmental risks of nanotechnology: in May 2006 CTA filed a legal petition with the Food and Drug Administration (FDA), calling on that agency to address the human health and environmental risks nanomaterials in consumer products, particularly nano-cosmetics and nano-sunscreens.

NRDC NATURAL RESOURCES DEFENSE COUNCIL
THE EARTH'S BEST DEFENSE

September 10, 2010

Comments from the NATURAL RESOURCES DEFENSE COUNCIL on the proposed conditional registration of a pesticide product HeiQ AGS-20, containing nanosilver

Docket ID # EPA-HQ-OPP-2009-1012

I. Background

EPA is proposing to conditionally register a pesticide product containing nanosilver as a new active ingredient for a period of 4 years. The antimicrobial pesticide product, HeiQ AGS-20, is a silver-based product that is proposed for use as a preservative for textiles. As a condition of registration, EPA is proposing to require product chemistry, toxicology, exposure, and environmental data. The data requirements are based on the regulations governing the registration of pesticides and on a November 2009, independent consultation EPA held with the Federal Insecticide, Fungicide, and Rodenticide Act ("FIFRA") Scientific Advisory Panel (SAP). In its final report, the SAP addressed a number of questions associated with assessing the hazard of and exposure to nanosilver and other nanoscale metal-based pesticides.[1]

The Agency states that it "will evaluate these data as they are submitted during the period of the conditional registration to confirm the product will not cause unreasonable adverse effects to human health and the environment." (Decision Doc at 4)

On August 12, 2010 EPA issued a 36-page Proposed Decision Document for the Registration of HeiQ AGS-20 as a Materials Preservative in Textiles which is available in the docket as ID# EPA-HQ-OPP-2009-1012-0014. Unless otherwise indicated, references are to this Decision Document.

In its Proposed Decision Document, EPA determined that "the nanosilver active ingredient in the product differed from currently registered silver-based antimicrobial products" and thus, "EPA

reclassified the application under the PRIA [Pesticide Registration Improvement Act] as one involving a "New Active Ingredient Registration"" (Decision Doc at 4).

II. Summary of comments

NRDC opposes registration of nanosilver, because its use as an antimicrobial in textiles may cause "unreasonable adverse effects on the environment."[2] Specifically, its use will result in human exposures and environmental releases which are likely to cause harm to beneficial microbes and other unintended targets; the impacts of these risks have not been evaluated. EPA acknowledges that it "lacks information to conduct a complete assessment of the potential risks to human health and the environment associated with the use of AGS-20," and so EPA determined that "more extensive product chemistry, toxicology, exposure, and environmental data are necessary." (Decision Doc at 3, 36, Appendix A). EPA therefore may not lawfully register this pesticide. Regrettably, despite this extensive and significant lack of data, rather than denying the registration until the data is submitted and reviewed, EPA is proposing to require these studies as a condition of registration. (Decision Doc at 3).

NRDC is opposed to the conditional registration. In general, we are concerned that conditional registrations, representing two-thirds of current product registrations, have been overused, possibly as a way for registrants to gain rapid market access while delaying, or even avoiding, the data requirements for product registration. But more specifically, EPA has failed to show that AGS-20 satisfies the conditions under which a conditional registration may be granted. First, the registrant for AGS-20 has failed to submit data that EPA regulations specifically identify as required to register an antimicrobial pesticide. These data requirements are clearly laid out in the Code of Federal Regulations. The registrant does not need and is not entitled to an additional period of time to generate the data because these are not supplemental requirements – they have always been required by EPA. Second, EPA has failed to show that registering it will not cause any unreasonable adverse effect on the environment. these uses, in addition to offering no measurable or documented benefits to the public, are likely to lead to occupational inhalation exposures, incidental dermal and oral exposure to children wearing treated clothing, and releases of silver ions to the environment. Silver ions are well-known to have non-specific microbe-killing activity, threatening beneficial microbes on our bodies as well as in the environment. Third, EPA has failed to show that conditional registration of AGS-20 is in the public interest. Therefore, the proposed registration of AGS-20 is a misuse of EPA's authority and is likely to lead to unsafe exposures to consumers and the environment. Instead of giving AGS-20 market access, EPA should be reining in companies that are marketing unregistered and therefore illegal nanosilver pesticide products.

III. Summary of silver toxicity and regulation: need for stringent registration review of nanosilver

Silver metal is a well-recognized non-specific antimicrobial metal. Silver ions (positively charged atoms, Ag+) are more toxic to aquatic organisms than any other metal except mercury.[3] Silver is toxic, persistent in the environment, and has the potential to bioaccumulate in ocean

plants at concentrations 10,000 to 70,000 times higher than in the surrounding sea water.[4] Its historical use in developing film for traditional photography proved that the release of silver into the waste stream is deadly for aquatic biota. Silver is acutely toxic to aquatic organisms at exquisitely low concentrations, as low as 50 ng/L (parts per trillion, ppt); a study in fish embryos reported toxicity down to 10 ng/L.[5] Because of its extreme toxicity to aquatic organisms, discharges of silver effluent into lakes, streams, ponds, or any public water is subject to National Pollutant Discharge Elimination System permit restrictions, and any water that has been treated with silver pesticide cannot be discharged into the sewage systems without first notifying the sewage treatment authorities.[6]

EPA's 1993 Reregistration Eligibility Decision (RED) for silver notes that in humans when it is inhaled or ingested, it can be absorbed from the lungs and the gastrointestinal tract into the blood stream, where it causes a permanent skin discoloring condition called argyria.[7] [8] The oral reference dose, considered the acceptable daily intake limit over a lifetime, established by EPA in 1991 for silver is 0.005 mg/kg/day.

Nanosilver, or silver nanoparticles, are made up of clusters of silver ions. Silver nanoparticles are intentionally engineered to release silver ions, which is the mechanism of their enhanced microbe-killing activity. In addition to releasing more antibacterial ions, silver nanoparticles appear to be able to penetrate into cells better than silver, or possibly, to deliver ions directly into cells. These are believed to be the properties that make nanosilver a much more efficient antimicrobial than silver, and much more toxic.[9] In cultured mouse sperm stem cells, a 48 hr treatment of nanosilver (15 nm diameter) was 45-fold more toxic than silver carbonate (EC50 of 8.75 v 408 ug/ml) in a concentration-dependent manner; nanosilver was the most toxic of the nanomaterials tested, and drastically reduced mitochondrial function and cell viability.[10] The Scientific Advisory Panel (SAP) in its 2010 report noted several major differences between silver and nanosilver that were likely to result in a distinct hazard profile for nanosilver. However, the SAP noted that there are no studies that are definitive regarding a comparison of silver and nanosilver toxicity, and more research is required.[11] The SAP report therefore provides an argument against the actions EPA is proposing here, to put nanosilver on the market essentially untested, with an inadequate hazard database, while knowing that it is likely to be more hazardous than silver.

IV. Specific comments

A. EPA has not satisfied the requirements for granting AGS-20 a conditional registration

FIFRA allows EPA to grant conditional registrations for active ingredients not contained in currently registered pesticides

> for a period reasonably sufficient for the generation and submission of required data (which are lacking because a period reasonably sufficient for generation of the data has not elapsed since the Administrator first imposed the data requirement) on the

> condition that by the end of such period the Administrator receives such data and the data do not meet or exceed risk criteria enumerated in regulations issued under this subchapter, and on such other conditions as the Administrator may prescribe. A conditional registration under this subparagraph shall be granted only if the Administrator determines that use of the pesticide during such period will not cause any unreasonable adverse effect on the environment, and that use of the pesticide is in the public interest.[12]

EPA proposes to grant the conditional registration for AGS-20 claiming that insufficient time has elapsed for the generation of data since the requirement for that data was imposed; use of the pesticide during the period that the newly required data is being developed and reviewed by the Agency will not cause unreasonable adverse effects; and use of the pesticide is in the public interest. (Decision Doc at 3) However, EPA has misapplied the standard and failed to make the requisite showing to grant a conditional registration.

1) The registrant has had sufficient time to generate and submit required data

FIFRA allows EPA to grant conditional registrations of active ingredients not contained in any currently registered pesticides to allow registrants to generate and submit required data.[13] However, that data can only be lacking "because a period reasonably sufficient for generation of the data has not elapsed since the Administrator *first imposed* the data requirement," (emphasis added). As further explained in the regulations

> EPA will not approve an application for conditional registration of a pesticide containing an active ingredient not contained in any currently registered product unless data required by this part are available for EPA to review except for:
>
> (i) Those data for which the requirement has been waived.
> (ii) Those data for which the requirement was imposed so recently that the applicant has not had sufficient time to produce the data.[14]

EPA regulations specify the types of data and studies that are required for EPA to evaluate the risks or benefits of a product having a particular use pattern.[15] Some studies are absolutely required, such as genetic toxicity studies, which are used to screen chemicals for mutagenic or carcinogenic potential.[16]

Other studies are conditionally required.[17] If certain conditions apply, then the conditionally required studies must also be submitted. The burden is on applicants to evaluate those conditions "to determine whether or not conditionally required data must be submitted as indicated by the conditions and criteria specified" in the regulations.[18] For example, a 90-day inhalation toxicity (rat) study is required if use of the pesticide product may result in repeated inhalation exposure at

a concentration likely to be toxic.[19] A 21-day dermal toxicity (rat) study is required if the intended use of the pesticide product is expected to result in human exposure via skin contact.[20]

In this case, EPA proposes conditionally registering AGS-20 and identifies a list several pages long of studies that the registrant will need to submit during the conditional period. EPA explains that the conditional registration is appropriate because it only recently reached a position about what types of data are needed to evaluate the potential risks to humans and to the environment.[21]

EPA has misapplied its authority to grant conditional registrations under FIFRA. Only when a data requirement is "first imposed" so recently that a registrant is unable to generate the data in time for the registration application may EPA grant a conditional registration. For example, there are situations where EPA may require additional information be provided because those data specified in the regulations are insufficient to permit EPA to evaluate the product.[22] When that new information is requested, a registrant should be given sufficient time to generate the data. That, however, is not the case here. In many instances, this application is completely missing data that are specifically required by the regulations and which are not new requirements.

For example, genetic toxicity tests are absolutely required under 40 CFR §161.340(a). The Decision Document indicates that there are "No Data" from genetic toxicity tests, which are used to determine whether the product is a potential mutagen or carcinogen. These tests have been required since the regulations were first established in 1984.[23] As such, all registrants have had over 25 years of notice that EPA has imposed this requirement, which is more than "sufficient for generation of the data...."[24] It is a violation of FIFRA to allow registration of this product in the complete absence of these required data.

The missing conditionally required data also mean that the conditional registration cannot be granted. For example, the application is missing, *inter alia*, two conditionally required studies: 90-day inhalation toxicity data and 21-day dermal toxicity data. The applicant should have known that these conditionally required studies must be completed and submitted based on the notes in the regulations.[25] [26] First, the formulation of AGS-20 as a powder will cause occupational inhalation exposures during handling during textile treatment and during manufacturing of clothing. It is reasonably foreseeable that inhalation exposure would occur (the condition requiring a 90-day inhalation toxicity test), and the registrant should have submitted that data. Second, the use of this product on clothing means that consumer dermal exposures could occur while wearing treated textiles. Again, this is a reasonably foreseeable occurrence, and should have been considered by the registrant. The registrant has the burden of identifying that these conditional data must be submitted. These are not new data requirements. The registrant's failure to provide these data in the application means the registration cannot be granted.

2) AGS-20 may cause "unreasonable adverse effects on the environment"

To grant a conditional registration, EPA must also determine that "use of the pesticide during such period will not cause any unreasonable adverse effect on the environment." 7 U.S.C.

§136a(c)(7)(C). Such a determination includes any "unreasonable risk to man or the environment, taking into account the economic, social, and environmental costs and benefits of the use of a pesticide." 7 U.S.C. §136(bb). EPA cannot make such a determination for AGS-20.

For HeiQ AGS-20 products, EPA has already properly determined that both dermal and incidental oral exposures will occur to consumers, and particularly children, through wearing treated clothing and mouthing of treated clothing (Decision Doc, App A at 10). NRDC agrees that people wearing treated clothing will have dermal contact with the chemical, and that some mouthing of the material is highly likely to occur with infants and young children who come into contact with the clothing, either on themselves or a parent, sibling, etc. The special considerations of the impact of these exposures on children and infants must be incorporated into EPA's assessment of these unique materials. Because EPA has failed to consider or evaluate these exposures, the Agency may not make the required safety finding under FIFRA.

When considering life-stage related sensitivities to nanoparticle toxicity, the elderly also represent a vulnerable subpopulation. The EPA-funded study by Gordon et al (2008) found a significant difference in the toxicity of inhaled zinc nanoparticles on young versus old mice which varied depending on the inbred strain of mice. The old (8-12 months) mice were more sensitive than the young (2-3 months) adult mice in three inbred strains, but the opposite was true in the BtBr strain.[27] These data suggest that age-related sensitivity and genetics may be a very significant factor in the toxicity of inhaled metal nanoparticles like zinc and silver, which EPA unlawfully failed to consider it its assessment of HeiQ AGS-20.[28]

Aggregation of nanosilver is likely to be a significant influence on toxicity, which the SAP noted in its report (SAP at 10). An EPA funded study by researchers from the New York University Nelson Institute of Environmental Medicine (Gordon et al, 2008) exploring the role of particle agglomeration on nanoparticle toxicity reported that particle composition as well as size affected toxic properties.[29] The researchers tested inbred BALB/c mice exposed by inhalation. Inhaled freshly generated carbon nanoparticles (11-60 nm range) produced much greater lung inflammation than the larger-sized aged carbon nanoparticles (150-250 nm). Under identical test conditions, copper and zinc nanoparticles showed less of a difference between fresh and aged, but both metals produced 3-fold more inflammation and lung injury (measured by protein) than carbon nanoparticles, demonstrating particular concerns with the toxicity of agglomerated metal-based nanoparticles. These effects seemed to override size differences, because even the larger-sized aged and agglomerated copper nanoparticles (approximately 200 nm diameter) produced significantly more lung inflammation than freshly generated carbon nanoparticles of a comparatively smaller size (11-60 nm). This demonstrates the exquisite immunotoxicity potential of metal-based nanoparticles like copper, zinc, and silver. Other published studies by independent scientists have also identified inflammation and immunotoxicity as a very sensitive, possibly the most sensitive endpoint of toxicity for metal-based nanomaterials including nanosilver.[30] EPA unlawfully failed to consider immunotoxicity in its assessment of HeiQ AGS-20, and therefore may not make the required safety finding under FIFRA.

3) Use of the pesticide is not in the public's interest.

In order to grant a conditional registration, EPA must also determine that use of the pesticide is in the public interest.[31] EPA has sided with the registrant that the registration of this product benefits the public based on four points: 1) conservation of the environment, 2) consumer benefits, 3) market equity and international trade, and 4) innovation. (Decision Doc 28-30) EPA provides no actual economic calculations or numerical data to support its finding for any of these points. Rather, the determination is based on supposition, conjecture, untested assumptions, and unproven claims. EPA also entirely ignores the public interest in not registering AGS-20 before its safety has been established, as required by law.

For the first claim, EPA notes that silver is already a registered pesticide, and that compared with normal-scale silver, the volume of silver in HeiQ's product is reduced. EPA argues that by making nanosilver available, less overall silver (by mass) will be released into the environment (Decision Doc at 28-29). This argument is false logic, a red herring, since nanosilver is much more potent (effective) – that is, less nanosilver kills more microbes. While AGS-20 may possibly lead to a reduction in the overall mass of silver released into the environment, its killing potential is greater and therefore the potential for environmental damage and non-target impacts is greater. In fact, the SAP noted in its 2010 report that the rate and concentration of deadly silver ions released from nanosilver is different and will likely affect the acute or chronic toxicity of nanosilver compared with silver.[32] The SAP referenced data showing that nanosilver, but not silver, can penetrate cell membranes and deliver toxic ions directly inside of cells and that this may be its mechanism for killing microbes so effectively.[33] The SAP also noted that "when compared as a function of silver ion concentration, the toxicity of silver nanoparticles appeared to be much higher than that of silver nitrate."[34] Moreover, the SAP noted that because of these differences in chemical properties, there are likely to be differences in exposure and environmental fate of nanosilver that should be considered.[35]

The second claim of consumer benefits is also false logic. EPA claims that consumers will benefit because the nanosilver product is a more effective antimicrobial, and therefore consumers owning textiles treated with the product will enjoy more durable, longer-lasting antimicrobial protection. But, EPA has not explained why consumers need antimicrobial textiles. In fact, the textiles that nanosilver products are being used in are mostly unnecessary and lead to potentially harmful exposures. For example, sports clothing that may stink less, camping clothing that may stink less, and towels and bed sheets that are touted to have less germs. This is a marketing campaign that targets consumers who mistakenly believe all microbes are harmful – not unlike the pre-1970s advertising campaigns of the leaded paint industry that marketed deadly leaded paints for children's toys and furniture by associating brighter colors and whiter whites with cleanliness and better health.[36] In fact, our bodies are covered with beneficial bacteria and microbes – little "germs" that eat away our dead hair and skin, help us digest food, and fight off other bacteria.

The third claim is market equity. EPA makes two arguments. First, it argues that the Agency may conditionally register pesticides that are identical or substantially similar to currently registered pesticides or pesticides that differ only in ways that would not significantly increase the risk of unreasonable adverse effects on the environment (Decision Doc at 29-30). This is inapposite. Compared with silver, nanosilver releases more ions and is therefore more toxic, more biologically active, more deadly to microbes, and more persistent in the environment

(Decision Doc at 28). Second, EPA argues that other products that are on the market were registered as silver, but are now known to contain nanosilver. EPA states that although the registrations were approved without EPA's knowledge that nanosilver was a component of the product, it would give HeiQ an unfair disadvantage to deny its registration while its competitors are already on the market. This has nothing to do with the public interest; it goes only to the private financial interest of the registrant. EPA should not allow *any* nanosilver pesticide to be in commercial products. It is an off-label use, and therefore illegal. EPA has stated that nanosilver is not silver, and therefore it must be reviewed and registered under FIFRA separately from silver.[37] William Jordan, Senior Policy Advisor, OPP, made clear at the April, 2010 public meeting of the Pesticide Program Dialogue Committee EPA's intention to issue a Federal Register notice clarifying the Agency's position that the presence of a nanoscale material is reportable under FIFRA Section 6(a)(2) and that an active or inert ingredient would be considered "new" if it is a nanoscale material.[38] Moreover, at the same meeting Mr. Jordan announced that the EPA would respond to the May 2008 Citizen's Petition on nanosilver that requested that the EPA take action on some 600 unregistered nanosilver pesticidal products.[39] The SAP also voiced its scientific opinion that the toxic profile of nanosilver is likely to differ from silver in many significant ways, including rate of ion release, environmental fate, chemical reactivity, agglomeration, and distribution in biological tissues.[40] EPA should be issuing fines for violation of FIFRA to those companies that keep their products on the market without a lawful registration, not giving companies a free pass.

Innovation is EPA's fourth argument that the registration of AGS-20 is in the public's interest. EPA bemoans the high costs of regulatory requirements, including new data generation, on the registrants and even frets that these regulatory costs may "discourage technology providers from pursuing the development of beneficial new applications of nanotechnology in the field of pesticides." (Decision Doc at 30). This argument is presented without any supporting data, or even "guesstimates" of the actual costs and benefits of this "innovative" technology. Pesticides are inherently hazardous. In passing FIFRA, Congress mandated that all pesticides go through a rigorous pre-market chemical risk assessment and product registration process, which must be repeated every fifteen years. The costs of research and development should include the development of statutorily mandated pre-market hazard data. EPA must apply and enforce these requirements under FIFRA.

The conditional registration of AGS-20 is not in the public interest. It provides no measurable medical or health benefits to consumers, but puts them in harm's way. There seems to be little doubt, including with EPA, that workers will inhale the powdered pesticide during manufacturing processes, that consumers will come in direct contact with the pesticide while wearing treated clothing, and that children have a high likelihood of ingesting the pesticide while mouthing the clothing in addition to direct dermal contact. Because the proposed application is to treat textiles that consumers will come into direct contact with, exposure will be unavoidable. The potential harm from such contact is poorly understood and untested, which is a direct violation of FIFRA requiring that safety findings be made. Moreover, release of the silver ions into the water waste stream from nanosilver-impregnated clothing through routine washing will pose a threat to aquatic ecosystems, aquatic food webs where bioaccumulation may occur, and embryonic fish that may be killed by even ppt levels of silver ions. It is also possible that nanosilver may impair the beneficial microbial systems that are used to treat sewage, leaving

waste water plant effluent highly contaminated and unsafe. These harms are more than speculative. They can be reasonably presumed to occur in at least some individuals and ecosystems, based on what we know about silver toxicity and about the strong likelihood that nanosilver is more harmful than silver to non-target aquatic species and beneficial microbes.[41]

B. Time to generate data should not be time on the market

In addition to violating the law, EPA's proposed conditional registration is also irresponsible. With the amount and the importance of the data that EPA is requiring (see Decision Doc, Appendix A for full list), it is unacceptable that EPA has given this product market access, conditionally or otherwise. EPA recognizes the inherent hazards of nanomaterials generally, and nanosilver specifically, commenting that the inhalation of other nanoparticles has led to pulmonary fibrosis, that workers could be exposed during handling of the powder, and consumers will be exposed dermally through wearing treated clothing. EPA comments that "there is a potential for children's incidental oral exposure to AGS-20 during the wearing and mouthing of treated clothing." triggering the requirement for reproductive/developmental studies (Decision Doc, Appendix A at 10).

In addition, EPA does not appear to have a reliable tracking system to identify when required data for a conditional registration are still missing, identify and sort data that has been received, review the data and record the Agency staff conclusions, and incorporate the incoming data into the chemical assessment and product registrations in a timely and appropriate manner that reflects any hazards identified in the data. Therefore, products and technical products that are conditionally registered float through the registration system without any transparent or public access to assurances that the registrant has supplied the data that the registration was conditioned upon, or that those data have been reviewed and appropriately incorporated into the chemical registration. As such, EPA cannot reliably assure the public or itself that the conditions of the registration will be met because the agency's conditional registration program is so disorganized.

C. Other nano-scale antimicrobials are on the market without having undergone a full chemical risk assessment on the nano-scale material

Nanosilver is not the only nano-scale antimicrobial that enjoys unregistered illegal widespread commercial use. There are other nano-metal pesticides that are commercialized, but not yet registered or safety tested. For example, Osmose, Inc., a wood preservation technologies company, advertizes nano-scale "micronized" copper-based biocides for wood treatment. A product report on their website confirms that an analysis of the treated wood "revealed the presence of nano-sized copper and iron particles (from grinding media) ranging from 10 to 700 nm in micronized treated wood...."[42] This product is already in widespread use; a 2009 media release from the manufacturer on the safety and performance of micronized copper technology boasts that "over 5 billion board feet of MicroPro treated wood has been sold since the product introduction in 2006."[43] The company claims that its micronized technologies are certified as an Environmentally Preferable Product (EPP), suggesting that its products are safe for the environment.[44] However, it appears that EPA has never been provided with any safety data for the nano-scale "micronized" formulation of this wood treatment biocide.

D. Problem with conditional registration generally

EPA has overused conditional registrations, as they now represent the majority of active registrations. The EPA Office of Pesticide Programs (OPP) has over 16,000 pesticide product active registrations (that is, currently registered). Of these, over 11,000 (68%) are conditionally registered.

Although the proportion of active registrations that are conditional is disturbing, it is not a new occurrence. This is a long-standing pattern with EPA's pesticide office. Based on a search through the registration database[45], of the 16,000 active products registrations:

- Almost 8,200 products have been conditionally registered ("CR status") since 2005,
- Approximately 5,400 products have had CR status since 2000,
- Over 3,200 products have had CR status since 1995, for 15 years.
- Over 2,100 products have had CR status since 1990, over 20 years.
- Over 800 technical products (that is, pure active ingredient), currently have CR status.

These astoundingly high numbers of product registrations that are still conditionally registered, even after so many years, raise several concerns.

First, it calls into question EPA's assertion that, "Ultimately, the Agency will use these data to determine whether the ingredient can be registered under FIFRA Section 3(c)(5)" when over 2000 active product registrations are conditionally registered for twenty years and almost 70% are conditionally registered at this time. (Decision Doc at 36).

Second, and more importantly, OPP may not be meeting its legal requirements under FIFRA to review each chemical every 15 years, address the hazards through mitigation measures, and incorporate the required mitigation through label amendments to address identified risks.[46] Instead, it appears that several thousand chemicals are "hiding out" in conditionally registered status, possibly avoiding registration review, while staying on the market despite significant data gaps. At a minimum, it shows an inexcusable lack of transparency and public accountability.

EPA must not register a pesticide until all the required data is provided, reviewed by EPA, and integrated into the chemical (ai) risk assessment and the product registration.

IV. Conclusion

EPA's proposed conditional registration of AGS-20 would violate FIFRA. EPA must cease allowing untested nanomaterials to flood consumer markets. EPA has misused its authority under FIFRA and has misinterpreted the use of conditional registrations. Further, EPA has failed to show that the use of AGS-20 will not cause unreasonable adverse effects and has failed to show that use of AGS-20 is in the public interest. In fact, the opposite is true – use of nanosilver-treated textiles is expected to lead to human exposures, environmental releases, and harm to non-

target beneficial microbes. EPA must withdraw this proposal, and instead issue fines against companies that are marketing nanosilver pesticidal products without having undergone the full registration process. If EPA were to do this, it is possible that the companies would work together to share the costs of generating the data required for a proper and complete registration application, leading to an even playing field for registrants, better data for EPA, and increased public confidence that pesticides are being regulated as the law requires.

Thank you for the opportunity to provide comments.

Respectfully,

Jennifer Sass, Ph.D., Senior Scientist, NRDC
Mae Wu, JD, Staff Attorney, NRDC

These comments are supported by the following organizations:

Alaska Community Action on Toxics (Pamela Miller, Exec Dir)
Beyond Pesticides (Jay Feldman)
Center for Environmental Health (Caroline Cox)
Center for Food Safety (George Kimbrell)
Consumers Union (Michael Hansen, PhD)
Environmental Working Group (Jane Houlihan, PhD)
ETC Group (Kathy Jo Wetter, PhD)
Food & Water Watch (Wenonah Hauter, Exec Dir)
Friends of the Earth US (Ian Illuminato)
International Center for Technology Assessment (Jaydee Hanson)
Madison Environmental Justice Organization (Maria Powell)
Nanotechnology Citizen Engagement Organization (Mathilde Colin)
Organic Consumers Association (Alexis Baden-Mayer, Esq.)
Pesticide Action Network North America (Karl Tupper)
TEDX The Endocrine Disruption Exchange (Theo Colborn, PhD)

---

[1] SAP report, 2010. Memorandum from the FIFRA Scientific Advisory Panel meeting held November 3-5, 2009 on the Evaluation of Hazard and Exposure Associated with Nanosilver and Other Nanometal Pesticide Products to EPA. January 26, 2010. http://www.epa.gov/scipoly/sap/meetings/2009/november/110309ameetingminutes.pdf

[2] 7 U.S.C. §136a(a).

[3] Luoma SN. Silver nanotechnologies and the environment: old problems or new challenges. A report by Woodrow Wilson International Center for Scholars and The Pew Charitable Trusts. 2008.

http://www.pewtrusts.org/uploadedFiles/wwwpewtrustsorg/Reports/Nanotechnologies/Nano_PEN_15_Final.pdf

[4] Luoma SN. Silver nanotechnologies and the environment: old problems or new challenges. A report by Woodrow Wilson International Center for Scholars and The Pew Charitable Trusts. 2008. http://www.pewtrusts.org/uploadedFiles/wwwpewtrustsorg/Reports/Nanotechnologies/Nano_PEN_15_Final.pdf

[5] Luoma SN. Silver nanotechnologies and the environment: old problems or new challenges. A report by Woodrow Wilson International Center for Scholars and The Pew Charitable Trusts. 2008. http://www.pewtrusts.org/uploadedFiles/wwwpewtrustsorg/Reports/Nanotechnologies/Nano_PEN_15_Final.pdf

[6] EPA RED Facts. Silver. June, 1993. http://www.epa.gov/oppsrrd1/REDs/factsheets/4082fact.pdf

[7] EPA RED Facts. Silver. June, 1993. http://www.epa.gov/oppsrrd1/REDs/factsheets/4082fact.pdf

[8] Papp T, Schiffmann D, Weiss D, Castranova V, Vallyathan V, Rahman O. Human health implications of nanomaterial exposure. Nanotoxicology, 2008. 2:1, 9 - 27

[9] SAP report at 10

[10] Braydich-Stolle L, Hussain S, Schlager JJ, Hofmann MC. In vitro cytotoxicity of nanoparticles in mammalian germline stem cells. Toxicol Sci. 2005 Dec;88(2):412-9. Epub 2005 Jul 13.

[11] SAP report at 10

[12] 7 U.S.C. § 136a(c)(7)(C).

[13] 7 U.S.C. § 136a(c)(7)(C).

[14] 40 CFR § 161.30(b)(2).

[15] 40 CFR § 161.101.

[16] 40 CFR §161.340.

[17] 40 CFR Part 161, Subpart D.

[18] 40 CFR § 161.101(b).

[19] 40 CFR 161.340(a), note (6).

[20] 40 CFR 161.340(a), note(18).

[21] Decision Doc at 3.

[22] 40 CFR § 161.35(c).

[23] 49 Fed. Reg. 42856 (Oct. 24, 1984).

[24] 7 U.S.C. § 136a(c)(7)(C).

[25] 40 CFR 161.340(a), note (6).

[26] 40 CFR 161.340(a), note(18).

[27] Gordon, T., Lung Chi, C., Cohen, Beverly S. Final Report: Role of particle agglomeration in nanoparticle toxicity. EPA Grant No. R832528. 2008. http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/7814/report/F

[28] Gordon, T., Lung Chi, C., Cohen, Beverly S. Final Report: Role of particle agglomeration in nanoparticle toxicity. EPA Grant No. R832528. 2008. http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/7814/report/F

[29] Gordon, T., Lung Chi, C., Cohen, Beverly S. Final Report: Role of particle agglomeration in nanoparticle toxicity. EPA Grant No. R832528. 2008. http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/7814/report/F

[30] UConn News. Scientists study health implications of products using nanosilver. February 18, 2009. http://news.uconn.edu/2009/February/rel09019.html

ScienceNews. Nanosilver disinfects – but at what price? Janet Raloff. November 30, 2008. http://www.sciencenews.org/view/generic/id/38913/title/Nanosilver_disinfects_%E2%80%94_but_at_what_price%3F

Perkins, C., M. Levin and S. DeGuise. 2008. The Immunomodulatory Effects of Nanosilver Upon in vitro Exposure. Society of Environmental Toxicology & Chemistry North American annual meeting: Tampa (Nov. 20).

Shin, S.-H., et al. 2007. The Effects of Nano-Silver on the Proliferation and Cytokine Expression by Peripheral Blood Mononuclear Cells. International Immunopharmacology 7(Dec. 15):1813

[31] 7 U.S.C. 136a(c)(7)(C).

[32] Memorandum from the FIFRA Scientific Advisory Panel meeting held November 3-5, 2009 on the Evaluation of Hazard and Exposure Associated with Nanosilver and Other Nanometal Pesticide Products to EPA. January 26, 2010, p 10. http://www.epa.gov/scipoly/sap/meetings/2009/november/110309ameetingminutes.pdf

[33] Ibid.

[34] Ibid.

[35] Ibid at 11.

[36] Cincinnati Children's Hospital. History of lead advertising: Do not forget the children. See as an example, the children's paint book issued by the lead paint industry titled. "The Dutch boy conquers old man gloom" (1929). http://www.cincinnatichildrens.org/research/project/enviro/hazard/lead/lead-advertising/children.htm

[37] Washington Post. EPA to regulate nanoproducts sold as germ-killing. Rick Weiss, Nov 23, 2006. http://www.washingtonpost.com/wp-dyn/content/article/2006/11/22/AR2006112201979.html

PPDC discusses nanotechnology and pesticides. Blog by Lynn L. Bergeson. May 5, 2010 http://nanotech.lawbc.com/2010/05/articles/united-states/federal/ppdc-discusses-nanotechnology-and-pesticides/

[38] Jordan, W. Nanotechnology and Pesticides. Pesticide Program Dialogue Committee, April 29, 2010. http://www.epa.gov/pesticides/ppdc/2010/april2010/session1-nanotec.pdf

[39] Jordan, W. Nanotechnology and Pesticides. Pesticide Program Dialogue Committee, April 29, 2010. http://www.epa.gov/pesticides/ppdc/2010/april2010/session1-nanotec.pdf

[40] Memorandum from the FIFRA Scientific Advisory Panel meeting held November 3-5, 2009 on the Evaluation of Hazard and Exposure Associated with Nanosilver and Other Nanometal Pesticide Products to EPA. January 26, 2010. http://www.epa.gov/scipoly/sap/meetings/2009/november/110309ameetingminutes.pdf, pg 6-9.

[41] Senjen R and Illuminato IS. Nano and Biocidal Silver: Extreme Germ Killers Present a Growing Threat to Public Health. Rep. Washington, DC: Report by Friends of the Earth, 2009.

[42] A comprehensive review of copper-based wood preservatives with a focus on micronized or dispersed copper systems. MH Freeman and CR McIntyre. 2008. Forest Prod. J. 58(11): 6-27. http://www.osmosewood.com/micropro/images/Nov08-FPJ-Feature.pdf

[43] Osmose and MicroPro media release. Consumer safety and product performance of micronized copper technology confirmed. February 10, 2009. www.osmosewood.com/pressroom/images/Osmose%20Press%20Release%20Feb%2010%202009.pdf

[44] Osmose and MicroPro media release. Consumer safety and product performance of micronized copper technology confirmed. February 10, 2009. www.osmosewood.com/pressroom/images/Osmose%20Press%20Release%20Feb%2010%202009.pdf

[45] National Pesticide Information Retrieval System, NPIRS Public. http://ppis.ceris.purdue.edu/

[46] 7 U.S.C. § 136a(g)

Published Online First on November 3, 2009 as 10.1158/0008-5472.CAN-09-2496

Titanium Dioxide Nanoparticles Induce DNA Damage and Genetic Instability *In vivo* in Mice

Benedicte Trouiller,[1] Ramune Reliene,[1,3] Aya Westbrook,[1,4] Parrisa Solaimani,[1,4] and Robert H. Schiestl[1,2,5]

[1]Departments of Pathology and Laboratory Medicine, [2]Radiation Oncology, and [3]Medicine, Center for Human Nutrition, David Geffen School of Medicine; [4]Interdepartmental Program in Molecular Toxicology; [5]Department of Environmental Health Sciences, School of Public Health, University of California-Los Angeles, Los Angeles, California

Abstract

Titanium dioxide (TiO_2) nanoparticles are manufactured worldwide in large quantities for use in a wide range of applications including pigment and cosmetic manufacturing. Although TiO_2 is chemically inert, TiO_2 nanoparticles can cause negative health effects, such as respiratory tract cancer in rats. However, the mechanisms involved in TiO_2-induced genotoxicity and carcinogenicity have not been clearly defined and are poorly studied *in vivo*. The present study investigates TiO_2 nanoparticles–induced genotoxicity, oxidative DNA damage, and inflammation in a mice model. We treated wild-type mice with TiO_2 nanoparticles in drinking water and determined the extent of DNA damage using the comet assay, the micronuclei assay, and the γ-H2AX immunostaining assay and by measuring 8-hydroxy-2′-deoxyguanosine levels and, as a genetic instability endpoint, DNA deletions. We also determined mRNA levels of inflammatory cytokines in the peripheral blood. Our results show that TiO_2 nanoparticles induced 8-hydroxy-2′-deoxyguanosine, γ-H2AX foci, micronuclei, and DNA deletions. The formation of γ-H2AX foci, indicative of DNA double-strand breaks, was the most sensitive parameter. Inflammation was also present as characterized by a moderate inflammatory response. Together, these results describe the first comprehensive study of TiO_2 nanoparticles–induced genotoxicity *in vivo* in mice possibly caused by a secondary genotoxic mechanism associated with inflammation and/or oxidative stress. Given the growing use of TiO_2 nanoparticles, these findings raise concern about potential health hazards associated with TiO_2 nanoparticles exposure. [Cancer Res 2009;69(22):8784–9]

Introduction

Titanium dioxide (TiO_2) accounts for 70% of the total production volume of pigments worldwide (1). It is widely used to provide whiteness and opacity to products such as paints, plastics, papers, inks, food colorants, and toothpastes. TiO_2 is also used in cosmetic and skin care products, particularly in sunblocks, where it helps to protect the skin from UV light, especially in the case of nanosized particles (<100 nm). Nevertheless, TiO_2 has recently been reclassified by the IARC as group 2B carcinogen: "possibly carcinogenic to humans".[5] The reason for this new classification stems from the fact that high concentrations of pigment-grade (<2.5 μm) and ultrafine (<100 nm) TiO_2 dust can cause respiratory tract cancer in exposed rats (2, 3). However, it should be noted that epidemiologic studies of workers exposed to pigment-grade TiO_2 conducted thus far have not been able to detect an association between occupational exposure to TiO_2 and an increased risk for lung cancer (4, 5). Genotoxicity studies that measure different types of DNA damage (e.g., gene mutations, chromosomal damage, and DNA strand break formation) are an important part of cancer research and risk assessment of potential carcinogens. These studies help to understand possible mechanisms causing tumor induction. As such, *in vivo* mechanisms underlying TiO_2 nanoparticles tumor induction are still unclear.

Because nanoparticle diameter does not exceed a hundred nanometers at maximum, they are able to penetrate cells (6) and interfere with several subcellular mechanisms. Indeed, some studies show that some nanoparticles can penetrate into cell nuclei and hence may directly interfere with the structure and function of genomic DNA (7). Additionally, after oral administration in mice, TiO_2 particles were shown to translocate to systemic organs such as liver and spleen as well as lung and peritoneal tissues (8). Genotoxicity studies have been done to understand the carcinogenic potential of TiO_2 nanoparticles using assays that measure mutations in genes (e.g., Ames/*Salmonella* and hypoxanthine guanine phosphoribosyl transferase (Hprt) assays; refs. 9–11), chromosomal damage representing possible clastogenic activity of the particles (e.g., micronuclei; refs. 10, 12–15), and DNA strand breakage (e.g., alkaline comet assay; refs. 10, 13). Except for one, these studies were conducted *in vitro* in cultured cells but conflict in their results. Half of the studies show that TiO_2 nanoparticles are genotoxic in cell lines (10, 12, 13, 15), whereas the other half show that TiO_2 nanoparticles are not (9, 11, 14). The rationale for these conflicting results is not clear because different cell types, doses, and nanoparticle sizes have been used. Some studies suggest possible mechanisms for TiO_2 nanoparticles genotoxicity. TiO_2 nanoparticles might damage DNA directly or indirectly via oxidative stress and/or inflammatory responses. Two recent studies show a direct chemical interaction between TiO_2 nanoparticles and DNA, through the DNA phosphate group, but a link to mutagenesis has not been proven (16, 17). On the other hand, other studies show that TiO_2 nanoparticles can cause DNA damage indirectly through inflammation (18–21) and generation of reactive oxygen species (12, 13, 22, 23).

Requests for reprints: Robert H. Schiestl, Department of Pathology, University of California-Los Angeles School of Medicine, 650 Charles E. Young Drive South, Los Angeles, CA 90095. Phone: 310-267-2087; Fax: 310-267-2578; E-mail: rschiestl@mednet.ucla.edu.

doi:10.1158/0008-5472.CAN-09-2496

[5]IARC. Monographs on the evaluation of carcinogenic risks to humans. Carbon black, titanium dioxide and non-asbestiform talc. Lyon (France): IARC. In press.

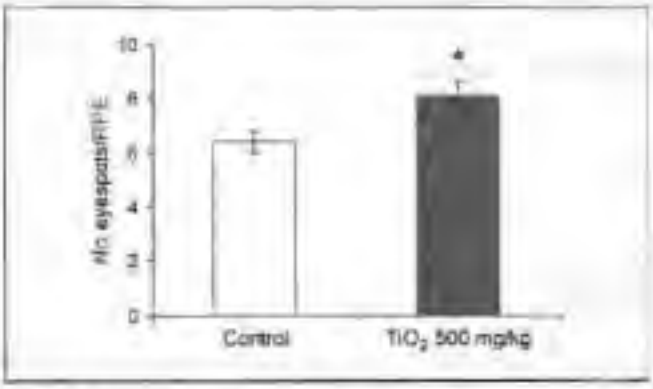

Figure 1. Frequency of DNA deletions in control and TiO_2 nanoparticles–treated mice. One RPE corresponds to one eye. Mice were treated with nanoparticles during embryonic development at a total dose of 500 mg/kg. Mean ± SE numbers of eyespots per RPE with $n = 42$ eyes for control and $n = 53$ eyes for TiO_2 nanoparticles–treated mice. *, $P < 0.05$.

Thus far, most nanoparticle genotoxicity studies have focused on cell culture systems, but confirmation from animal experiments, more relevant to human exposure, is required. To further understand TiO_2 nanoparticles toxicity *in vivo*, we studied the effect of TiO_2 nanoparticles exposure on genotoxicity, DNA damage, and inflammation in mice. To evaluate inflammation in mice, we determined mRNA expression of both proinflammatory and anti-inflammatory cytokines. To assess DNA damage, we used the γ-H2AX and the comet assays to evaluate DNA strand breaks, the micronuclei assay to estimate chromosomal damage, and the measure of 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels using high-performance liquid chromatography to determine oxidative DNA damage. We also used an *in vivo* DNA deletion assay, which allows visual detection of DNA deletion events within the *pink-eyed unstable* (p^{un}) locus in developing mouse embryos (24), which can detect environmental as well as genetic cancer predisposing factors (24). Our results show that TiO_2 P25 nanoparticles can induce 8-OHdG, γ-H2AX foci, micronuclei, DNA deletions, and inflammation markers in a mice model. Therefore, this study suggests that TiO_2 nanoparticles are genotoxic *in vivo*.

Materials and Methods

Mouse care and breeding. C57BL/6Jp^{un}/p^{un} mice were obtained from The Jackson Laboratory. The C57BL/6Jp^{un}/p^{un} background is essentially identical to C57BL/6J, with the exception of a naturally occurring 70-kb internal duplication in the *pink-eyed dilution* (*p*) gene, termed the p^{un} allele. Mice were housed and cared for under standard specific pathogen-free conditions and according to the Animal Rescue Coalition and Institutional Animal Care and Use Committee regulations. Mice were given a standard, autoclaved diet (from Harlan Teklad (Harlan Teklad No. 8656) and sterilized water *ad libitum*. Mice were housed in a 12 h light/dark cycle. Pregnancy was timed by checking for vaginal plugs, with noon of the day of discovery counted as 0.5 days post-coitum. Four- to 5-month-old mice were used for all experiments.

TiO_2 nanoparticles preparation and exposure. "Aeroxide" P25 TiO_2 (Degussa, now Evonik) nanoparticles were chosen for this study. The crystal structure is a mixture of 75% anatase and 25% rutile TiO_2, purity was at least 99.5% TiO_2, and primary particle size was 21 nm with a specific surface area of 50 ± 15 m^2/g. These nanoparticles have been used in many of the previous mammalian studies (13, 14, 22, 25–28). Using dynamic light scattering in water revealed that the size of TiO_2 nanoparticles agglomerates ranged from 21 to 1,446 nm and the mean size was 160 ± 5 nm. About 70% of particles have a size of 160 nm. Solutions of dispersed TiO_2 nanoparticles were prepared by ultrasonication (Solid State/Ultrasonic FS-14, Fisher Scientific) for 15 min in drinking water at 60, 120, 300, and 500 μg/ml concentrations just before use. We measured TiO_2 nanoparticles–supplemented water intake at the end of experiments in each cage, which housed 2 to 3 mice, and calculated an average daily water intake per mouse. Daily TiO_2 nanoparticles–supplemented water intake ranged from 3 to 7 ml/mouse, consistent with normal daily water intake. Doses were calculated using a 30 g average weight per mouse, and an average of 5 mL water intake per day. The exposure was 5 days in adult males. For *in utero* exposure, pregnant dams were given nanoparticles-supplemented drinking water for 10 days from 8.5 to 18.5 days post-coitum at a concentration of 300 μg/mL. Water was used as negative control.

***In vivo* DNA deletion assay.** To evaluate genotoxicity of TiO_2 nanoparticles, we employed an intrachromosomal duplication of 70-kb fragment spanning exons 6 to 18 of the *p* gene in mice (termed p^{un} mutation). When a DNA deletion event occurs between these duplications, the p^{un} allele reverts to the wild-type *p* gene. Reconstitution of the wild-type *p* gene can be seen as a single pigmented cell or a clone of pigmented cells on the unpigmented retinal pigment epithelium (RPE) in the transgenic mice and represents a DNA deletion as a permanent genotoxic event (29). Pregnant mice were treated with TiO_2 nanoparticles, and offspring were sacrificed at age 20 days. Their eyes were extracted and dissected to display the RPE for the deletion/eyespot assay as described previously (24). One RPE corresponds to one eye.

Alkaline comet assay. Peripheral blood was collected by submandibular vein puncture (before treatment and after treatment) in an EDTA-coated tube. The comet assay was done as described previously (30). On average, from three slides, 150 to 200 randomly captured comets per sample were analyzed. Results were expressed as average ± SE tail moment.

Micronuclei assay. The micronuclei assay was done as described elsewhere (31). Three microliter aliquots of the peripheral blood were collected as described above and smeared on slides and stained into Giemsa stain for 15 min. Approximately 2,000 erythrocytes were scored per animal to estimate the frequency of micronucleated erythrocytes.

Bone marrow preparation. Animals were sacrificed with an overdose of isoflurane after 5 days of treatment with TiO_2 nanoparticles in drinking water. Both femurs were dissected, and marrow cells were flushed out with 1 mL PBS and pipetted several times. The cell suspension was centrifuged at 3,000 rpm for 5 min, the supernatant was withdrawn, and the cell pellet was resuspended and placed on a clean glass slide.

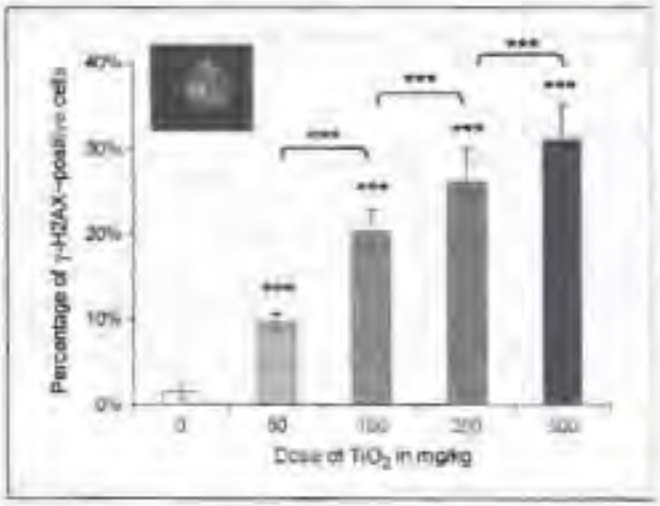

Figure 2. Percentage of γ-H2AX–positive cells in bone marrow in untreated and TiO_2 nanoparticles–treated mice and a picture of a γ-H2AX–positive cell with more than four foci. Columns, mean of 5 mice; bars, SE. ***, $P = 0.001$. TiO_2 nanoparticles–treated versus control.

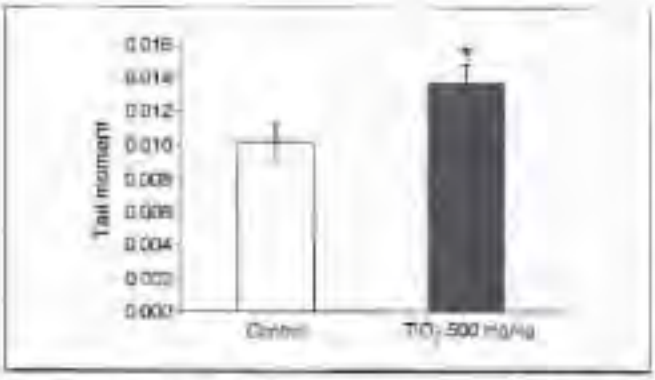

Figure 3. Frequency of DNA strand breaks in mice before and after treatment with 500 mg/kg TiO_2 nanoparticles. DNA damage is represented by the tail moment. Mean ± SE. ($n = 5$ mice/group). *, $P < 0.05$, compared with untreated mice.

γ-H2AX assay, RNA isolation, and quantitative real-time PCR. The γ-H2AX assay was done with bone marrow cells and the RNA isolation for quantitative real-time PCR was carried out on peripheral blood. These assays were done as described elsewhere (31).

Determination of oxidative DNA damage by measuring 8-OHdG. Mouse livers were isolated just after 5 days of treatment with nanoparticles and immediately frozen in liquid nitrogen and homogenized under liquid nitrogen. 8-OHdG level was measured using high-performance liquid chromatography with electron capture detection system as described previously (32).

Statistical analysis. For the deletion assay, the comet assay, the micronuclei assay, and 8-OHdG and mRNA levels of cytokines, we used the Student's t test to compare untreated mice with treated mice. For the γ-H2AX experiment, the percentage of positive cells for control groups versus treated groups was compared via χ^2 test. In addition to the t test, for comet assay data, the Wilcoxon test for matched paired data was also used to compare the effect of TiO_2 nanoparticles on the tail moment before and after nanoparticle treatment. The difference was considered significant at the 95% confidence level ($P < 0.05$) and highly significant at the 99% confidence level ($P < 0.01$).

Results

TiO_2 nanoparticles increased the frequency of DNA deletions. We used the DNA deletion assay to evaluate *in vivo* genotoxicity of TiO_2 nanoparticles. We quantified the number of eyespots per RPE as a measure of DNA deletions in *in utero* exposed mice. TiO_2 nanoparticles–treated mice had an average of 8.13 ± 1.70 eyespots per RPE versus 6.42 ± 1.47 eyespots per RPE in untreated mice (Fig. 1). TiO_2 nanoparticles–exposed mice displayed a significant increase in eyespots (27%) compared with unexposed mice ($P = 0.019$), suggesting that, after maternal oral exposure, TiO_2 nanoparticles increased DNA deletion frequency in fetuses.

TiO_2 nanoparticles induced γ-H2AX foci. Phosphorylation of histone H2AX on serine 139 occurs at sites flanking DNA double-strand breaks (DSB), providing a measure of the number of DSBs within a cell (33). We used this assay to compare DSB formation in bone marrow of mice with and without TiO_2 nanoparticles treatment.

The γ-H2AX foci formation increased by ~10%, 20%, 25%, and 30% following treatment with 50, 100, 250, and 500 mg/kg TiO_2 nanoparticles, respectively, compared with untreated mice (Fig. 2). Percentage of γ-H2AX–positive cells increased with TiO_2 nanoparticles concentration in a clear dose-dependent manner ($P < 0.001$). These data provided evidence that, after oral administration TiO_2 nanoparticles induce DSBs in bone marrow cells.

TiO_2 nanoparticles increased DNA strand breaks. DNA strand breaks (DSBs, single-strand breaks, and/or strand breaks induced by alkali-labile sites) were measured by the alkaline comet assay in mice peripheral blood before and after treatment. Tail moment significantly increased after TiO_2 nanoparticles treatment (Fig. 3). The average tail moment was 0.0102 ± 0.001 before treatment and 0.0137 ± 0.0011 after TiO_2 nanoparticles treatment. TiO_2 nanoparticles increased DNA strand breaks in WBCs from peripheral blood by 34% ($P = 0.001$, t test, and $P = 0.04$, Wilcoxon test).

TiO_2 nanoparticles induced micronuclei. The micronuclei assay was used to detect chromosomal damage in erythrocytes from peripheral blood. The incidence of micronuclei serves as an index of clastogenicity. Micronuclei frequency increased significantly only at the highest (500 mg/kg) dose of TiO_2 nanoparticles used ($P = 0.009$; Fig. 4). At this dose, the average micronuclei frequencies for untreated mice were 4.3 ± 0.93 versus 9.2 ± 1.07 per 2,000 RBC for TiO_2 nanoparticles–treated mice, which resulted in a 2.1-fold increase in micronuclei formation. This result showed that, at high dose, TiO_2 nanoparticles induced detectable clastogenicity in mice peripheral blood.

TiO_2 nanoparticles induced oxidative DNA damage. We examined the degree of oxidative DNA damage by measuring the level of 8-OHdG in DNA isolated from TiO_2 nanoparticles–treated and untreated mouse livers. The level of 8-OHdG was significantly higher in TiO_2 nanoparticles–treated than untreated mice ($P = 0.04$; Fig. 5). The average number of 8-OHdG per 10^6 dG was 4.25 ± 0.66 for untreated mice and 6.43 ± 0.58 for TiO_2 nanoparticles–treated mice resulting in a 1.5-fold increase at 500 mg/kg TiO_2 nanoparticles. This suggested that TiO_2 nanoparticles induced oxidative DNA damage in liver.

TiO_2 nanoparticles induced a proinflammatory response. We quantified mRNA transcripts of three Th1/proinflammatory cytokines (T-helper cell type 1) and three Th2/anti-inflammatory cytokines (T-helper cell type 2) in the peripheral blood. After treatment, the proinflammatory cytokines tumor necrosis factor-α, IFN-γ, and the mouse orthologue of interleukin-8 (KC) were significantly upregulated ($P = 0.01$, 0.02, and 0.05, respectively; Fig. 6A).

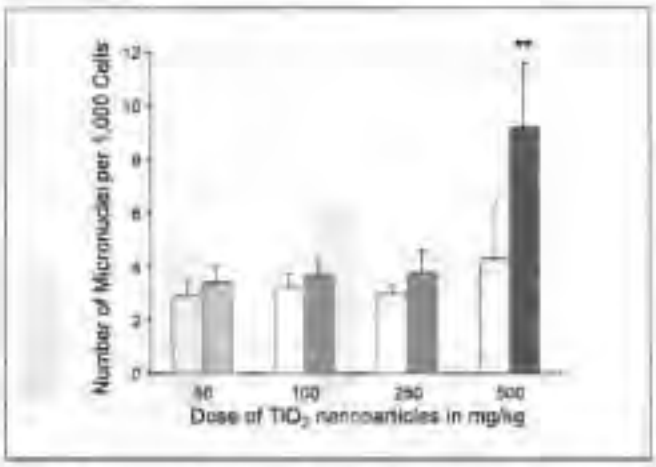

Figure 4. Frequency of micronuclei in mice before and after TiO_2 nanoparticles treatment in peripheral blood erythrocytes. Open columns, untreated controls; gray columns, TiO_2 nanoparticles–treated mice. Columns, mean of 5 mice; bars, SE. *, $P < 0.01$, compared with untreated mice.

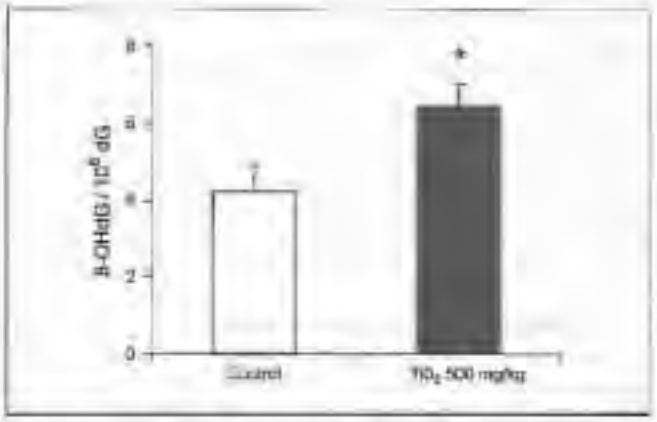

Figure 5. Level of 8-OHdG in untreated and 500 mg/kg TiO_2 nanoparticles–treated mouse livers. Mean ± SE ($n = 5$ mice/group). *, $P < 0.05$ compared with untreated mice.

A general upregulation of these cytokines may be due to the effects of circulating TiO_2 nanoparticles directly in the peripheral blood, suggesting systemic distribution, and direct activation of a proinflammatory response. In the contrary, anti-inflammatory cytokines with generally opposing function were not upregulated, including transforming growth factor-β, interleukin-10, and interleukin-4 (Fig. 6*B*). TiO_2 nanoparticles did not induce an anti-inflammatory response, which mean they did not inhibit the production and release of proinflammatory mediators.

Discussion

Here, we report for the first time that TiO_2 nanoparticles are genotoxic and clastogenic *in vivo* in mice. We showed that TiO_2 nanoparticles (500 mg/kg) induce not only DNA single-strand breaks and DSBs but also chromosomal damage. The formation of γ-H2AX foci, which show DSB formation, was the most sensitive parameter and showed a consistent dose-dependent response. Concerning health relevance, DSBs are much more damaging in terms of genetic instability than single-strand breaks and oxidative DNA damage, which are transient. Our results extend previous *in vivo* findings with the micronuclei and comet assays in several human cells and Syrian hamster embryo cells (10, 12, 13, 15), although they have not been detected in some studies (11, 14). Differences in response between studies may be due to how TiO_2 nanoparticles differ in terms of TiO_2 production, particle size, degree of aggregation, preparation method (sonication), incubation conditions, dose, and susceptibility between cell types (34, 35), implying that more studies are needed to determine the conditions in which TiO_2 nanoparticles genotoxicity occurs.

To date, very few *in vivo* genotoxicity studies have been carried out with nanoparticles. A chronic exposure to TiO_2 nanoparticles at concentrations that produce chronic pulmonary inflammation was associated with an increased incidence of tumors in rat lungs (3). Thus far, only two *in vivo* genotoxicity studies have been reported, which showed that *in vivo* TiO_2 nanoparticles increased *Hprt* mutation frequency in alveolar epithelial cells (9) but did not induce DNA adduct formation in rat lungs (26). Our study showed for the first time that, *in vivo* after oral exposure, TiO_2 nanoparticles induce DNA strand breaks and chromosomal damage in bone marrow and/or peripheral blood, which may help to further understand potential mechanisms of TiO_2 nanoparticles carcinogenicity. We also found that maternal exposure to 500 mg/kg TiO_2 nanoparticles during gestation results in significantly elevated frequencies of DNA deletions in offspring. This result is a major finding because it shows for the first time that *in utero* exposure of fetuses via the mother causes an increase in large deletions in offspring. Taken together, our findings show that TiO_2 nanoparticles, orally administrated, induce genotoxicity systemically in organs, such as blood, bone marrow, and even the embryo.

Surprisingly, human studies have not been able to detect any relation between TiO_2 occupational exposure and cancer risk (4, 5, 36), but these studies have methodologic and epidemiologic limitations as reviewed by the National Institute of Occupational Safety and Health (37). In addition, these studies were not designed to investigate the relationship between TiO_2 particle size and lung cancer risk, an important question for assessing the potential occupational carcinogenicity of TiO_2. Indeed, Donaldson and colleagues, comparing several tumor studies, pointed out that TiO_2 nanoparticles produced lung tumors in rats at a much lower dose than fine particles (<250 nm; 10 and 250 mg/m^3 for nanoparticles and fine particles, respectively; ref. 3). Although TiO_2 nanoparticles are prone to forming agglomerates of >100 nm in solution, these agglomerates are apparently not stable and appear to dissociate in

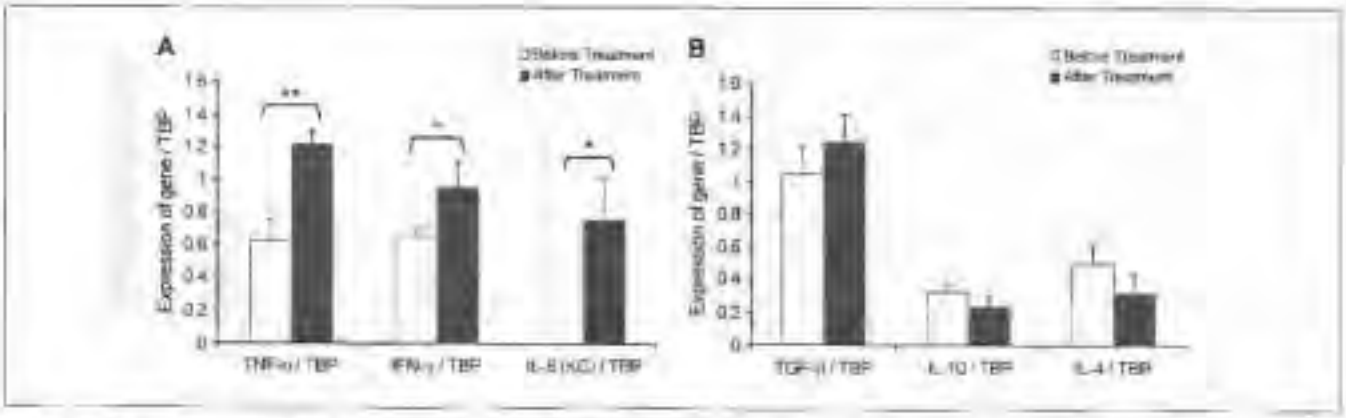

Figure 6. TiO_2 nanoparticles at 500 mg/kg induce proinflammatory but not anti-inflammatory cytokines. Open columns, untreated controls; black columns, TiO_2 nanoparticles–treated mice. *A*, expression of proinflammatory cytokine panel relative to TBP, the internal control gene. *, $P < 0.05$; **, $P < 0.01$, Student's *t* test for treatment comparisons. *B*, expression of anti-inflammatory cytokine panel relative to TBP. Student's *t* test revealed no significant differences. For each graph, $n = 5$ mice/group.

bodily fluids and tissues, which could be an explanation for TiO_2 nanoparticles higher toxicity. It has also been reported that TiO_2 nanoparticles surface interactions are weak (20). In addition, an inhalation study showed that TiO_2 nanoparticles agglomerates of ~700 nm dissociate into smaller units after deposition in the lung (21). Even if nanoparticles aggregate into larger-sized agglomerates, their primary particle sizes remain a significant trait that affects their toxicity. Further human studies would be necessary to understand nanoparticle health effects. For instance, one could use blood-based assays similar to those done in this study in a future molecular epidemiology study in occupational settings. Furthermore, TiO_2 is also used in food colorants and toothpaste. This supports the notion that nanoparticle ingestion constitutes a relevant route of exposure in humans and underscores the significance of the findings of our study. In addition, given the capability of nanoparticles to enter the systemic blood circulation, nanoparticles may pose hazard to a variety of other organs as we have shown here.

As suggested previously, a possible mechanism for nanoparticles-caused genotoxicity might be via oxidative stress (38). Indeed, previous studies showed that TiO_2 nanoparticles have hydroxyl radical activity (39–41) and can also trigger reactive oxygen species production in different cell lines (13, 42) on interaction with the cell membrane or even in cell-free environment (23, 43). We confirmed these results in our *in vivo* experiment by showing oxidative DNA damage (8-OHdG) increase in mouse livers after TiO_2 nanoparticles treatment. Also, TiO_2 nanoparticles–increased DNA deletions during fetal development might be a result of oxidative genome damage. As discussed previously (44), oxidative stress is particularly hazardous in replicating cells. For instance, oxidative DNA lesions (e.g., 8-OHdG, single-strand breaks, or stalled replication forks) can lead to DSBs after replication, which can result in recombination, thus producing permanent genome rearrangements. As shown in yeast, oxidative mutagens might be powerful inducers of DNA deletions (45). Because embryonic cells are generally characterized by a high replication index, they might be particularly susceptible to oxidative genome damage.

We have also observed an inflammatory reaction as shown by changes in cytokine expression in peripheral blood, in which TiO_2 nanoparticles could be exerting direct inflammatory effects on circulating innate cells and Th1 effector cells. The inflammatory response involving recruitment and activation of phagocytes is capable of causing oxidative bursts that may serve as a possible explanation for the observed genotoxicity to peripheral leukocytes, micronuclei formation, oxidative DNA damage in liver cells, and DNA deletion induction in fetal RPE. Because we showed that in mice TiO_2 nanoparticles induce an inflammatory reaction and oxidative DNA damage, it is tempting to speculate that the mechanism underlying TiO_2 nanoparticles genotoxicity might be a secondary (indirect) genotoxicity pathway as suggested by Dankovic and colleagues (3). Secondary genotoxicity is considered to be the key aspect of some particle toxicity (e.g., quartz and silica) because of their ability to elicit persistent inflammation *in vivo* (9, 46). This implies that particles have prooxidant and proinflammatory activity, leading to genotoxicity.

In summary, our study showed for the first time that TiO_2 nanoparticles induce clastogenicity, genotoxicity, oxidative DNA damage, and inflammation *in vivo* in mice. These results have been observed after only 5 days of treatment via drinking water and in multiple organs suggesting a systemic effect. We also showed that *in utero* exposure to TiO_2 nanoparticles results in an increased frequency in DNA deletions in the fetus. These results represent the first comprehensive *in vivo* genotoxicity study of TiO_2 nanoparticles. These data suggest that we should be concerned about a potential risk of cancer or genetic disorders especially for people occupationally exposed to high concentrations of TiO_2 nanoparticles and that it might be prudent to limit ingestion of TiO_2 nanoparticles through nonessential drug additives, food colors, etc.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 7/30/09; revised 8/22/09; accepted 9/10/09; published OnlineFirst 11/3/09.

Grant support: NIH grants RO1 ES09519 (R.H. Schiestl) and 1R03 CA133928-01 (R. Reliene).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Susanne Henning (University of California Los Angeles Center for Human Nutrition) for performing high-performance liquid chromatography, Dr. Gopal Iyer (Department of Chemistry and Biochemistry, University of California Los Angeles) for dynamic light scattering measurements, and Drs. Fabien Nogué, Norman Arnheim, and, especially, Jean-Louis Ploutinoc for very interesting comments on the article.

References

1. Baan R, Straif K, Grosse Y, et al. Carcinogenicity of carbon black, titanium dioxide, and talc. Lancet Oncol 2006;7:295–6.
2. Baron PJ, Schins RP, Albrecht C. Inhaled particles and lung cancer, part B: paradigms and risk assessment. Int J Cancer 2004;110:3–14.
3. Dankovic D, Kuempel E, Wheeler M. An approach to risk assessment for TiO_2. Inhal Toxicol 2007;19 Suppl 1:205–12.
4. Boffetta P, Soutar A, Cherrie JW, et al. Mortality among workers employed in the titanium dioxide production industry in Europe. Cancer Causes Control 2004;15:697–706.
5. Fryzek JP, Chadda B, Marano D, et al. A cohort mortality study among titanium dioxide manufacturing workers in the United States. J Occup Environ Med 2003;45:400–9.
6. Park S, Lee YK, Jung M, et al. Cellular toxicity of various inhalable metal nanoparticles on human alveolar epithelial cells. Inhal Toxicol 2007;19:59–65.
7. Chen M, von Mikecz A. Formation of nucleoplasmic protein aggregates impairs nuclear function in response to SiO_2 nanoparticles. Exp Cell Res 2005;305:51–62.
8. Jani PU, McCarthy DE, Florence AT. Titanium dioxide (rutile) particle uptake from the rat GI tract and translocation to systemic organs after oral administration. Int J Pharm 1994;105:157–68.
9. Driscoll KE, Deyo LC, Carter JM, et al. Effects of particle exposure and particle-elicited inflammatory cells on mutation in rat alveolar epithelial cells. Carcinogenesis 1997;18:423–30.
10. Wang JJ, Sanderson BJ, Wang H. Cyto- and genotoxicity of ultrafine TiO_2 particles in cultured human lymphoblastoid cells. Mutat Res 2007;628:99–106.
11. Warheit DB, Hoke RA, Finlay C, et al. Development of a base set of toxicity tests using ultrafine TiO_2 particles as a component of nanoparticle risk management. Toxicol Lett 2007;171:99–110.
12. Gurr JR, Wang AS, Chen CH, Jan KY. Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. Toxicology 2005;213:66–73.
13. Kang SJ, Kim BM, Lee YJ, Chung HW. Titanium dioxide nanoparticles trigger p53-mediated damage response in peripheral blood lymphocytes. Environ Mol Mutagen 2008;49:399–405.
14. Linnainmaa K, Kivipensaa P, Vainio H. Toxicity and cytogenetic studies of ultrafine titanium dioxide in cultured rat liver epithelial cells. Toxicol In Vitro 1997;11:329–35.
15. Rahman Q, Lohani M, Dopp E, et al. Evidence that ultrafine titanium dioxide induces micronuclei and apoptosis in Syrian hamster embryo fibroblasts. Environ Health Perspect 2002;110:797–800.
16. Li S, Zhu H, Zhu B, et al. Impact and mechanism of TiO_2 nanoparticles on DNA synthesis *in vitro*. Sci China Ser B Chem 2008;51:367–72.
17. Zhu R-R, Wang S-L, Zhang R, Sun X-Y, Yao S-D. A novel toxicological evaluation of TiO_2 nanoparticles on DNA structure. Chin J Chem 2007;25:958–61.
18. Chen HW, Su SF, Chien CT, et al. Titanium dioxide nanoparticles induce emphysema-like lung injury in mice. FASEB J 2006;20:2393–5.
19. de Haar C, Hassing I, Bol M, Bleumink R, Pieters R. Ultrafine but not fine particulate matter causes airway

inflammation and allergic airway sensitization to co-administered antigen in mice. Clin Exp Allergy 2006; 36:1469–79.

20. Grassian VH, O'Shaughnessy PT, Adamcakova-Dodd A, Pettibone JM, Thorne PS. Inhalation exposure study of titanium dioxide nanoparticles with a primary particle size of 2 to 5 nm. Environ Health Perspect 2007;115: 397–402.

21. Oberdorster G, Ferin J, Lehnert BE. Correlation between particle size, *in vivo* particle persistence, and lung injury. Environ Health Perspect 1994;102 Suppl 5:173–9.

22. Federici G, Shaw BJ, Handy RD. Toxicity of titanium dioxide nanoparticles to rainbow trout (*Oncorhynchus mykiss*): Gill injury, oxidative stress, and other physiological effects. Aquat Toxicol 2007;84:415–30.

23. Veranth JM, Kaser EG, Veranth MM, Koch M, Yost GS. Cytokine responses of human lung cells (BEAS-2B) treated with micron-sized and nanoparticles of metal oxides compared to soil dusts. Part Fibre Toxicol 2007;4:2.

24. Behrens R, Bishop AJ, Aubrecht J, Schiestl RH. *In vivo* DNA deletion assay to detect environmental and genetic predisposition to cancer. Methods Mol Biol 2004;262: 125–39.

25. Dick CA, Brown DM, Donaldson K, Stone V. The role of free radicals in the toxic and inflammatory effects of four different ultrafine particle types. Inhal Toxicol 2003;15:39–52.

26. Gallagher J, Heinrich U, George M, et al. Formation of DNA adducts in rat lung following chronic inhalation of diesel emissions, carbon black and titanium dioxide particles. Carcinogenesis 1994;15:1291–9.

27. Rehn B, Seiler F, Rehn S, Bruch J, Maier M. Investigations on the inflammatory and genotoxic lung effects of two types of titanium dioxide: untreated and surface treated. Toxicol Appl Pharmacol 2003;189:84–95.

28. Xia T, Kovochich M, Brant J, et al. Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. Nano Lett 2006;6:1794–807.

29. Behrens R, Schiestl RH. Mouse models for induced genetic instability at endogenous loci. Oncogene 2003;22: 7000–10.

30. Behrens R, Pollarda J, Sobol Z, et al. *N*-acetyl cysteine protects against ionizing radiation-induced DNA damage but not against cell killing in yeast and mammals. Mutat Res 2009;665:37–43.

31. Westbrook AM, Wei B, Braun JR, Schiestl RH. Intestinal mucosal inflammation leads to systemic genotoxicity in mice. Cancer Res 2009;69:4827–34.

32. Behrens R, Fischer E, Schiestl RH. Effect of *N*-acetyl cysteine on oxidative DNA damage and the frequency of DNA deletions in atm-deficient mice. Cancer Res 2004;64:5148–53.

33. Ismail IH, Wadhra TI, Hammarsten O. An optimized method for detecting γ-H2AX in blood cells reveals a significant interindividual variation in the γ-H2AX response among humans. Nucleic Acids Res 2007;35:e36.

34. Tao F, Kobzik L. Lung macrophage-epithelial cell interactions amplify particle-mediated cytokine release. Am J Respir Cell Mol Biol 2002;26:499–505.

35. Thevenot P, Cho J, Wavhal D, Timmons RB, Tang L. Surface chemistry influences cancer killing effect of TiO_2 nanoparticles. Nanomed Nanotechnol Biol Med 2008;4:226–36.

36. Garabrant DH, Fine LJ, Oliver C, Bernstein L, Peters JM. Abnormalities of pulmonary function and pleural disease among titanium metal production workers. Scand J Work Environ Health 1987;13:47–51.

37. National Institute of Occupational Safety and Health. Evaluation of health hazard and recommendations for occupational exposure to titanium dioxide. NIOSH Current Intelligence Bulletin. Cincinnati: National Institute of Occupational Safety and Health, DHHS publication; 2005.

38. Schins RP, Knaapen AM. Genotoxicity of poorly soluble particles. Inhal Toxicol 2007;19 Suppl 1:189–98.

39. Donaldson K, Borwick PH, Gilmour PS. Free radical activity associated with the surface of particles: a unifying factor in determining biological activity? Toxicol Lett 1996;88:293–8.

40. Gilmour P, Brown DM, Borwick PH, et al. Surface free radical activity of PM10 and ultrafine titanium dioxide: a unifying factor in their toxicity? Ann Occup Hyg 1997; 41:52–8.

41. Zhang Q, Kusaka Y, Sato K, et al. Differences in the extent of inflammation caused by intratracheal exposure to three ultrafine metals: role of free radicals. J Toxicol Environ Health Part A 1998;53:423–38.

42. Helfenstein M, Miragoli M, Rohr S, et al. Effects of combustion-derived ultrafine particles and manufactured nanoparticles on heart cells *in vitro*. Toxicology 2008;253:70–8.

43. Sayes CM, Wahi R, Kurian PA, et al. Correlating nanoscale titania structure with toxicity: a cytotoxicity and inflammatory response study with human dermal fibroblasts and human lung epithelial cells. Toxicol Sci 2006;92:174–85.

44. Behrens R, Schiestl RH. Antioxidant *N*-acetyl cysteine reduces incidence and multiplicity of lymphoma in Atm deficient mice. DNA Repair (Amst) 2008;3: 872–9.

45. Brennan RJ, Swoboda RE, Schiestl RH. Oxidative mutagens induce intrachromosomal recombination in yeast. Mutat Res 1996;308:139–67.

46. Schins RPF. Mechanisms of genotoxicity of particles and fibers. Inhal Toxicol 2002;14:57–78.

Reproductive Toxicology 30 (2010) 343–352

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Review

Reproductive and developmental toxicity studies of manufactured nanomaterials

Makoto Ema*, Norihiro Kobayashi, Masato Naya, Sosuke Hanai, Junko Nakanishi

Research Institute of Science for Safety and Sustainability, National Institute of Advanced Science and Technology (AIST), 16-1 Onogawa, Tsukuba, Ibaraki 305-8569, Japan

ARTICLE INFO

Article history:
Received 4 December 2009
Received in revised form 27 April 2010
Accepted 16 June 2010
Available online 25 June 2010

Keywords:
Nanomaterials
Nanoparticles
Titanium dioxide
Fullerenes
Metallic particles
Luminescent particles
Reproductive and developmental toxicity
Testicular toxicity

ABSTRACT

This paper reviews studies *in vivo* and *in vitro* on the reproductive and developmental toxicity of manufactured nanomaterials including metallic and metal oxide-based particles, fullerenes (C_{60}), carbon black (CB), and luminescent particles. Studies *in vivo* showed increased allergic susceptibility in offspring of mouse dams intranasally insufflated with respirable-size titanium dioxide (TiO_2), adverse effects on spermatogenesis and histopathological changes in the testes and changes in gene expression in the brain of mouse offspring after maternal subcutaneous injection of TiO_2 nanoparticles, transfer to rat fetuses of radiolabeled gold nanoparticles and C_{60} after maternal intravenous injection, death and morphological abnormalities in mouse embryos after maternal intraperitoneal injection of C_{60}, and adverse effects on spermatogenesis in mouse offspring after maternal intratracheal instillation of CB nanoparticles. Studies *in vitro* revealed that TiO_2 and CB nanoparticles affected the viability of mouse Leydig cells, that gold nanoparticles reduced the motility of human sperm, that silver, aluminum, and molybdenum trioxide were toxic to mouse spermatogonia stem cells, that silica nanoparticles and C_{60} inhibited the differentiation of mouse embryonic stem cells and midbrain cells, respectively, and that cadmium selenium-core quantum dots inhibited pre- and postimplantation development of mouse embryos. Although this paper provides initial information on the potential reproductive and developmental toxicity of manufactured nanomaterials, further studies, especially *in vivo*, using characterized nanoparticles, relevant routes of administration, and doses closely reflecting expected levels of exposure are needed.

Contents

* Corresponding author. Tel.: +81 29 861 8021; fax: +81 29 861 8330.
E-mail address: ema-makoto@aist.go.jp (M. Ema).

0890-6238/$ – see front matter
doi:10.1016/j.reprotox.2010.06.002

1. Introduction

Nanomaterials are defined as materials having a physicochemical structure on a scale greater than typical atomic/molecular dimensions but less than 100 nm (nanostructure), which exhibit physical, chemical and/or biological characteristics associated with a nanostructure [1]. Nanoparticles are defined as particles with at least one dimension smaller than 100 nm and include manufactured nanoparticles, ambient ultrafine particles and biological nanoparticles [1,2]. Humans have been exposed to airborne nanoparticles throughout evolution, but exposure has increased dramatically because of anthropogenic factors including combustion engines, power plants, and other sources of thermodegradation [2]. The rapidly developing field of nanotechnology, which is creating materials with size-dependent properties, is likely to become another source of exposure to nanomaterials. The surface and interface of particles are particularly important components of nanoparticles. Nanomaterials have an increased surface area: mass ratio thereby greatly enhancing their chemical/catalytic reactivity compared to normal-sized forms of the same substance. Surface coatings can be utilized to alter surface properties of nanoparticles to prevent aggregation or agglomeration with different particle-types, and/or serve to passivate the particle-type to migrate the effects of ultraviolet radiation-induced reactive oxidants [1]. The distinctive and often unique properties of nanomaterials offer the promise of broad advances for a wide range of technologies. Nanomaterials are used in a variety of areas including advanced materials, electronics, magnetics and optoelectronics, biomedicine, pharmaceuticals, cosmetics, energy, and catalytic and environmental detection and monitoring [3,4]. At present, there are relatively few environments where exposures are known to occur. However, if the commercialization of products using nanomaterials develops as anticipated, the potential for exposure is likely to increase notably over the coming decade [1]. Despite growing concern over the possible risk that nanomaterials pose, there is a lack of information on their potential toxicity. At this moment, there is a knowledge gap between the increasing development and use of nanomaterials and the prediction of possible health risks.

In recent years, reproductive and developmental toxicity has increasingly become recognized as an important part of overall toxicology. In fact, adverse effects of environmental chemicals on the reproductive success of wildlife populations have been noted [5]. It is reported that nanoparticles can pass through biological membranes [6,7]; raising fears that they can affect the physiology of any cell in the body. The possibility of chemicals entering biological systems is of great concern to the public with regard to possible reproductive and developmental toxicity. In this paper, we review studies on the reproductive and developmental toxicity of nanomaterials, published in openly available scientific literature.

2. Reproductive and developmental toxicity of manufactured nanomaterials

The literature on manufactured nanomaterials was searched using TOXNET/TOXLINE for studies *in vivo* and *in vitro* of reproductive and developmental toxicity, excluding abstracts. Although no information was available on the reproductive and developmental toxicity of single- or multi-wall carbon nanotubes, articles on metallic and metal oxide-based particles, fullerenes (C_{60}), and carbon black (CB) and luminescent particles were found. In this paper, we review studies using mammalian animals and cells on the reproductive and developmental effects of nanomaterials. The final search of the literature was conducted in March, 2010.

2.1. Metallic and metal oxide-based particles

In vivo and *in vitro* studies of titanium dioxide (TiO_2) nanoparticles, and *in vitro* studies of silver, aluminum, molybdenum trioxide (MoO_3), gold, magnetic iron oxide (Fe_3O_4), cobalt–chromium (CoCr) and silica nanoparticles have been published.

2.1.1. Titanium dioxide (TiO_2)

TiO_2 is widely used as a white pigment in paints, plastics, inks, paper, creams, cosmetics, drugs and foods. TiO_2 was previously classified as biologically inert in animals and humans [8–10] and has been used as a negative control particle in a variety of toxicological studies. Recently, concern has been raised on possible adverse effects of TiO_2 on human health because exposure to high concentrations of ultrafine TiO_2 was involved in the induction of lung inflammatory responses [11] and tumors [12]. Very recently, the International Agency for Research on Cancer (IARC) Monograph Working Group classified TiO_2 as possibly carcinogenic to humans (i.e., group 2B) based on results from studies in which the inhalation and intratracheal instillation of TiO_2 provided sufficient evidence in animals for carcinogenicity [13]. As for genotoxicity, the results of studies on TiO_2 nanoparticles are inconclusive [14,15]. *In vivo* and *in vitro* studies of TiO_2 are summarized in Table 1.

2.1.1.1. In vivo study of titanium dioxide (TiO_2). Pregnant BALB/c mice on gestational day (GD) 14 or nonpregnant control mice were administered respirable-size TiO_2 [16], that is less than 10 μm in particle size [17], suspended in phosphate-buffered saline (PBS) at 50 μg/mouse by a single intranasal insufflation. Pups obtained by spontaneous delivery received a single intraperitoneal injection of ovalbumin (OVA) with alum on postnatal day (PND) 4. These pups were exposed to aerosolized OVA on PNDs 12–14, and subjected to an examination of pulmonary function and a pathological analysis. Airway responsiveness to increasing concentrations of aerosolized methacholine was measured using whole body plethysmography. Bronchoalveolar lavage (BAL) differential cell counts and histopathological examinations of the lung were also performed. Lung inflammatory responses were determined 48 h postadministration in nonpregnant and pregnant mice ($n > 9$/group). TiO_2-treated nonpregnant mice exhibited minimal increases in BAL polymorphonuclear leukocyte counts, whereas pregnant mice showed acute neutrophilic inflammation. Pregnant mice exposed to TiO_2 had higher serum levels of cytokines, including interleukin-1β, tumor necrosis factor-α, interleukin-6 and chemokine, 48 h after exposure compared with nonpregnant mice ($n = 9$/group). Offspring of dams exposed to TiO_2 showed increased airway hyperresponsiveness, increased percentage of eosinophils, and pulmonary inflammation ($n = 17–21$/group). These findings indicate that TiO_2 caused acute cellular inflammation in pregnant mice and increased allergic susceptibility in their pups.

A TiO_2 nanopowder (25–70 nm in particle size, 20–25 m^2/g in surface area, anatase, Sigma–Aldrich Japan, Inc.) suspended in saline with 0.05% Tween 80 was subcutaneously injected into pregnant Slc:ICR mice ($n = 15$) on GDs 6, 9, 12 and 15 at

Table 1
In vivo and *in vitro* reproductive and developmental toxicity studies of titanium dioxide (TiO_2) particles.

In vivo/in vitro	Material characteristics	Animal/cells	Exposure			Findings	References
			Route/method	Duration/time	Dose/concentration		
In vivo	Respirable-size	BALB/c mice	Intranasal instillation	Single on GD 14	50 μg/mouse	↑ Acute cellular inflammation in pregnant mice; ↑ Susceptibility to allergy in pups	[17]
In vivo	25–70 nm in particle size, 20–25 m²/g in surface area, anatase	Slc:ICR mice	Subcutaneous injection	GDs 6, 9, 12, and 15	100 μg/mouse/day (10–15 mice/group)	Changes in gene expression related to development and function of central nervous system in male pups	[18]
In vivo	25–70 nm in particle size, 20–25 m²/g in surface area, anatase	Slc:ICR mice	Subcutaneous injection	GDs 3, 7, 10, and 14	100 μg/mouse/day (6 mice/group)	↓ Body weight of pups; ↓ DSP of pups; ↓ Epididymal sperm motility in pups; ↓ Number of Sertoli cells in pups; Histopathological changes in testis of pups	[19]
In vitro	25–70 nm in particle size	Mouse testis Leydig cell line TM3	Incubation	16, 24, or 48 h	1–1000 μg/mL	↓ Viability of TM3 at 100 μg/mL; ↓ Proliferation of TM3 cells at 100 μg/mL; No changes in HO-1 or StAR mRNA expression at up to 100 μg/mL	[20]

100 μg/mouse/day as the exposure group, and 100 μl of vehicle alone was injected into pregnant mice (n = 14) as the control group [18]. Brain tissue was obtained from male offspring on embryonic day 16 (n = 8/group) or on PND 2 (n = 10/group), PND 7 (n = 10/group), or PND 21 (n = 9/group), total RNA was extracted from whole brain, and gene expression was analyzed. Maternal exposure to TiO_2 caused changes in the expression of genes associated with brain development, cell death, response to oxidative stress, and mitochondria in the brain during the prenatal period, and genes associated with inflammation and neurotransmitters in the later stages. However, this study did not investigate how maternal behavior toward the pups changed and how this in turn altered gene expression. It is difficult to evaluate the change in gene expression using the toxicogenomic data of this study, because not enough microarray data was provided in the paper.

Slc:ICR mice (n = 6/group) were subcutaneously injected with TiO_2 nanoparticles (25–70 nm in particle size, 20–25 m^2/g in surface area, anatase, Sigma–Aldrich) suspended in saline with 0.05% Tween 80 at 100 μg/mouse/day on GDs 3, 7, 10 and 14 [19]. Male offspring were autopsied on PND 4 or postnatal week (PNW) 6. Lower body weights were found among offspring of dams exposed to TiO_2. Aggregates of TiO_2 nanoparticles (100–200 nm) were detected in Leydig cells, Sertoli cells, and spermatids in the testes of pups on PND 4 and PNW 6. Disorganized and disrupted seminiferous tubules, tubule lumens with few mature sperm, and decreases in daily sperm production (DSP), epididymal sperm motility, and numbers of Sertoli cells were observed at PNW 6 in pups of the TiO_2-treated group (n = 8/group). TiO_2 particles were detected in cells of the olfactory bulb and cerebral cortex of pups at PNW 6. There were many cells positive for caspase-3, an enzymatic marker of apoptosis, in the olfactory bulb of pups on PNW 6 in the TiO_2-exposed group. Although the possibility of adverse effects of TiO_2 nanoparticles on brain development is noted, the behavioral effects of nanoparticles were not investigated. There was a lack of description on the maternal findings in this report.

2.1.1.2. In vitro study of titanium dioxide (TiO_2). The direct effects of TiO_2 (25–70 nm in particle size, Aldrich) on testis-constituent cells was determined using the mouse Leydig cell line TM3, testosterone-producing cells of the testis [20]. TiO_2 was suspended in a balanced salt solution [0.05% Tween 80–0.25% DMSO in PBS(−)], and sonicated for 10 min immediately prior to use in the assay. TiO_2 was added to the culture system for 16, 24, or 48 h. The uptake of TiO_2 nanoparticles by Leydig cells was detected after incubation of cells with TiO_2 at 30 μg/mL for 48 h. Following incubation of cells with TiO_2 at 10 or 100 μg/mL, a remarkable inhibition of viability and transient reduction in proliferation of TM3 cells were observed at 100 μg/mL after 24 h. No effect of TiO_2 was found on the expression of heme oxygenase-1 (HO-1), a sensitive marker of oxidative stress, or steroidogenic acute regulatory (StAR) mRNA in TM3 cells treated for 16 h at up to 100 μg/mL or for 48 h at up to 30 μg/mL. These findings suggest that TiO_2 nanoparticles have no direct effect on the induction of oxidative stress or synthesis of testosterone in Leydig cells.

2.1.2. Gold

Colloidal gold has been used in medical applications and gold nanoparticles are used commercially in a wide array of catalytic applications and optical and electrical applications as components of various probes, sensors, and optical devices [21]. *In vivo* and *in vitro* studies of gold particles are shown in Table 2.

2.1.2.1. In vivo study of gold. The distribution of ^{198}Au-colloidal particles (4–200 nm) was determined after a single injection into the iliac artery of pregnant SD rats on GDs 16–18 [22]. Although more than 90% of the radiocolloid was found in the maternal liver

Table 3
In vivo and in vitro reproductive and developmental toxicity studies of gold particles.

In vivo/in vitro	Material/characteristics	Animals/cells	Exposure		Dose/concentration	Findings	Reference
			Route/method	Duration/frequency			
In vivo	^{198}Au-colloidal particles (4–20 nm in diameter)	10 rats	Intrauterine injection	Single during GDs 16–18	~200 μCi/rat	No detection of radioactivity in amniotic fluid, fetal membranes, or fetus	[22]
Review	^{198}Au-colloidal nanoparticles (5 or 30 nm in diameter)	Wistar rats	Intravenous injection	Single on GD 19	0.5 mL/rat of solution containing 20 μg of gold (7–10 rats/group)	Transfer rate to fetus: 0.018% for 5 nm particles; Transfer rate to fetus: 0.005% for 30 nm particles	[23]
In vivo	Colloidal gold nanoparticles (2 or 40 nm in diameter)	C57BL/6 mice	Intravenous injection	Once on GDs 16–18	1 mL/rat of solution containing gold particles (5 mice/group)	No transfer to fetus	[24]
In vitro	Gold nanoparticles (10, 15, or 30 nm in diameter) coated with polyethylene glycol	Human placenta	Recirculating perfusion	6 h	7.9 × 10^{11} for 15 nm particles and 7.8 × 10^{10} for 30 nm particles	Detection of high levels of nanoparticles even after perfusion in maternal circuit; No detection of nanoparticles in fetal outflow	[25]
In vitro	Gold nanoparticles (10, 15, or 30 nm in diameter) coated with polyethylene glycol	Human placenta	Recirculating perfusion	6 h	~5.1 × 10^{9} for 10 nm particles and 2.0 × 10^{9} for 15 nm particles	In compartmental transfer of nanoparticles	[25]
In vitro	Gold nanoparticles (9 nm in size)	Human sperm	Mixed with semen	4 h	44 ppm	Spermicidality	[26]

at 15 min after injection, no radioactivity was detected in the amniotic fluid, fetal membranes, or fetus. These findings indicate the impermeability of the rat placenta to colloidal gold. Detailed experimental conditions including concentrations of gold particles and numbers of rats used were not described in this report.

Pregnant Wistar rats (n = 7–10/group) were injected intravenously with ^{198}Au-colloidal particles (5 and 30 nm in diameter, Daichi Radio Isotope Co., Ltd. and Hoext Japan Co., Ltd., respectively) into the tail vein on GD 19 (vaginal plug = GD 1) and sacrificed 1 or 24 h later [23]. The 0.5 mL of solution injected contained 20 μg of gold. The clearance of ^{198}Au-colloid from blood was faster in dams injected with the 30 nm particles than in dams injected with the 5 nm particles, and, therefore, the radioactivity remaining in maternal blood was greater in the 5 nm-group. Fetal radioactivity was detected in pregnant rats sacrificed at 1 and 24 h after the injection of 5 nm particles and at 24 h after the injection of 30 nm particles. The transfer rate to the fetus was very small, being approximately 0.018 and 0.005% for the 5 and 30 nm particles, respectively. The levels of radioactivity in the fetal membrane and placenta were greater in the 5 nm-group that in 30 nm-group, and 100–300 times greater than the levels in the fetus for either group. The authors described that the transfer or deposition of ^{198}Au-colloid was directly affected not by particle size, but by the average concentration in maternal blood.

Pregnant C57BL/6 mice were intravenously injected into the tail vein with 1 mL of a solution containing 2 or 40 nm colloidal gold nanoparticles (n = 5/goroup) or 1 mL of saline (n = 3 as controls) on GDs 16–18 and killed 24 h after the last injection [24]. The 2 and 40 nm gold nanoparticles (Fitzgerald Industry Inc.) contained 15 × 10^{13} particles (12.13 μg) and 9 × 10^{10} particles (58.21 μg), respectively. The gold nanoparticles had a negative surface charge and were monodispers and spherical in shape. No particles were detected in the fetuses and placentae. These findings suggest that gold nanoparticles do not penetrate the placental barrier.

2.1.2.2. In vitro study of gold. The transplacental transfer of monodispersed gold particles (10, 15, and 30 nm in diameter before coating) coated with polyethylene glycol (PEG) was examined using placentae from healthy, nonsmoking mothers [25]. In the open perfusion as a "once-through" perfusion, nanoparticles (7.9 × 10^{11} for 15 nm particles and 7.8 × 10^{10} for 30 nm particles) were suspended in 5 mL of physiological saline and injected into the maternal artery within 5 min, and the maternal and fetal outflow were collected at 3-min intervals for 18 min. In the maternal outflow, the nanoparticles of 15 and 30 nm were detected at 570 and 678 ppb within 3–6 min of injection, and only 9.3 and 18.0 ppb, respectively, at the end of perfusion. No nanoparticles were detected in the fetal outflow. Recirculating perfusion was performed with 10 and 15 nm nanoparticles only. Both the maternal and fetal sides were recirculated. The nanoparticles (9.1 × 10^{9} for 10 nm particles and 2.0 × 10^{9} for 15 nm particles) were added to the maternal reservoir and the perfusion was continued for 6 h. Samples were taken from the maternal and fetal reservoirs every 30 min for the first 2 h, and once per hour thereafter. Nanoparticles did not cross the placenta regardless of particle size. At the end of the perfusion, concentrations of nanoparticles in maternal perfusate samples decreased 41 and 64% giving final concentrations of 24.2 and 22.2 ppb for the 10 and 15 nm nanoparticles, respectively. The gold aggregates were located in syncytiotrophoblasts and trophoblasts, but no gold particles were detected in the fetal capillary endothelium in perfused tissue. These findings indicate that PEGylayed gold nanoparticles do not cross the human placenta from the maternal to fetal circulation.

The effect of gold nanoparticles (9 nm) at a concentration of 44 ppm on human sperm was determined using a single, fresh, donor semen sample from a healthy male [26]. In a mixture of 500 μL of the gold nanoparticle solution and semen, 25% of sperm

were not motile. The rate of motility among the control sperm was 95%. The penetration of sperm heads and tails by gold nanoparticles, and fragmentation of sperm were found in the mixture. Toxicity parameters, except for motility, were not investigated in this study.

2.1.3. Silver, aluminum, and molybdenum trioxide (MoO_3)

Nanoscaled silver powder is used in biocides, transparent conductive inks and pastes, and various consumer and industrial products that need enhanced antimicrobial properties [21]. Nanoscaled aluminum powder is used in various electronic circuits and as a scratch-resistant coating for plastic lenses, antimicrobial agents, and new tissue-biopsy tools [21]. MoO_3 nanoparticles have electrochromic, photochromic, and gas-sensing properties [27]. *In vitro* studies of silver, aluminum, and MoO_3 particles are listed in Table 3.

2.1.3.1. In vitro study of silver, aluminum, and molybdenum trioxide (MoO_3). *In vitro* studies of silver (15 nm in diameter), aluminum (30 nm in diameter), and MoO_3 (30 nm in diameter) nanoparticles were performed using the C18-4 cell line, which was established from type A spermatogonia isolated from 6-day-old mouse testes [28]. The cells were immortalized and exhibited phenotypic characteristics of germline stem cells *in vivo*, were adherent, and responded to the growth factor glial cell line-derived neurotrophic factor. Nanoparticles were dispersed in PBS at final concentrations of 5, 10, 25, 50, and 100 μg/mL culture medium, and the C18-4 cells were incubated with nanoparticles for 48 h. Silver nanoparticles caused necrosis and apoptosis at 10 μg/mL and above. Aluminum nanoparticles did not induce shrinkage, necrosis, or apoptosis below 10 μg/mL. No distinct changes in cell morphology were observed at any concentration of MoO_3 nanoparticles. Reduced mitochondrial function and cell viability were noted after incubation with silver nanoparticles at 10 μg/mL, and the EC_{50} was calculated at 7.75 μg/mL. The effects of aluminum nanoparticles on mitochondrial function could not be determined because the particles accumulated in the cells and formed cytoplasmic aggregates at low concentrations. MoO_3 nanoparticles reduced mitochondrial function at 50 μg/mL and above, and the EC_{50} was 90 μg/mL. Silver nanoparticles slightly increased lactase dehydrogenase (LDH) leakage at 5 μg/mL, and the EC_{50} was 2.5 μg/mL. The leakage of LDH was increased by aluminum nanoparticles at 5 μg/mL and above, values reaching a plateau at around 25 μg/mL and the EC_{50} being 4.7 μg/mL. An increase in LDH leakage was observed with MoO_3 nanoparticles at 5 μg/mL and above, and the value reached a plateau at 10 μg/mL. The EC_{50} was 5 μg/mL. An increased number of apoptotic C18-4 cells were found after incubation with silver nanoparticles at 5 μg/mL, aluminum nanoparticles at 5 and 10 μg/mL, and MoO_3 nanoparticles at 50 μg/mL. These results indicate that silver nanoparticles are most toxic and MoO_3 nanoparticles are least toxic to this cell line. The authors noted that this cell line provides a valuable model to assess the cytotoxicity of nanoparticles in the germ line *in vitro*.

2.1.4. Magnetic iron oxide (Fe_3O_4)

The magnetic properties of magnetic iron oxide nanoparticles may lead to a range of new biomedical and diagnostic applications including cellular therapy by cell labeling and targeting, tissue repair, drug delivery, magnetic resonance imaging, and magnetofection [29]. An *in vivo* study of magnetic Fe_3O_4 particles is presented in Table 3.

2.1.4.1. In vitro study of magnetic iron oxide (Fe_3O_4). The effect of Fe_3O_4 on sperm was determined after incubation of bovine sperm in glucose-free modified Tyrode solution with an aqueous colloid solution of Fe_3O_4 nanoparticles coated with poly(vinyl alcohol) for 2 h at 37 °C [29]. The final concentration of Fe ions was 7.35 mM. In

Table 3
In vitro reproductive and developmental toxicity studies of silver, aluminum, molybdenum trioxide (MoO_3), magnetic iron oxide (Fe_3O_4), cobalt–chromium–CuO, and silica (SiO_2) particles.

Material/characteristics	Cells	Exposure		Findings	Reference
		Route/increase	Duration/time	Dose/concentration	
Silver nanoparticles (15 nm in diameter)	Mouse spermatogonial stem cell line C18-4	Incubation	48 h	5–100 μg/mL	Necrosis and apoptosis at 10 μg/mL and above ↓ Mitochondrial function and cell viability at 10 μg/mL ↑ LDH leakage at 5 μg/mL ↑ Apoptotic cells at 5 μg/mL	[28]
Aluminum nanoparticles (30 nm in diameter)	Mouse spermatogonial stem cell line C18-4	Incubation	48 h	5–100 μg/mL	No shrinkage, necrosis, or apoptosis at or below 10 μg/mL ↑ LDH leakage at 5 μg/mL ↑ Apoptotic cells at 5 and 10 μg/mL No distinct change in cell morphology	[28]
MoO_3 nanoparticles (30 nm in diameter)	Mouse spermatogonial stem cell line C18-4	Incubation	48 h	5–100 μg/mL	Mitochondrial function at 50 μg/mL and above ↑ LDH leakage at 5 μg/mL ↑ Apoptotic cells at 50 μg/mL No adverse effect on sperm motility or acrosome reaction	[28]
Magnetic Fe_3O_4 nanoparticles coated with poly(vinyl alcohol)	Bovine sperm	Incubation	2 h	7.35 mM (at Fe ions)	↓ DNA damage of fibroblasts by indirect exposure	[29]
Gold nanoparticles (20–5 nm in diameter)	Human fibroblasts, plasmacytoma cells, and layer of bovine oviductal cells	Direct and indirect exposure	24 h	0.005 mg/mL	↓ DNA damage of fibroblasts by indirect exposure	[30]
Spherical amorphous silica nanoparticles (10, 30, 60, or 400 nm in average primary particle size)	D1 mouse embryonic stem cells	Incubation	10 days	1–100 μg/mL	↓ Differentiation of embryonic stem cells after exposure to 10 and 30 nm, but not 60 and 400 nm, particles	[31]

the first 20 min of incubation, 23% of the particles were taken up by sperm cells. Later on, about 60% of these particles were released from the cells and a further linear uptake was observed for an additional 1.5 h of incubation. Particles were bound to the acrosome in the head of the sperm, and to mitochondria in the tail of the sperm. The sperm was further incubated for 4 h. Motility and the ability to undergo an acrosome reaction, i.e. the ability to fertilize an egg, were not affected by the presence of the magnetic nanoparticles.

2.1.5. Cobalt–chromium (CoCr) nanoparticles

Internal exposure to CoCr nanoparticles can occur by wear mechanism associated with metal-on-metal (CoCr) orthopaedic joint replacements [30]. An *in vitro* study of CoCr particles is presented in Table 3.

2.1.5.1. In vitro study of cobalt–chromium (CoCr) nanoparticles. The cellular toxicity of CoCr nanoparticles (29.5 ± 6.3 nm in diameter, Osprey Metals) when located on the other side of a fully confluent cellular barrier was assessed using BeWo b30 cells, a human trophoblast choriocarcinoma-derived cell line, which were grown as a multi-layered (3–4 cells thick) barrier to simulate tight barriers in the body like the placental barrier [30]. Human fibroblast cells were placed on one side of this layer of cells, and CoCr particles on the other. The fibroblasts were checked for DNA damage using the alkaline comet assay after introduction of the particles. Indirect exposure to CoCr nanoparticles caused DNA damage. Indirect exposure to micrometer-sized CoCr (2.9 ± 1.1 μm in diameter) also damaged DNA. More than 95% of the nanoparticles were located within the cells of the superficial layer after 24 h of exposure, indicating that nanoparticles were internalized by the BeWo cells and did not pass through the barrier. The authors of this paper noted that the DNA damage was mediated by a novel mechanism involving pannexin and connexin hemichannels and gap junctions and purinergic signaling. These findings suggest that there is some possibility of placental transfer of particles.

2.1.6. Silica (SiO_2)

Industrial silica products are widely used in the electronics industry and as a food additive, and nanosized amorphous silica is used in a wide variety of applications including catalytic supports, photonic crystals, gene delivery, photodynamic therapy, and biomedical imaging [31]. An *in vitro* study of silica particles is presented in Table 3.

2.1.6.1. In vitro study of silica (SiO_2). The embryonic stem (ES) cell test using the D3 murine ES cell line was performed to determine the potential of spherical amorphous silica nanoparticles (10, 30, 80 and 400 nm in average primary particle size, Glantreo Ltd.) to inhibit the differentiation of ES cells into spontaneously contracting cardiomyocytes [32]. Silica nanoparticles were dialyzed against pure MilliQ water and diluted in distilled water, and the ES cells were exposed at 1–100 μg/mL throughout the entire 10-day test period. Transmission electron microscopy revealed that the dried silica particles were spherical and showed no substantial aggregation, except for the 10 nm particles, and measured diameters of the particles specified as 10, 30, 80, and 400 nm by the manufacturer were 11, 34, 34, and 248 nm, respectively. Silica particles of 30, 80 and 400 nm were observed in cells of the embryonic body. A concentration-dependent inhibition of the differentiation of ES cells into contracting cardiomyocytes was observed after exposure to 10 and 30 nm particles while the 80 and 400 nm particles did not inhibit the differentiation at up to 100 μg/mL. The inhibitory effect of the 30 nm particles was greater than that of the 10 nm particles as evidenced by the estimated ID_{50} values, 29 and 59 μg/mL, respectively. Inhibition of the differentiation of ES cells occurred below cytotoxic concentrations, suggesting a specific effect of the 10 and 30 nm particles on the differentiation of the ES cells.

2.2. Fullerenes (C_{60})

A fullerene is any molecule entirely in the form of a hollow sphere, ellipsoid, or tube. The first fullerene to be discovered is known as buckminsterfullerene C_{60}. Fullerenes have unique physicochemical properties that have been exploited for use in cosmetics, lubricants, dietary supplements, building materials, clothing treatment, electronics, and fuel cells [33]. *In vivo* and *in vitro* studies on fullerenes are listed in Table 4.

2.2.1. In vivo study of fullerenes (C_{60})

[60]Fullerene (C_{60}, purity > 99.9%, Terms Co.) was solubilized with poly(vinylpyrrolidone) (PVP). Pregnant Slc mice (n = 2/group) were intraperitoneally injected with C_{60} at 25, 50, or 137 mg/kg, PVP, or distilled water on DG 10, and their embryos were examined 18 h after injection [34]. No effects were observed in embryos of dams injected with PVP or distilled water. After the injection of C_{60}, all embryos died at 137 mg/kg. At 50 mg/kg, C_{60} was clearly distributed into the yolk sac and embryos and 50% of embryos were abnormal in shape predominantly in the head and tail regions. At 25 mg/kg, one pregnant mouse had all normal embryos and the other had only one abnormal embryo. The authors of this study speculated that C_{60} was incorporated into the concepts and the severely disrupted the function of the yolk sac and embryonic morphogenesis.

The distribution of [^{14}C]$_{60}$ was determined in rat dams and their pre- and postnatal offspring [35]. C_{60}, with an average particle size of less than 10 nm and estimated at 2 nm, was suspended in PVP. SD rats were given an intravenous injection of a suspension of approximately 0.3 mg [^{14}C]$_{60}$/kg into the tail vein on GD 15 or lactational day (LD) 8, and tissues of dams were collected 24 h (n = 4) and 48 h (n = 3) later. In pregnant dams at 24 h after injection, radioactivity was found in the liver (43% of the injected radioactivity), spleen (4%), reproductive tract (3%), and placenta (2%). Radioactivity was also detected in the digest of fetuses (0.87%). In lactating dams, radioactivity was detected in the liver (35%), spleen (4%), reproductive tract (0.10–0.42%), mammary tissue (0.48–0.94%), and milk at 24 h after injection. Radioactivity transferred to pups via lactation was found in the gastrointestinal tract (0.28%) in pups sacrificed at 24 h after injection, with an increase in distribution to the gastrointestinal tract of pups (0.43%) by 48 h after injection. The authors of this study noted that C_{60} distributed to the placenta and fetuses of exposed pregnant dams and to the milk and pups of exposed lactating dams.

2.2.2. In vitro study of fullerenes (C_{60})

Midbrain tissue samples of embryos of pregnant Slc–ICR mice on GD 11 were dissociated into individual cells, cell suspensions were prepared in culture medium, and a midbrain micromass culture was performed to evaluate the toxicity of C_{60} solubilized with PVP [34]. The C_{60} solution in the medium was incorporated into the midbrain culture plates, and further cultured for 6 days. The IC_{50} values of C_{60} for cell differentiation and proliferation were 0.43 and 0.47 mg/mL, respectively. Differentiation was inhibited as cytotoxicity increased. C_{60} was assumed to decrease cell proliferation via active oxygen species, because cell proliferation inhibited by C_{60} was partly restored by the addition of antioxidative enzymes.

2.3. Carbon black (CB)

CB is a low solubility particle produced industrially from incomplete thermal decomposition of hydrocarbons, a process controlled

Table 4
In vivo and *in vitro* reproductive and developmental toxicity studies of fullerenes (C_{60}), carbon black (CB), cadmium selenium-core quantum dots (CdSeQDs) and polystyrene-based fluorescent particles.

In vivo/*in vitro*	Materials/characteristics	Animals/cells	Exposure			Findings	References
			Route/method	Duration/time	Concentration		
In vivo	C_{60} (purity > 99.9%)	Slc mice	Intraperitoneal injection	Single on GD 10	25–137 mg/kg	Deaths of all embryos at 138 mg/kg Abnormalities in 50% of embryos at 50 mg/kg	[34]
In vivo	[^{14}C]C_{60} (>10 nm, estimated 2 nm in particle size)	SD rats	Intravenous injection	Single on DG 15 or LD 8	Approx. 0.3 mg/kg (3–4 rats/group)	Distribution of C_{60} in placentae and fetuses of exposed pregnant dams Distribution of C_{60} to milk and offspring of exposed lactating dams	[35]
In vitro	C_{60} (purity > 99.9%)	Midbrain cells of Slc:ICR mouse embryos at GD 11	Incubation	6 days	10–1000 μg/mL	IC_{50} for cell differentiation = 430 μg/mL IC_{50} for cell proliferation = 470 μg/mL	[34]
In vivo	CB Printex 90 (14 nm in particle size, 300 m^2/g in surface area) Printex 25 (56 nm in particle size, 45 m^2/g in surface area) Flammruss 101 (95 nm in particle size, 20 m^2/g in surface area)	ICR mice	Intratracheal instillation	10 times at weekly intervals	0.1 mg/mouse	No effect of 14, 56 or 95 nm particles on body weight or reproductive organs ↑ Serum testosterone levels after instillation of 14 and 56 nm particles ↓ DSP after instillation of 14, 56, and 90 nm particles	[37]
In vitro	CB (14 nm in particle size, Printex 90)	Mouse testis Leydig cell line TM3	Incubation	16, 24, or 48 h	1–1000 μg/mL	↓ Viability of TM3 at 1000 μg/mL No effect on proliferation of TM3 cells No changes in HO-1 mRNA expression at up to 100 μg/mL ↑ StAR mRNA expression at 30 μg/mL for 48 h-incubation	[20]
In vitro	CdSeQDs (approx. 3.5 nm in diameter) ZnS coating CdSeQDs	ICR mouse morulas and blastocysts	Incubation	24 h	125, 250, or 500 nmol/L	↓ Development of morulas into blastocysts at 250 and 500 nmol/L ↑ Number of apoptotic cells of blastocysts at 250 and 500 nmol/L ↓ Cell proliferation of blastocysts at 250 and 500 nmol/L ↓ Blastocyst development at 125 nmol/L and higher No cytotoxicity of ZnS coating CdSeQDs	[38]
In vitro	CdSeQDs (approx. 3.5 nm in diameter)	Female ICR and male C57BL/6J mice	Blastocysts were preincubated with CdSeQDs and transfered to pseudopregnat mice	Preincubation of blastocysts for 24 h	500 nmol/L	↓ Implantation rate ↑ Resorptions ↑ Embryos with abnormal development ↓ Fetal weight	[38]
In vitro	Polystyrene-based fluorescent nanoparticles (microspheres 40 to over 120 nm in size, Molecular Probes Inc.)	Two-cell stage mouse embryos	Incubation	4 days for 2-cell embryos 48 h for blastocysts	11.0 million/mL	No effect on development of 2-cell embryos No effect on hatching, implantation, or degeneration after exposure up to the blastocyst stage	[41]

to achieve pre-defined and reproducible particle sizes and properties suitable for a diverse range of industrial applications [36]. The CB particles so formed are complex, with a degenerated graphitic crystallite structure and high-power electron micrographs clearly show irregular layered graphitic plates. The most common use of CB is as a pigment and reinforcing phase in automobile tires. CB helps conduct heat away from the tread and belt area of the tire, reducing thermal damage and increasing tire life. CB is also employed in some radar-absorbent materials and in photocopiers and laser printer toner. *In vivo* and *in vitro* studies of CB are listed in Table 4.

2.3.1. In vivo study of carbon black (CB)

The effect of CB nanoparticles with a primary size of 14 nm (300 m^2/g in surface area, Printex 90, Degussa), 56 nm (45 m^2/g in surface area, Printex 25, Degussa), and 95 nm (20 m^2/g in surface area, Flammruss 101, Degussa) on the male reproductive system was determined [37]. Six-week-old male ICR mice (n = 15–16/group) were intratracheally instilled with CB particles suspended in normal saline containing 0.05% Tween 80 at 0.1 mg/mouse for the 14, 56, and 95 nm CB particles and 1.56 μg/mouse for the 14 nm CB (particle number concentration of 14 nm CB is the same as that of 56 nm CB). Mice received 10 weekly instillations and were killed on day after the last instillation. No effect of the 14, 56, or 96 nm particles was observed on body weight or male reproductive organ weights. Vacuolation of the seminiferous tubules and decreased DSP were found in mice instilled with all three sizes of CB particles. Levels of serum testosterone were increased after instillation of all three particles. The group exposed to the 14 nm particles, with approximately the same number of particles per unit volume as the 56 nm particles, showed fewer effects than did the group exposed to the 56 nm particles. The authors noted that CB nanoparticles impaired the function of Leydig cells, and the consequent fluctuation of sperm testosterone levels caused a reduction of DSP. These findings suggest that CB nanoparticles adversely affect mouse spermatogenesis and the effect depends on particle mass rather than particle number.

2.3.2. In vitro study of carbon black (CB)

The direct effects of CB (14 nm in particle size, Printex 90, Degussa) on testis-constituent cells was determined using the mouse Leydig cell line TM3 [20]. The test was performed using the procedure described above in the TiO_2 section. The uptake of CB nanoparticles by Leydig cells was detected after 48 h. Cell viability was markedly inhibited at 1000 μg/mL, but CB did not affect the proliferation of TM3 cells. No effect of CB was found on the expression of HO-1 mRNA in TM3 cells at up to 100 μg/mL. StAR mRNA expression was increased at 30 μg/mL after incubation for 48 h. These findings suggest that CB nanoparticles have no direct effect on the induction of oxidative stress but affect the production of steroid hormones in Leydig cells.

2.4. Luminescent particles

In vitro studies of cadmium selenium-core quatum dots (CdSeQDs) and polystyrene-based fluorescent particle have been published.

2.4.1. Cadmium selenium-core quantum dots (CdSeQDs)

Quantum dots are colloidal nanocrystalline semiconductors that have unique light-emitting properties and can be used as a novel luminescent material [38]. CdSeQDs are useful as an alternative to fluorescent dyes for use in biological imaging, due to their bright fluorescence, narrow emission, broad UV excitation, and high photostability [39]. An *in vitro* study of CdSeQDs is shown in Table 4.

2.4.1.1. In vitro study of cadmium selenium-core quantum dots (CdSeQDs). The developmental effect of CdSeQDs (approximately 3.5 nm in diameter) was determined using mouse embryos [38]. For water solubilization, the CdSeQDs were surface coupled with mercaptoacetic acid and suspended in PBS. Morulas and blastocysts were obtained from superovulating ICR female mice, which were mated with fertile males of the same strain, by flushing the fallopian tubes on GD 3 and flushing the uterine horns on GD 4, respectively. After incubation of morulas or blastocysts with CdSeQDs for 24 h, an inhibition of the preimplantation development of morulas into blastocysts, increased number of apoptotic cells in the inner cell mass (ICM) of blastocysts (n = 200/group) and inhibition of cell proliferation, primarily in the ICM, of blastocysts (n = 180/group) at 250 nmol/L and above, and inhibition of the postimplantation development of blastocysts at 125 nmol/L and above were observed. To examine the effect of CdSeQDs on the piostimplantation development of blastocysts, blastocysts (n = 200/group) exposed to 0 or 500 nmol/L for 24 h were transferred to recipient ICR mice (n = 25/group), which were mated with vasectomized C57BL/6J male mice, on pseudopregnant day (PD) 4 and killed on PD 18. A decreased implantation rate and fetal weight, and increased numbers of embryos with abnormal development and resorptions were observed in the CdSeQDs-treated group. CdSeQDs coated with ZnS had no significant cytotoxic effect on blastocyst development. These findings indicate that CdSeQDs affect adversely pre- and postimplantation embryonic survival and development and the ZnS coating alters the CdSeQD-induced toxicity.

2.4.2. Polystyrene-based fluorescent particles

Fluorescent nanoparticles are promising tools for optical data storage and other technical applications in biochemical, bioanalytical, and medical areas, and were successfully used for immunoassays [40]. An *in vitro* study of fluorescent nanoparticles is shown in Table 4.

2.4.2.1. In vitro study of polystyrene-based fluorescent particles. The effect of ultrafine polystyrene-based fluorescent particles (Molecular Probes Inc.), ranging from 40 nm to over 120 nm in size with different fluorescence colors corresponding to particle size, on mouse embryos was examined [41]. Two-cell stage embryos were incubated with fluorescent nanoparticles at 11.0 million/mL for 4 days, and development was assessed. Untreated embryos incubated for 4 days were further incubated with fluorescent nanoparticles at 11.0 million/mL for 48 h, and the developmental stages of the blastocysts were assessed. No effect of nanoparticles was found on the development of 2-cell stage embryos to the blastocyst stage. There was no effect of nanoparticles on hatching, implantation on the culture dish, or degeneration after additional exposure until the blastocyst stage. Although nanoparticles were internalized, the development of embryos was not affected. Nanoparticles were predominantly found in the trophoblast cells with a few located in the inner cell mass in hatched blastocysts. These findings show that fluorescent nanoparticles did not affect the development of mouse early embryos and suggest that internalized nanoparticles did not affect cellular processes or the expression of factors needed for development.

3. Discussion and conclusions

This paper reviewed the *in vivo* and *in vitro* studies on the reproductive and developmental toxicity of nanomaterials. Although it provides initial information on the potential toxicity of nanomaterials, it should be followed up by relevant hazard studies of nanomaterials.

In vivo studies have showed increased allergic susceptibility in offspring of mouse dams intranasally insufflated with respirable-

size TiO_2, adverse effects on spermatogenesis and histopathological changes in the testes, and changes in gene expression in the brain in mouse offspring after maternal subcutaneous injections of TiO_2 nanoparticles, transfer to rat fetuses of radiolabeled gold nanoparticles and C_{60} after maternal intravenous injection, death and morphological abnormalities in mouse embryos after maternal intraperitoneal injections of C_{60}, and adverse effects on spermatogenesis in mouse offspring after maternal intratracheal instillations of CB nanoparticles. However, these studies were performed with 1–10 administrations of a large bolus and/or a route of exposure not relevant to humans using relatively small numbers of animals. *In vivo* studies should be performed that include doses that closely reflect expected exposure levels. Major routes of exposure to nanoparticles are the respiratory tract, skin, eyes, and gastrointestinal tract. Studies using relevant routes of exposure are needed to clarify the toxicity of nanoparticles. The number of animals per group should be sufficient to allow meaningful interpretation of the data for reproductive and developmental toxicity studies, and a dose–response analysis is also needed to allow more realistic comparisons with actual human exposure. In the studies presented in this review paper, there was a lack of information regarding maternal toxicity. The investigation of maternal toxicity is essential for reproductive and developmental toxicity studies, because the toxicity to offspring may be modified or influenced by toxicity to the mother, and toxicity to offspring often occurs in conjunction with maternal toxicity in animal studies.

Radioactivity was detected in rat fetuses of dams intravenously injected with gold nanoparticles or C_{60}, but unlabeled gold nanoparticles were not detected in mouse fetuses of dams injected intravenously or in the fetal outflow of human placenta. *In vitro* study also revealed some possibility of placental transfer of CoCr particles mediated by a novel mechanism. In terms of developmental toxicity, information on the placental transfer of nanomaterials to offspring of dams given during gestation and lactation is of great interest in interpretation of the data. Measurements of the placental transfer of nanoparticles are an important source of information on the mechanism of action and the risk of nanoparticles, and may help to clarify the reproductive and developmental toxicity of nanoparticles.

As for the effect of nanoparticles on embryonic development, maternally administered C_{60} impaired embryonic development and the results of micromass culture suggest a dysmorphogenic effect of C_{60}. The C_{60} was clearly distributed into the yolk sac. These findings resemble those of developmental toxicity studies of trypan blue, which was teratogenic in mice, rats, hamsters, and guinea pigs [42]. It is generally accepted that teratogenic action of trypan blue in rats is due to its accumulation and interference in the function of the yolk sac, an organ of histotrophic nutrition that provides the principal source of nutrients before the initiation of functional chorio-allantoic placentae. Mice and rats have a yolk sac placenta, which plays a significant role during early in organogenesis. This is not the case for humans and monkeys in which the yolk sac placenta is of insignificant importance. Trypan blue produces malformations in rats and mice due to its accumulation in the yolk sac. This is not possible in humans and monkeys [43].

It is noted that test conducted and reported according to international accepted test guidelines and in compliance with the principles of Good Laboratory Practice (GLP) should have the highest grade of reliability and data for hazard identification must be evaluated considering their quality and adequacy for risk assessment [44]. At present, however, such studies are not available for reproductive and developmental toxicity of nanomaterials. Oberdörster et al. [1] described that studies to assess reproductive effects following pulmonary exposure to nanomaterials should follow protocols similar to OECD guideline 422 for the Testing of Chemicals (Combined Repeated Dose of Toxicity Study with the Reproduction/Developmental Toxicity Screening Test). The OECD guideline 421 for Testing of Chemicals (Reproduction/Developmental Toxicity Screening Test) is also useful to obtain initial information on possible effects on reproduction and development. In these tests, test materials are given to male rats for a minimum of 4 weeks beginning before mating and to females beginning before mating to shortly after parturition of pups. These screening tests are performed using relatively small numbers of animals in the dose groups and do not provide complete information on all aspects of reproduction and development due to the limitation of the exposure period and selectivity of endpoints. The two-generation study, which covers the whole reproductive cycles of at least one generation, may be adequate to evaluate the reproductive and developmental toxicity of nanomaterials. However, the concentrations, populations, and duration of exposure to nanomaterials are different from one another. It is required to modify the exposure period and the endpoints correlated with the exposure period. To further evaluate the reproductive and developmental toxicity of nanomaterials, a more specific test should be designed on a case-by-case basis according to the characterization of human exposure.

In vitro studies revealed high concentrations of TiO_2 nanoparticles to affect the viability and proliferation of mouse Leydig cells, but not the gene expression associated with spermatogenesis. Gold nanoparticles decreased the motility of human sperm, silver, aluminum, and MoO_3 were toxic to mouse spermatogonia stem cells, CoCr nanoparticles damaged DNA of human fibroblast cells, silica nanoparticles inhibited the differentiation of mouse ES cells, C_{60} inhibited the differentiation of mouse midbrain cells, CB decreased the viability of mouse Leydig cells, and CdSeQDs inhibited the pre- and postimplantation development of mouse embryos. In these studies, the concentrations of nanoparticles were very high and unlikely to occur in animal studies. The mechanistic pathways that operate at low realistic concentrations are likely to be different from those operating at very high concentrations when the cell's or organism's defenses are overwhelmed [2]. The findings of these *in vitro* studies are difficult to evaluate because of differences in the chemical composition and sizes of particles, target cells, duration of exposure, endpoints, and exposure concentrations among experiments. *In vivo* studies correlated with results obtained from *in vitro* studies should be performed.

Oxidative stress as a common mechanism for cell damage induced by nanoparticles is well known and a wide range of nanomaterial species have been shown to create reactive oxygen species (ROS) both *in vivo* and *in vitro*. It is suggested that a free radical-induced mechanism or another form of oxidative stress played a role in the developmental toxicity of C_{60} in zebrafish, in which C_{60} caused decreases in the embryonic survival rate, the hatching rate, heartbeat and pericardial edema, and the toxicity was effectively attenuated by adding glutathione, an antioxidant [45]. In mammals, TiO_2 nanoparticles in Leydig cells, Sertili cells, spermatids, and cells of the olfactory bulb and cerebral cortex of pups, and C_{60} in embryos and yolk sac were noted after a maternal administration. In *in vitro* studies, TiO_2 and CB nanoparticles in Leydig cells, Fe_3O_4 and gold nanoparticles in sperm cells, silica nanoparticles in cells of the embryonic body, CoCr nanoparticles in BeWo cells, and fluorescent nanoparticles in trophoblast cells were observed. Determination of the oxidative stress in these cells may help us to understand the reproductive and developmental toxicity of nanoparticles.

The contradicting results obtained from the studies presented in this review may be attributed to the use of different nanomaterials and experimental models, the exposure during different stages of offspring development, and evaluations with different endpoints. It is likely that the size, shapes, chemistry, crystallinity, surface properties, concentration, agglomeration, and dose of nanoparticles are all involved in detecting biological activity. The characterization of

administered materials in toxicity studies is fundamental, and characterizing delivered nanomaterials after administration in a test system or model provides the best quality data on dose and material properties that are related to observed responses, but this is limited by current methodological capabilities [2]. Further studies, especially *in vivo*, using different types of characterized materials, relevant routes of administration, and doses closely reflecting expected levels of exposure are needed to adequately evaluate the reproductive and developmental toxicity of nanomaterials.

Conflict of interest

None.

Acknowledgements

This study was conducted under the "Evaluating Risks Associated with Manufactured Nanomaterials" Project funded by the New Energy and Industrial Technology Development Organization (NEDO), Japan.

References

[1] Oberdörster G, Maynard A, Donaldson K, Castranova V, Fitzpatric J, Ausman K, et al. Principles for characterizing the potential human health effects from exposure to nanoparticles: elements of a screening strategy. Part Fibre Toxicol 2005;2:8, doi:10.1186/1743-8977-2-8.

[2] Oberdörster G, Oberdörster E, Oberdörster J. Nanotoxicology: an emerging discipline evolution from studies of ultrafine particles. Environ Health Perspect 2005;113:823–39.

[3] Penn SG, He L, Natan MJ. Nanoparticles for bioanalysis. Curr Opin Chem Biol 2003;7:609–15.

[4] Liu WT. Nanoparticles and their biological and environmental applications. J Biosci Bioeng 2006;102:1–7.

[5] Colborn T, vom Saal F, Soto A. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect 1993;101:378–84.

[6] Brooking J, Davis SS, Illum L. Transport of nanoparticles across the rat mucosa. J Drug Target 2001;9:267–79.

[7] Wang J, Liu Y, Jiao F, Lan F, Li W, Gu Y, et al. Time-dependent translocation and potential impairment on central nervous system by intranasally instilled TiO_2 nanoparticles. Toxicology 2008;254:82–90.

[8] Lee KP, Trochimowicz HJ, Reinhardt CF. Pulmonary response of rats exposed to titanium dioxide (TiO_2) by inhalation for two years. Toxicol Appl Pharmacol 1985;79:179–92.

[9] Lindenschmidt RC, Driscoll KE, Perkins MA, Higgins JM, Maurer JK, Belfiore KA. The comparison of a fibrogenic and two nonfibrogenic dusts by bronchoalveolar lavage. Toxicol Appl Pharmacol 1990;102:268–81.

[10] Muhle H, Bellmann B, Creutzenberg O, Dasenbrock C, Ernst H, Kilpper R, et al. Pulmonary response to toner upon chronic inhalation exposure in rats. Fundam Appl Toxicol 1991;17:280–99.

[11] Warheit B, Sayes CM, Reed KL, Swain KA. Health effects related to nanoparticle exposures: environmental, health and safety considerations for assessing hazards and risks. Pharmacol Ther 2008;120:35–42.

[12] Heinrich U, Fuhst R, Rittinghausen S, Cretzenberg O, Bellmann B, Koch W, et al. Chronic inhalation exposure of Wistar rats and two different strains of mice to diesel engine exhaust, carbon black, and titanium dioxide. Inhal Toxicol 1995;7:533–56.

[13] Baan R, Staif K, Grosse Y, Secretan B, Ghissassi F, Cogliano V, on behalf of the WHO International Agency for Research on Cancer Monograph Working Group. Carcinogenicity of carbon black, titanium dioxide, and talk; 2006 [cited June 22, 2009], available from: http://oncology.thelancet.com.

[14] Landsiedel R, Kapp MD, Schulz M, Wiench K, Oesch F. Genotoxicity investigations on nanomaterials: methods, preparation and characterization of test material, potential artifacts and limitations-many questions, some answers. Mutat Res 2009;681:241–58.

[15] Ema M, Kobayashi N, Naya M, Hanai S, Nakanishi J. Genotoxicity evaluation of titanium dioxide nanoparticles. Jpn J Environ Toxicol 2009;12:71–84 [in Japanese].

[16] Fedulov AV, Leme A, Yang Z, Dahl M, Lim R, Mariani TJ, Kobzik L. Pulmonary exposure to particles during pregnancy causes increased neonatal asthma susceptibility. Am J Respir Mol Biol 2008;38:57–67.

[17] CEPA/OEHHA (California Environmental Protection Agency/Office of Environmental Health Hazard Assessment). Safe drinking water and toxic enforcement ACT of 1986 (Position 65). Notice to interested parties, February 21, 2003. Chemical listed effective February 21, 2003 as known to the State of California to cause cancer; 2003 [cited October 13, 2009], available from: http://oehha.ca.gov/prop65/prop65_list/files/22103not.pdf.

[18] Shimizu M, Tainaka H, Oba T, Mizuo K, Umezawa M, Takeda K. Maternal exposure to nanoparticle titanium dioxide during the prenatal period alters gene expression related to brain development in the mouse. Part Fibre Toxicol 2009;6:20, doi:10.1186/1743-8977-6-20.

[19] Takeda K, Suzuki K, Ishihara A, Kubo-Irie M, Fujimoto R, Tabata M, et al. Nanoparticles transferred from pregnant mice to their offspring can damage the genital and cranial nerve system. J Health Sci 2009;55:95–102.

[20] Komatsu T, Tabata M, Kubo-Irie M, Shimizu T, Suzuki K, Nihei Y, et al. The effects of nanoparticles on mouse testis Leydig cells *in vitro*. Toxicol in Vitro 2008;22:1825–31.

[21] Shelley SA. Nanotechnology: turning basic science into reality. In: Theodore L, Kunz RG, editors. Nanotechnology-environmental implications and solution. Hoboken: John Wiley and Sons, Inc. Publication; 2005. p. 61–107.

[22] Challier JC, Panigel M, Meyer E. Uptake of colloidal ^{198}Au by fetal liver in rats, after direct intrafetal administration. Int J Nucl Med Biol 1973;1:103–6.

[23] Takahashi S, Matsuoka O. Cross placental transfer of ^{198}Au-colloid in near term rats. J Radiat Res 1981;22:242–9.

[24] Sadauskas E, Wallin H, Stoltenberg M, Vogel U, Doering P, Larsen A, et al. Kupper cells are central in the removal of nanoparticles from the organism. Part Fibre Toxicol 2007;4:10, doi:10.1186/1743-8977-r-r4-10.

[25] Myllynen P, Loughran MJ, Howard CV, Sormunen R, Walsh A, Vähäkangas KH. Kinetics of gold nanoparticles in the human placenta. Reprod Toxicol 2008;26:130–7.

[26] Wiwanitkit V, Sereemaspun A, Rojanathanes R. Effect of gold nanoparticles on spermatozoa: the first world report. Fertil Steril 2009;91:e7–8.

[27] Kim WS, Kim HC, Hong SH. Gas sensing properties of MoO_3 nanoparticles synthesized by solvothermal method. J Nanopart Res 2009, doi:10.1007/s11051-009-9751-6.

[28] Braydich-Stolle L, Hussain S, Schlager JJ, Hofmann MC. *In vitro* cytotoxicity of nanoparticles in mammalian germline stem cells. Toxicol Sci 2005;88:412–9.

[29] Makhluf SBD, Qasem R, Rubinstein S, Gedanken A, Breibart H. Loading magnetic nanoparticles into sperm cells does not affect their functionality. Langmuir 2006;22:9480–2.

[30] Bhabra G, Sood A, Fisher B, Cartwright L, Saunders M, Evans WH, et al. Nanoparticles can cause DNA damage across a cellular barrier. Nat Nanotechnol 2009;4:876–83.

[31] Lin YS, Haynes CL. Impact of mesoporous silica nanoparticle size, pore ordering, and pore integrity on hemolytic activity. J Am Chem Soc 2010;132:4834–42.

[32] Park MVDZ, Annema W, Salvati A, Lesniak A, Elsaesser A, Barnes C, et al. In vitro developmental toxicity test detects inhibition of stem cell differentiation by silica nanoparticles. Toxicol Appl Pharmacol 2009;240:108–16.

[33] Loutfy RO, Lowe TP, Moravsky AP, Katagiri S. Commercial production of fullerenes and carbon nanoparticles. In: Perspectives of fullerene nanotechnology. Netherlands: Springer; 2002. p. 35–46.

[34] Tsuchiya T, Oguri I, Yamakoshi YN, Miyata N. Novel harmful effects of [60]fullerene on mouse embryos in vitro and in vivo. FEBS Lett 1996;393:139–45.

[35] Sumner SCJ, Fennell TR, Snyder RW, Taylor GF, Lewin AH. Distribution of carbon-14 labeled C_{60}([^{14}C]C_{60}) in the pregnant and in the lactating dam and the effect of C_{60} exposure on the biochemical profile of urine. J Appl Toxicol 2009, doi:10.1002/jat.1503.

[36] Donaldson K, Tran L, Jimenez LA, Duffin R, Newby DE, Mils N, et al. Combustion-derived nanoparticles: a review of their toxicology following inhalation exposure. Part Fibre Toxicol 2005;2:10, doi:10.1186/1743-89772-2-10.

[37] Yoshida S, Hiyoshi K, Ichinose T, Takano H, Oshio S, Sugawara I, et al. Effect of nanoparticles on the male reproductive system of mice. Int J Androl 2008;32:337–42.

[38] Chan WH, Shiao NH. Cytotoxic effect of CdSe quantum dots on mouse embryonic development. Acta Pharmacol Sin 2008;28:259–66.

[39] Derfus AM, Chan WCW, Bhatia SN. Probing the cytotoxicity of semiconductor quantum dots. Nano Lett 2004;4:11–8.

[40] Bangs LB. New developments in particle-based immunoassays: introduction. Pure Appl Chem 1996;68:1873–9.

[41] Bosman SJ, Niet SP, Patton WC, Jacobson JD, Corselli JU, Chan PJ. Development of mammalian embryos exposed to mixed-size nanoparticles. Clin Exp Obst Gyn 2005;32:222–4.

[42] Shepard TH, Lemire RJ. Catalog of teratogenic agents. 11th ed. Baltimore: The Johns Hopkins University Press; 2004.

[43] Nielsen E, Thorup I, Schnipper A, Hass U, Meyer O, Ladefoged J, et al. Children and the unborn child. Exposure and susceptibility to chemical substances—an evaluation. Environmental project No. 589, Miljøproject; 2001 [cited October 18, 2009], available from: http://www2.mst.dk/udgiv/Publications/2001/87-7909-574-7/pdf/87-7909-333-7.pdf.

[44] Klimisch HJ, Andreae M, Tillmann U. A systemic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regl Toxicol Pharmacol 1997;25:1–5.

[45] Zhu X, Zhu L, Li Y, Duan Z, Chen W, Alvarez PJJ. Developmental toxicity in zebrafish (*Danio rerio*) embryos after exposure to manufactured nanomaterials: buckminsterfullerene aggregates (nC_{60}) and fullerol. Environ Toxicol Chem 2007;26:976–9.

The Release of Nanosilver from Consumer Products Used in the Home

Troy Benn* and Bridget Cavanagh Arizona State University
Kiril Hristovski Arizona State Univ.-Polytechnic Campus
Jonathan D. Posner and Paul Westerhoff Arizona State University

Nanosilver has become one of the most widely used nanomaterials in consumer products because of its antimicrobial properties. Public concern over the potential adverse effects of nanosilver's environmental release has prompted discussion of federal regulation. In this paper, we assess several classes of consumer products for their silver content and potential to release nanosilver into water, air, or soil. Silver was quantified in a shirt, a medical mask and cloth, toothpaste, detergent, a towel, a cup, baby bottle, and two food bottles. Silver concentrations ranged from 1.4 to 270,000 μg Ag g product^{-1}. Products were washed in 500 mL of tap water to assess the potential release of silver into aqueous environmental matrices (wastewater, surface water, saliva, etc.). Silver was released in quantities up to 45 μg Ag g product^{-1}, and size fractions were both larger and smaller than 100 nm. Scanning electron microscopy confirmed the presence of nanoparticle silver in most products as well as in the wash water samples. Four products were subjected to a toxicity characteristic leaching procedure to assess the release of silver in a landfill. The medical cloth released an amount of silver comparable to the results characterized here. This paper presents methodologies that can be used to quantify and characterize silver and other nanomaterials in consumer products. The quantities of silver in consumer products can in turn be used to estimate real-world human and environmental exposure levels.

SILVER HAS LONG BEEN USED in products as an antimicrobial agent. Particles of silver can be administered in products to release ionic silver (Ag^+), which is often attributed with antimicrobial efficacy (Percival et al., 2005; Wijnhoven et al., 2009). Nanosilver, defined here as particles of silver having at least one dimension in the 1- to 100-nm size range, is increasingly being used in consumer products to control the growth of microorganisms on surfaces and in solutions. Properties of nanosilver, such as a low redox potential (Ivanova and Zamborini, 2009), could increase the capacity of smaller nanosilver particles to release Ag^+ compared with bulk silver. In addition, the generation of reactive oxygen species has been suggested as a mechanism for nanosilver toxicity (Kim et al., 2007). Regardless of the toxicity mechanism(s), increased antimicrobial behavior of nanosilver is often observed at particle sizes under 30 nm (Auffan et al., 2009) and allows manufacturers to minimize the total silver used in a product compared with other forms such as silver nitrate or microscale silver (Ki et al., 2007).

However, it is unclear whether the novel properties of nanosilver will lead to adverse human and/or environmental health effects. The production, use, and disposal of products containing nanosilver can lead to the release of increased amounts of silver into various environmental compartments (air, water, soil). For example, nanosilver released from clothing could enter the environment in the effluent and/or biosolids resulting from wastewater treatment (Benn and Westerhoff, 2008). The possible adverse effects of increased environmental exposure to nanosilver include the development of silver-resistant bacteria (Gupta et al., 1999; Percival et al., 2005, 2008; Silver 2003), the impairment of aquatic (Choi and Hu, 2008; Griffitt et al., 2008, 2009; Navarro et al., 2008) and soil (Roh et al., 2009) organisms, and the impairment of human health (El-Ansary and Al-Daihan, 2009; Greulich et al., 2009; Hussain et al., 2005; Ji et al., 2007; Mirsattari et al., 2004; Takenaka et al., 2001).

The possible adverse effects have prompted 14 organizations, led by the International Center for Technology Assessment, in

J. Environ. Qual. 39:1875–1882 (2010)
doi:10.2134/jeq2009.0363
Published online 12 July 2010.
Supplemental data file available online for this article.
Received 12 Sept. 2009.
*Corresponding author (Troy.Benn@asu.edu).

T. Benn, B. Cavanagh, and P. Westerhoff, School of Sustainable Engineering and the Built Environment, Arizona State Univ., PO Box 875306, Tempe, AZ 85287-5306; K. Hristovski, College of Technology and Innovation, Arizona State Univ.-Polytechnic Campus, 6073 S. Backus Mall, Mesa, AZ 85212; J.D. Posner, Mechanical Engineering and Chemical Engineering Programs, Arizona State Univ., PO. Box 876106, Tempe, AZ 85287-6106. Assigned to Associate Editor Joel Pedersen.

Abbreviations: EDX, energy dispersive X-ray; ICP-OES, inductively coupled plasma-optical emission spectroscopy; SEM, scanning electron microscopy; TCLP, toxicity characterization leaching procedure; WWTP, wastewater treatment plant.

from the toothpaste. The size fractionation data on the silver released from the toothpaste into tap water also suggest that the majority of silver is retained by the 100-nm-pore-size filter (see Table 2 and next section). A heterogeneous distribution of silver in the toothpaste may explain the discrepancy between the advertised concentration (100 ppm) and the measured concentration (8 μg g^{-1} [8 ppm]). Figure 2a shows 500-nm-diam. agglomerates of nanosilver that are typical of the medical mask, and SEM at lower magnification shows them unevenly distributed over the mask surface (Supplementary Fig. S2). Furthermore, precipitates were visually identified at the bottom of the detergent bottle. The SEM analysis of these particulates showed micrometer-scale agglomerates comprised of nanoscale particles that EDX analysis suggests are silver (Fig. 3). These unique crystal-like structures may be formed during the evaporation of the detergent during SEM stub preparation. However, these agglomerates were not detected with SEM in the bulk of the detergent. The microscale silver that settled to the bottom of the bottle was probably not sampled during the acid digestion analysis, and the low variability in the silver content analysis of the detergent could be attributed to dissolved silver ions. Such heterogeneous silver distributions could result in varying levels of silver released during use of the product and/or impair product performance.

Release of Silver into Water

Table 2 summarizes the quantity and particle size fractionation of silver released from consumer products that were washed in tap water for 1 h. The products released highly variable amounts of their silver. For example, the face mask, which contained approximately 27% silver by weight, released <0.01% (15.8 μg) of its silver into the wash water. In contrast, the shirt, which contained 44 μg g^{-1} of silver, released about 2% (34 μg) of its silver. The toothpaste, shampoo, and detergent were assumed to release all of their silver into the wash water.

The 100-nm-pore-size filter removed the majority of the silver released by the toothpaste, shampoo, and detergent. This suggests that the silver is released as particles, or is associated with particles, larger than 100 nm. Conversely, the majority of the silver released from the shirt, mask, and medical cloth passed through the 20-nm filter. Scanning electron microscopy was used to confirm the size of the nanosilver released from the medical cloth and mask. Figure 2a shows three large agglomerates (~500 nm in diam.) of silver nanoparticles on the surface of the face mask, which is completely coated with smaller nanoparticles (<20 nm in diam.). The small nanoparticles on the surface were confirmed with an EDX analysis at a location on the face mask surface away from the 500-nm agglomerates, which also yielded a dominant silver signal. This suggests that the silver passing through the 20-nm filter could be nanoparticles in addition to dissolved silver ions. Figure 2b shows agglomerations of particles with sizes <100 nm that were released into the tap water. These agglomerations are similar to those seen on the mask fabric. However, they could be an artifact of the centrifugation step during preparation of the SEM sample; forced settling during centrifugation may increase the probability of particles colliding and forming larger agglomerates.

The SEM analysis was also conducted on other products and their subsequent wash waters. The typical particles found in the wash water of the toothpaste (Fig. 1) are much larger in size (100–500 nm) than those from the medical cloth and mask. This concurs with the size fraction data in Table 2, in which only about 10% of the toothpaste silver passed through a 20-nm filter. Although not advertised to contain nanosilver, the toothpaste appears to release at least some nanosize silver particles into tap water. The SEM/EDX also confirmed the presence of silver nanoparticles in the wash water of the shirt (Supplementary Fig. S4).

A calculation of the silver released per capita from the use of daily household products (i.e., washing one 178-g shirt and using 5 g of shampoo, 100 g of detergent, and 1 g of toothpaste), assuming that all of the released silver reaches the sewer, suggests that one consumer could be responsible for releasing about 470 μg Ag into the sewer system per day. This calculation uses the wash water data for the shirt and toothpaste and assumes that the shampoo and detergent release 100% of their silver contents. This calculation can be used to comprehend the influx of silver to sewage as a result of the use of nanosilver in the home. If 10,000 people released 470 μg Ag per capita d^{-1} into a million-gallon-per-day capacity WWTP, the biosolids silver concentration might increase by 0.7 mg Ag kg^{-1} (using model from Benn and Westerhoff, 2008). This value is comparable to

Table 2. Mass and size fractionation of silver released from consumer products washed in tap water for 1 h.

Product	Mass of product washed	Size fraction of silver released into 500 mL of tap water			Total silver released per product mass
		Total	<100 nm	<20 nm	
	g	μg			μg Ag g product^{-1}
Athletic shirt†	41 ± 9.6	27 ± 1.4	20 ± 0.5	11 ± 1.2	0.56 ± 0.01
Unfinished cloth fabric‡	45 and 46	22 and 47	12 and 16	12 and 13	0.5 and 1.1
Medical mask	1.4	15.8	14.6	14.8	11
Medical cloth	0.3	13.8	11.3	13.3	46
Toothpaste	2.1	17.3	14.6	4.3	18
Shampoo	13.2	11.8	4.8	3.8	0.9
Detergent	23.9	43.3	4.8	1.7	1.8
Yellow cloth (towel)	5	<5	<5	<5	<1.0
Teddy bear	26	<5	<5	<5	<0.2

† Three samples of the athletic shirt were washed and analyzed.

‡ Two samples of the unfinished cloth fabric were washed and analyzed.

the 1.29 mg kg^{-1} sludge silver concentration predicted by a probability density function model (Gottschalk et al., 2009). Furthermore, a survey of U.S. WWTPs in 2006 to 2007 quantified silver in biosolids ranging from 1.94 to 856 mg kg^{-1} (USEPA, 2009b). Therefore, a worst-case scenario where everybody in a population leached 470 μg Ag d^{-1} might lead to increases in WWTP biosolids silver concentrations comparable to concentrations currently observed. This demonstrates that an increase in the use of nanosilver could noticeably increase the amount of silver in some wastewater systems. The environmental impact of this increase in wastewater silver is yet to be determined. It should be noted that other consumer products containing silver were not tested that could add to an estimation of silver released per capita per day. Also, the release of silver over product lifetimes could be investigated to justify this calculation.

Release of Silver from Humidifiers into Air

Silver was quantified in the water emitted from two humidifiers. The plastic tank of the small humidifier, which contained 60 μg Ag g plastic^{-1}, released water containing 1.1 ± 0.4 μg Ag L^{-1} (± represents standard deviation of eight samples) into the air at a flow rate of 100 mL h^{-1}. Therefore, the mass flow rate of silver released into the air is estimated to be 0.11 ± 0.04 μg Ag h^{-1}. Similarly, the large humidifier released water containing 0.19 μg Ag L^{-1} at a flow rate of 420 mL h^{-1}, which is a release rate into the air of 0.08 μg Ag h^{-1}. Because the silver concentrations in the collected water were low, filter fractionation and SEM analyses could not be conducted. The SEM/EDX analysis was attempted on the plastic of the small humidifier, but the presence of nanosilver could not be confirmed due to the instability of the plastic sample under the electron beam.

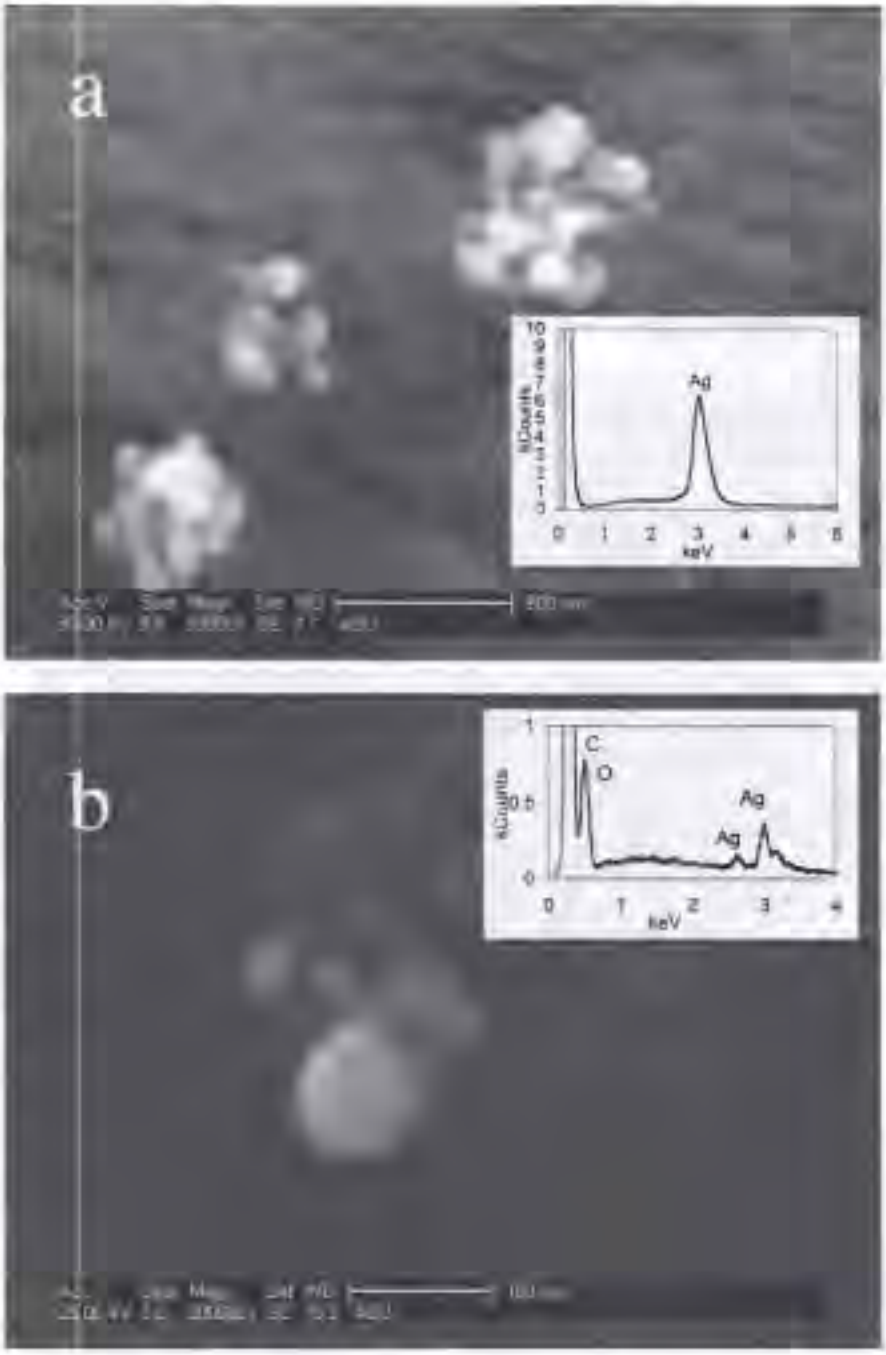

Fig. 2. (a) Scanning electron micrograph (SEM) of three nanosilver agglomerations (~200–500 nm diam.) on the fabric surface of a medical face mask that is also completely coated with nanosilver particles <20 nm in diameter (underlying particles). Inset: Energy dispersive X-ray (EDX) analysis indicating the agglomerations and surface of the mask contain silver. (b) SEM of a tap water wash of the face mask. Inset: EDX analysis showing silver. Carbon and oxygen peaks are attributed to the background carbon tape of the SEM stub.

Release of Silver into Landfills

It is probable that most of the silver released from these products into wastewater will enter municipal and septic sewage systems. At municipal WWTPs most of the silver can be removed either by adsorption to particulates (Benn and Westerhoff, 2008; Wang et al., 2003) or precipitation with chloride (Wang et al., 2003), after which it is settled out within the biosolids to be disposed of as agricultural fertilizer, incinerated, or landfilled. Silver not removed at a WWTP will reenter the aquatic environment on discharge of the treated effluent. Although this amount of silver is estimated to be low (Benn and Westerhoff, 2008; Mueller and Nowack, 2008), it must not be neglected when considering fate

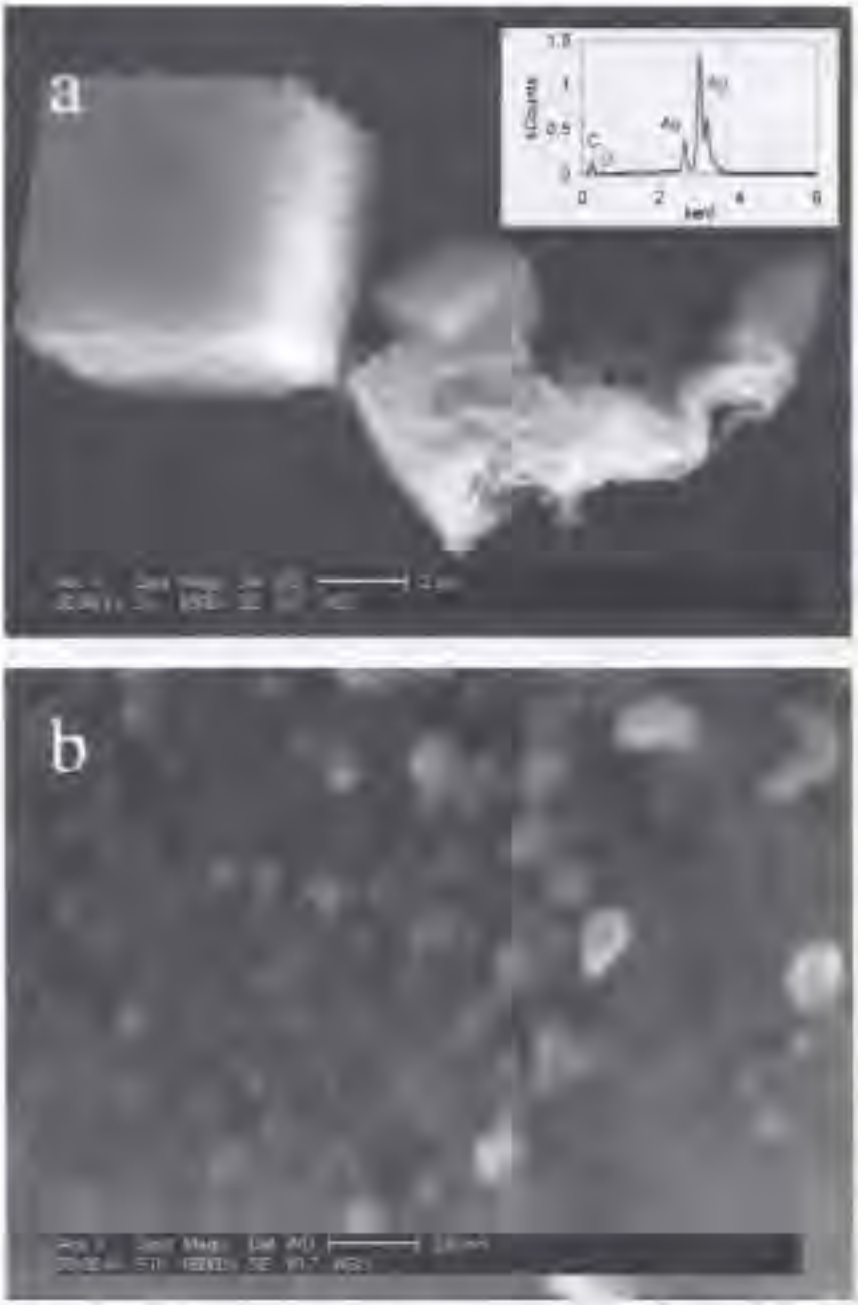

Fig. 3. (a) Micron-sized, cubic agglomerates of nanosilver particles collected from the bottom of the detergent bottle. Inset: Energy dispersive X-ray analysis of the cubic structure showing dominant silver presence. (b) Surface of a cubic structure showing the arrangement of silver nanoparticles.

and transport over long time scales and potential adverse environmental effects due to bioaccumulation.

Several products in this study will end up in landfills at the end of their useful life. The medical mask and cloth, towel, teddy bear, and humidifier did not release a significant portion of their silver in the tap water wash, so it is reasonable to assume that these products will still contain silver when they are disposed of in landfills. Moreover, if one assumes that a majority of the silver released into wastewater is collected in WWTP biosolids and eventually deposited in a landfill, the flux of silver entering landfills can be estimated as the total silver contained in the products. The total combined estimated silver in the consumer products investigated here is approximately 1450 mg (Table 1).

Since silver-containing products will be disposed of in landfills, TCLP experiments were performed on four products to simulate the release of silver within a landfill. Table 3 presents the results. The medical mask and cloth were chosen for TCLP experiments because of their high silver content, and the humidifiers were chosen because their low silver release rate suggested that the product might contain silver at the time of disposal. At 2900 μg L^{-1}, the medical cloth TCLP silver concentration was on the same order as the toxicity characterization limit of 5000 μg L^{-1} (USEPA, 1992). The medical mask and cloth released 1.7 and 54 μg Ag g $product^{-1}$, respectively. These values are similar to the mass of silver released into water (Table 2). Conversely, the humidifiers released silver faster into the TCLP solution than into water droplets during product use. The plastic and filter resin from the small and large humidifiers released 0.22 and 0.13 μg Ag g $product^{-1}$, respectively. Therefore, based on a 24-h evaluation, the humidifiers would release silver in a landfill at a rate about 10 times faster than into the air during use. This example illustrates the complexity of understanding and predicting fluxes of nanomaterials into the environment.

Implications

The research presented here quantifies the silver that is currently being used in consumer products and could be released into sectors of the environment. These data, when coupled with future nanosilver toxicity data, can be used to estimate the environmental and human health risks resulting from the use of nanosilver. This work is thus useful for consumers, product manufacturers, and policymakers, although some limitations are worth consideration. The wash experiments were designed to investigate silver leaching into tap water as opposed to simulating "real-world" use scenarios. Silver leaching in real-world scenarios will be affected by water quality parameters such as pH, detergents, and oxidants (Geranio et al., 2009) as well as consumer behaviors (washing habits, disposal, use of product, etc.). Lower pH values, higher temperatures, mechanical stress, and oxidants such as bleaching agents would cause a general trend toward increased silver release rates compared with the conditions of these experiments. Despite higher pH values (~10) in "real-world" washing conditions, silver release rates

Table 3. Silver released during toxicity characterization leaching procedure (TCLP).†

Sample‡	Sample mass	TCLP solution volume	TCLP silver content	Mass of silver leached
	g	mL	μg L^{-1}	μg Ag g product^{-1}
Medical mask	0.7	28	88	1.7
Medical cloth	0.9	17.5	2900	54
Small humidifier (plastic)	1.5–2.0	30–39	10 ± 0.9	0.2 ± 0.02
Large humidifier (resin)	1.9–2.4	39–48	7 ± 3.5	0.1 ± 0.07

† The ± values represent standard deviations of three repetitions.

‡ Medical mask and medical cloth were analyzed only once due to limited sample quantity.

may be higher than those observed here if oxidizing agents such as bleach are used (Geranio et al., 2009). The quantities of silver passing 100- and 20-nm-pore-size filters most likely consist of both ionic and nanoparticle silver. Some of the silver retained by the 100-nm-pore-size filter may be ionic and/or nanoparticles associated with material larger than 100 nm. Detection methods specific to nanosilver are needed to provide a more accurate characterization of silver. Finally, the quantities of silver released from these products may be considered as an initial leaching characterization due to the lack of replication in the leaching experiments. The validity of assumptions made for WWTP and landfill influxes could be assessed by replicating leaching experiments and quantifying silver release rates over product life cycles.

Because government does not specifically regulate the use of nanosilver in products, the onus of protecting human and environmental health from potential adverse effects currently falls on individuals. This research demonstrates that consumers will subject themselves and/or the environment to some exposure of silver (nanoparticle, ionic, or microscale) by using and/or disposing of silver-containing products. Although many factors contribute to perceptions of nanotechnology risks (Kahan et al., 2008), these data allow individuals to conceptually weigh the potential adverse effects of these quantities of silver against the perceived benefits from use of these products.

It has been demonstrated that silver particles can agglomerate and settle out of some products, such as the detergent. This suggests that some silver products may not perform as designed. Manufacturers may want to consider validating the function of the nanosilver in their products using some of the methods described here. For example, these characterization techniques can verify the properties of nanosilver (e.g., concentration, size) being varied in a product while the impact on antimicrobial efficacy is monitored.

The silver being used in products clearly will be released into the environment at some point of the product life cycle. Knowing this, society can begin to take an earth systems engineering and management approach to the design of nanosilver products (Allenby, 2000, 2007). That is, we should recognize that by engineering a product to be antimicrobial, we are also engineering the concentration of silver in various environments. This allows for the potential human and environmental effects to be factored into an improved design or a regulatory framework for nanosilver products.

Environmental occurrence and toxicity data related to engineered nanomaterials are needed before a regulatory direction can be established (Morris and Willis, 2007). This research provides an example of occurrence and environmental release data for nanomaterials that the USEPA's voluntary Nanomaterial Stewardship Program was designed to produce (USEPA, 2009a). The efficiency of that program has been questioned (Maynard and Rejeski, 2009), and one possible explanation for the lack of participation is the cost, in time and money, of producing such data. The methods presented here indicate that gathering basic data regarding the environmental transport of nanomaterials from consumer products can be achieved with relative ease at the laboratory scale. But it will be a challenge to scale up this approach to acquire occurrence and environmental release data for all consumer products containing nanomaterials.

The uncertainty regarding the potential negative impacts from the use of nanosilver in consumer products makes it unclear whether the government should regulate the growing economic market for these items. The research presented here is evidence for consumers, product manufacturers, scientists, and policymakers that nanomaterials will enter our environment as a result of their use in consumer products. Because these data only specify quantities of silver in products and possible releases into the environment, not toxicity, the nanosilver regulation debate remains open.

Acknowledgments

Funding was provided by the NSF through the Urban Ecology IGERT program at Arizona State University (Award Abstract no. 0504248), the USEPA (Grant no. RD833322), and the Fulton Undergraduate Research Initiative at Arizona State University. Additional funding was provided from the Paul L. Busch Award for Innovation by the Water Environment Research Foundation. The authors gratefully acknowledge the use of facilities within the LeRoy Eyring Center for Solid State Science and the Goldwater Environmental Laboratory at Arizona State University. This work was supported in part by the Department of Energy (DE-FG02-08ER64613) with Daniel Drell as contract monitor. The authors would like to thank Mr. McMillan at Puckskin for his generous donation of fabrics as well as Jameson Wetmore and Brad Allenby at Arizona State University for their input on the implications of the research. The opinions expressed here are those of the authors.

References

Allenby, B. 2000. Earth systems engineering and management. IEEE Technol. Soc. Mag. 19:10–24.

Allenby, B. 2007. Earth systems engineering and management: A manifesto. Environ. Sci. Technol. 41:7960–7965.

Auffan, M., J. Rose, J. Bottero, G. Lowry, J. Jolivet, and M. Wiesner. 2009. Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. Nature Nanotechnol. 4:634–641.

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

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

City of Tempe. 2010. Typical water quality values. Available at http://www.tempe.gov/waterquality/typical_values.htm (verified 15 June 2010). City of Tempe, Tempe, AZ.

Clesceri, L.S., A.E. Greenberg, and A.D. Eaton. 1998. Standard methods for the examination of water and wastewater. Am. Public Health Assoc., Am. Water Works Assoc., Water Environ. Fed., Washington, DC.

El-Ansary, A., and S. Al-Daihan. 2009. On the toxicity of therapeutically used nanoparticles: An overview. J. Toxicol. doi:10.1155/2009/754810.

Geranio, L., M. Heuberger, and B. Nowack. 2009. The behavior of silver nanotextiles during washing. Environ. Sci. Technol. 43:8113–8118.

Gottschalk, F., T. Sonderer, R. Scholz, and B. Nowack. 2009. Modeled environmental concentrations of engineered nanomaterials (TiO_2, ZnO, Ag, CNT, Fullerenes) for different regions. Environ. Sci. Technol. 43:9216–9222.

Greulich, C., S. Kittler, M. Epple, G. Muhr, and M. Koller. 2009. Studies on the biocompatibility and the interaction of silver nanoparticles with human mesenchymal stem cells (hMSCs). Langenbecks Arch. Chir. 394:495–502.

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

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

Gupta, A., K. Matsui, J.F. Lo, and S. Silver. 1999. Molecular basis for resistance to silver cations in Salmonella. Nat. Med. 5:183–188.

Hussain, S.M., K.L. Hess, J.M. Gearhart, K.T. Geiss, and J.J. Schlager. 2005. In vitro toxicity of nanoparticles in BRL 3A rat liver cells. Toxicol. In Vitro 19:975–983.

Ivanova, O., and F. Zamborini. 2009. Size-dependent electrochemical oxidation of silver nanoparticles. J. Am. Chem. Soc. 132:70–72.

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

Kahan, D.M., P. Slovic, D. Braman, J. Gastil, G. Cohen, and D. Kysar. 2008. Biased assimilation, polarization, and cultural credibility: An experimental study of nanotechnology risk perceptions. Woodrow Wilson Int. Cent. for Scholars, Washington, DC.

Ki, H.Y., J.H. Kim, S.C. Kwon, and S.H. Jeong. 2007. A study on multifunctional wool textiles treated with nano-sized silver. J. Mater. Sci. 42:8020–8024.

Kim, J.S., E. Kuk, K.N. Yu, J.H. Kim, S.J. Park, H.J. Lee, S.H. Kim, Y.K. Park, Y.H. Park, C.Y. Hwang, Y.K. Kim, Y.S. Lee, D.H. Jeong, and M.H. Cho. 2007. Antimicrobial effects of silver nanoparticles. Nanomed. Nanotechnol. Biol. Med. 3:95–101.

Maynard, A., and D. Rejeski. 2009. Too small to overlook. Nature 460:174.

Miesztari, S.M., R.R. Hammond, M.D. Sharpe, F.Y. Leung, and G.B. Young. 2004. Myoclonic status epilepticus following repeated oral ingestion of colloidal silver. Neurology 62:1408–1410.

Morris, J., and J. Willis. 2007. U.S. Environmental Protection Agency nanotechnology white paper. EPA 100/B-07/001. USEPA, Washington, DC.

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

Navarro, E., F. Piccapietra, B. Wagner, F. Marconi, R. Kaegi, N. Odzak, L. Sigg, and R. Behra. 2008. Toxicity of silver nanoparticles to *Chlamydomonas reinhardtii*. Environ. Sci. Technol. 42:8959–8964.

Percival, S.L., P.G. Bowler, and D. Russell. 2005. Bacterial resistance to silver in wound care. J. Hosp. Infect. 60:1–7.

Percival, S.L., E. Woods, M. Nuttelpor, P. Bowler, A. Radford, and C. Cochrane. 2008. Prevalence of silver resistance in bacteria isolated from diabetic foot ulcers and efficacy of silver-containing wound dressings. Ostomy Wound Manage. 54:30–40.

Roh, J.Y., S.J. Sim, J. Yi, K. Park, K.H. Chung, D.Y. Ryu, and J. Choi. 2009. Ecotoxicity of silver nanoparticles on the soil nematode *Caenorhabditis elegans* using functional ecotoxicogenomics. Environ. Sci. Technol. 43:3933–3940.

Silver, S. 2003. Bacterial silver resistance: Molecular biology and uses and misuses of silver compounds. FEMS Microbiol. Rev. 27:341–353.

Takenaka, S., E. Karg, C. Roth, H. Schulz, A. Ziesenis, U. Heinzmann, P. Schramel, and J. Heyder. 2001. Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. Environ. Health Perspect. 109:547–551.

USEPA. 1992. Test Method 1311: Toxicity characterization leaching procedure. *In* Test methods for evaluating solid waste: Physical/chemical methods. SW-846. USEPA, Washington, DC.

USEPA. 2009a. Nanoscale Materials Stewardship Program interim report. p. 38. Available at http://www.epa.gov/oppt/nano/nmsp-interim-report-final.pdf (verified 15 June 2010). USEPA Office of Pollution Prevention and Toxics, Washington, DC.

USEPA. 2009b. Targeted National Sewage Sludge Survey sampling and analysis technical report. EPA-822-R-08-016. USEPA Office of Water (4301T), Washington, DC.

Wang, J., C.P. Huang, and D. Pirestani. 2003. Interactions of silver with wastewater constituents. Water Res. 37:4444–4452.

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

Woodrow Wilson International Center for Scholars. 2009. Nanotechnology consumer product inventory. Available at http://www.nanotechproject.org/inventories/consumer/ (verified 15 June 2010). Project on Emerging Nanotechnologies, Washington, DC.