Nanoscale Materials
[no specified CAS]

Nomination
and
Review of Toxicological Literature

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Prepared by the Chemical Selection Working Group,
U.S. Food & Drug Administration

NANOSCALE MATERIALS

1.0 Basis for nomination

Nanoscale materials are being nominated for toxicological studies by the U.S. Food & Drug Administration based on *(a)* increasing widespread use in drug, food and cosmetic products, and *(b)* the general lack of data on the toxicology and pharmacokinetics of these materials.

2.0 Introduction

Nanoscale materials are typically defined as substances specifically synthesized with at least one dimension less than 100 nm (equals 0.1 micrometers) (National Nanotechnology Initiative , 2006; ASTM, 2006); however, there is not universal acceptance of the 100 nm cutoff for upper size limit. The interest in nanoscale materials is due to unique properties that exist in some particles in the 10-100 nm size range that do not exist in larger dimension particles of the same chemical and physical makeup. As a result, this nomination refers to nanoscale particles with dimensions less than micrometer scale that exhibit unique properties not recognized in micron or larger sized particles.

Nanotechnology and nanoscale materials have been touted as the "next industrial revolution", resulting in considerable investment of industrial and government financial resources for development and marketing.

Coordination of the U.S. Government investment in nanotechnology has been the focus of the National Nanotechnology Initiative (NNI; www.nano.gov) which reports to the White House Office of Science and Technology Policy (OSTP). The NNI, through the OSTP, informs the President and Congress regarding the investment in nanotechnology to *(a)* keep the U.S. at the forefront of this important and emerging industry, and *(b)* reduce duplication of efforts across the many U.S. Agencies.

While investment into the development of nanotechnology surpassed $1-billion (www.nano.gov) in 2006, funding for toxicological studies has not been as robust (Davies, 2006; Maynard, 2006). There was a recent U.S. Congressional Hearing on Nanotechnology, chaired by Congressman S. Boehlert (House Science Committee Chairman). He stated regarding the U.S. Government's role in protecting both the American public and businesses, specifically in efforts to implement and prioritize a research agenda and fund it adequately *"…we haven't done that, and 'time's a wasting.'"* (Boehlert, 2006). Congressman's Boehlert impression was that investment into the safety of nanotechnology is lagging behind new nanotechnology discoveries.

The NNI identified areas of health related research that are on-going in the US. Government, and pointed out areas where additional resources should be invested (National Nanotechnology Initiative, 2006). Identification of the potential toxicological hazards of nanoscale materials within the U.S. Government is being funded within and through the following agencies:

- National Toxicology Program funded toxicological studies
  - Inhalation toxicology of carbon nanotubes (conducted as IAG with NIOSH)
  - Fullerene toxicity (conducted through NTP contract mechanism)
  - Topical penetration and phototoxicity of TiO_2 and ZnO (conducted as IAG with NCTR/FDA)
  - Nanomaterials Characterization Laboratory (NCL) at National Cancer Institute (characterization and toxicity of cancer therapeutics; IAG with NTP regarding *in vitro* toxicity methods)
- National Science Foundation grant program
- National Institutes of Health extramural grant program
- Environmental Protection Agency grants program (STAR)

Nanoscale materials are being included in a variety of products in the U.S. market and are included in cosmetics, human and animal drugs, devices and biologics either based on their inherent properties (*e.g.* bactericidal nano-silver, UV reflective nano-TiO2) or as a platform for transport of other molecules (*e.g.* fullerenes and dendrimers). The NCL is conducting material characterization and toxicology assessment for the development of cancer therapeutic drugs, and will provide the eventual drug sponsor with data to understand the toxicological risk which could be included as part of a New Drug Application (NDA) to the FDA. In summary, there are few published studies detailing the safety of nanomaterials.

3.0 Production Processes and Import Volumes

Nanoscale materials are being produced at many levels within US manufacturing. This includes, for instance, nanofibers for strengthening polymers (*e.g.* tennis rackets and tires, nano-metallic fibers and crystals for electronics, and nanocrystalline materials for photonic processes (*e.g.* solar cells, self-cleaning windows, bioremediation). To date there are no reports on the volume of production of nanoscale gold or nanoscale silver, nor is there information available regarding import of nanoscale materials.

4.0 Uses

The use of nanoscale materials is widespread across much of industrial commerce. Nanoscale materials are being used in may consumer products (e.g. in tires as a enhancement/replacement for latex, in glass or ceramics as a self cleaning surface coatings, in solar cells as superior photocatalysts, in sporting equipment such as tennis rackets and baseball bats to increase strength, in soccer and tennis balls, to provide a more consistent bounce and extend their useful life, and in textiles to provide stain, water and wrinkle resistance). Manufactured nanoscale materials are also being used in cosmetics, sunscreens, combination products (drug/device), and in food packing material. The potential for manufactured nanoscale materials to be used in future regulated products is great.

Nanoscale gold is being marketed as nanorods and nanowires for commercial applications, especially in the electronics and catalysis industries. Nanoscale gold with derivative surfaces [*e.g.* proteins, (Burt et al., 2004; Shenoy et al., 2006)] is also being developed as a drug for targeted chemotherapy and targeted photodynamic therapy.

Nanoscale silver is being marketed in many products in the U.S. as an antibacterial and antiviral chemical. Silver has been used for many years as an antibacterial agent in wound and burn dressings; however, the enhanced antibacterial properties of nanoscale silver have resulted in its inclusion in catheters and wound dressings.

5.0 Environmental Occurrence and Persistence

Nanoscale materials arise from natural and anthropomorphic sources. Natural sources include volcanic emissions, fires, and ocean mist. Inadvertent man-made sources for nanoscale materials include the exhaust of internal combustion engines, milling and grinding, and wear debris from artificial joints.

The U.S. Environmental Protection Agency has highlighted a need for data on environmental fate of nanoscale materials in its STAR grant program (*http://es.epa.gov/ncer/rfa/2005/2005_star_nano.html*) and is funding research in this area.

However, there is a paucity of information regarding the environmental disposition (*i.e.* fate) of nanoscale materials. Since washing machines containing nanoscale silver release small amounts into the washed clothes, the U.S. EPA has recently requested manufacturers of these devices provide information regarding the environmental fate and impact of nanoscale silver (Weiss, 2006).

6.0 Human Exposure

Unintended exposure to nanoscale materials has occurred over the past several years through wear of materials implanted into the body (*e.g.* artificial joints) and through inhalation of internal combustion engine exhausts and fire smoke. Intended exposure is now occurring due to the inclusion of nanoscale materials in cosmetics, foods, sunscreens, some prescription drug products, and some commercial products. The most commonly used nanoscale materials in cosmetics and sunscreens are the oxides of titanium (TiO_2) and zinc (ZnO), which act as physical blocking materials for ultraviolet light. Data regarding the dermal penetration of these oxides are currently being generated by the National Toxicology Program.

Human exposure to nanoscale gold occurs through contact with either electronic or catalytic devices containing nanoscale wires or tubes, inhalation or dermal contact during the manufacture of these devices, or during chemotherapy or photodynamic therapy. In the latter two cases, the nanoscale binds to proteins or antibodies to direct targeting of specific cells or organs (e.g. Burt et al., 2004; Shenoy et al., 2006).

Human exposure to nanoscale silver may occur through contact with burn and wound dressings containing nanoscale silver as an antibiotic, or through contact with surfaces treated with nanoscale silver as an antibiotic. It is believed that the antibacterial properties of nanoscale silver will result in its inclusion in more products, resulting in increased human exposure (e.g. masks to reduce transmission of infectious agents, home water or food sanitizing kits, kitchen towels, counter-tops or cutting boards to reduce food-born infections).

7.0 Research and Testing Needs

The FDA is taking steps to address the safety and dermal penetration of nanoscale materials used in sunscreens and cosmetics, studies sponsored by the National Toxicology Program; however, there are currently few known toxicology or ADME (absorption, distribution, metabolism, elimination) studies being conducted on nanoscale material in general and no known such studies with colloidal gold (Au^0) or nanoscale silver (topical, gastrointestinal or inhaled). Moreover, there is a paucity of information in the literature regarding the effects of size, surface chemistry, surface physics, and route of administration on the toxicity and ADME of nanoscale materials in general and nanoscale gold and silver specifically, information vitally important to the agency. In addition, the agency is in need of assessing the ability of nanoscale material, based on size and surface coating, to bridge the blood–brain barrier and enter the CNS.

It is also important for the FDA to obtain biological and toxicological data on nanoscale materials in an effort to be assured that the current assays and tests that the agency requires sponsors to conduct in support of product safety are adequate to detect adverse biological and toxicological events.

Based on the above reasons, the FDA is submitting the following Scientific Issues Nomination to the National Toxicology Program: 1) examine the role of nanoscale size and nanoscale surface coating on the fate (ADME) of nanoscale material in a rodent animal model; 2) examine the ability of nanoscale material to pass through the blood-brain barrier and enter the CNS; 3) examine a select set of required (Human and Animal Drug ICH Guidelines) / CFSAN RedBook Guidelines) test to determine their ability to detect adverse biological or toxicological effects when challenged with nanoscale material; and 4) utilize nanoscale colloidal gold and nanoscale silver as test agent nanoscale materials.

8.0 Toxicology Data

8.1 Colloidal gold Nanoparticles

A distinction must be made between the various forms of gold. Metallic ground state gold (Au^0) is the soft malleable gold that has been coveted for millennia. Au^0 is also the predominant form of nanoscale gold that is synthesized in the 1-100 nm range. Gold also exists in two oxidation states. Au^I is the form that is usually associated with "soft" ligands such as sulfur or phosphorous donor ligands. Au^{III} is usually stabilized by "hard" ligands such as oxygen or nitrogen donors. In the presence of reducing agents (*e.g.* ascorbate) Au^I and Au^{III} can be

reduced to form nanoscale Au^0. There have been a considerable number of publications on the bio-distribution and toxicity of Au^I and Au^{III} compounds (reviewed in Merchant, 1998; Eisler, 2004); however, this nomination focuses on the nanoscale Au^0 state of gold.

The toxicity of colloidal nanoscale gold (Au^0) inside biological systems is of considerable concern. Gold is being considered for use in many biomedical applications, including biodiagnostic devices, drug/DNA delivery, and biosensing. Several published studies have shown cytotoxicity and immunotoxicology related to exposure to complexes of Au^I and Au^{III} (reviewed in Merchant, 1998, and Eisler, 2004); however, very few studies have evaluated the *in vivo* toxicology of colloidal gold (Au^0).

One characteristic that has made use of colloidal gold particles in histology and immunodiagnostics popular was the discovery that these particles could bind proteins without altering their activity. Recently, CytImmune proposed to use colloidal gold as a nanoparticle vector that targets delivery of tumor necrosis factor (TNF) to solid tumors growing in mice (*e.g.* see discussion of Paciotti *et al*, 2004, or Visaria et al., 2006). The particles were characterized for size and zeta potential using TEM/light scatter and zeta potential analyzer, respectively. They demonstrated that their PEG-ylated TNF-conjugate colloidal gold nanoplatform rapidly accumulates in tumors and shows little to no accumulation in the liver, spleen, or other organs of the animals. Gold-TNF conjugates without PEG showed extensive liver and splenic uptake and retention for greater than 1 month, without any behavioral signs of distress. Therefore it appears that changes in the surface of the particle (that is, deleting the PEG coating) can have a dramatic effect on the biodistribution.

Hillyer and Albrecht (2001) dosed Balb/c mice with water for 7 days containing 2 ppb of 4, 10, 28, or 58 nm colloidal gold (without any coating). The dosed water was removed and the mice sacrificed immediately or 12 hrs later. In general, the smaller the gold nanoparticle, the higher the levels that were obtained in the blood, brain, heart, kidney, spleen, and liver. The 58 nm colloidal gold particles did not cross the gut and accumulate in the internal organs. Transmission electron microscopy showed persorption of the 4 nm gold through single degrading enterocytes in the process of being extruded from the villus. This work suggests there is a cutoff between 28 nm and 58 nm for intestinal absorption of colloidal gold.

Balb/c mice bearing intramuscular 50-90 mm^3 EMT-6 mammary carcinomas were injected (*i.v.*) with 1.9 nm colloidal gold (no coating; 1.3 g/kg bw) particles for radiotherapy of the tumors (Hainfeld et al., 2004). The level of gold in the serum rose rapidly following injection with a slow clearance (values not given). Gold in the tumors peaked at 7 min, and fell to 50% peak value in 41 min, while the gold levels in the adjacent normal muscle tissue peaked at 5 min and decreased to 50% peak value in 24 min. The gold preferentially accumulated in 5 min into the tumor, tumor periphery, blood, and kidney. While this was not an

ADME study, it does suggest that very small nanoscale colloidal gold is rapidly excreted, but preferentially retained in vascular tissue, in agreement with the studies of Hillyer and Albrecht (2001).

In a study by Paciotti et al. (2004) synthesized colloidal gold (33 nm) and coated it with either tumor necrosis factor-α (TNF) or TNF and thiol-containing polyethylene glycol (PEG) . These were injected (i.v.) into MC-38 tumor-bearing C57/Bl6 mice and the nano-gold-TNF was cleared faster than TNF from the serum, while the nano-gold-TNF-PEG was cleared much slower than TNF from the serum and had preferential uptake in the tumor but not liver, brain, or lung. Similar results were obtained by Visaria et al. (2006) with nano-gold-TNF-PEG in A/J mice bearing SCK mammary carcinomas. These results demonstrate that with nanoscale gold as a delivery platform, surface changes in the nanoscale particles can have a dramatic effect on biodistribution.

In the only report on carcinogenicity of gold (Furst and Schlauder, 1978) a single intramuscular injection of 300 mesh (40-50 micrometer or smaller particles) gold did not result in induction of any cancer in lifetime study with Fischer-344 rats.

The basic questions of biocompatibility, distribution, and excretion of unmodified colloidal gold particles remain unclear. In response to the recent efforts to utilize colloidal gold in drug delivery devices and other biomedical applications, *in vivo* toxicity testing is required to better our understanding of this novel class of nanoparticles.

8.2 Nanoscale Silver

The antimicrobial properties of silver have been recognized for a long time, explaining its inclusion in traditional ocular treatment of newborns with silver nitrate and use in burn wound dressings (Russell *et al.* , 1994; Silver, 2003; Klasen, 2000; Fan and Bard, 2002; Balogh et al., 2001). Silver sulphadiazine and ionic silver (Ag^{+1}) have been used for many years as broad spectrum antibacterial, antifungal, antiviral agents for treatment of cuts, burns, and amputation sites (Russell *et al.*, 2004; Balogh et al., 2001). The absorption of silver following ingestion (either food or water) occurs primarily in the small intestine (Hill and Pillsbury, 1939). Harrison (1979), using ^{110}Ag-sulfadiazine, demonstrated poor absorption in wounded human skin, and the silver did not distribute in rats from damaged skin into internal organs. Others (Sano et al, 1982; Wang et al., 1985) showed that the silver dissociated from the sulfadiazine and was present in the body for longer periods (also confirmed in autopsy samples; Klasen, 2000). In the study of Wang et al. (1985) patients treated with silver sulfadiazine retained silver in the oral lips, gingival, and cheeks for up to a year following cessation of treatment. The blue discoloration of tissue is due to deposits of silver (*i.e.* argyria) and has been reported since 980 AD following treatment with silver preparations.

Nanoscale silver has been recently recognized as a more potent antimicrobial form of silver (Baker et al., 2005; Aymonier et al., 2002; Wright et al., 2002; Melaiye et al., 2005; Sondi et al., 2004; Alt et al., 2004). As an example, Wright *et al* (2002) demonstrated that wound dressing coated with sputtered nanoscale silver reduced infections in burns.

The antibacterial action of Ag^{+1} may have several mechanisms. Recent observations have suggested the primary mechanism of action is cell death due to the uncoupling of oxidative phosphorylation (Holt and Bard, 2005), which confirms work from other investigators; however, others have reported interaction with membrane-bound enzyme and protein thiol groups that may results in compromised cell wall integrity that would lead to deterioration of proton gradient-driven oxidative phosphorylation (Bragg and Rainnie, 1974; Liau et al., 1997; Silver, 2002; Zeiri et al., 2004).

Lok et al. (2006) have recently examined the effect of nanoscale silver on *E. coli* using proteomics and measurement of membrane properties. They have extended the previous observations that silver mechanism of action is disruption of proton motive force and decoupling of oxidative phosphorylation resulting in loss of intracellular ATP. They reported the effective concentration of nanoscale silver was considerably lower than that for Ag^{+1} ions.

Recently a case report was published regarding elevated liver enzymes following topical use of a nanoscale silver preparation on a young burn victim (Trop et al., 2006). 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 ug/kg, respectively) were still elevated at 7 weeks. In preclinical studies with pigs, no elevated plasma levels or adverse reactions were reported with the same nanoscale silver preparation (Burrell, 1997). While clinical studies contrasted the efficacy of the nanoscale silver versus other silver forms, there was no measurement of serum levels or reports of adverse reactions (Tredget et al., 1998; Yin et al., 1999; Innes et al., 2001).

The plasma levels of silver in the patient in Trop et al. (2006) were higher than the modest levels reported by Boosalis et al. (1987) and comparable to the rapid increase reported by Coombs et al (1992) following topical application of silver sulfadiazine; however, in the silver sulfadiazine studies there were no reports of hepatotoxicity, although others have reported allergic reactions (McKenna et al., 1995), erythema multiform (Lockhart et al., 1983), mental deterioration (Iwasaki et al., 1997), and transient leucopenia (Caffee and Bingham, 1982). It is not clear at this time if the report of hepatotoxicity by Trop et al. (2006) with nanoscale

silver is an isolated incidence (they ruled out any other causative factors), or the beginning of a trend with nanoscale versus other forms of silver.

In the only report on carcinogenicity of silver (Furst and Schlauder, 1978) a single intramuscular injection of 300 mesh (40-50 micrometer or smaller particles) silver did not result in induction of any cancer in lifetime study with Fischer-344 rats.

In the studies outlined below, we propose to examine the *in vivo* toxicity and the role of size on toxicity of nanoscale silver in rodents.

9.0 Recommended Studies

This nomination focuses on nanoscale gold (Au^0) and nanoscale silver because of increasing use and the gap in knowledge that cannot be filled by information submitted by manufacturers. There are possible toxicity concerns due to the lack of understanding of various aspects of the nanoscale materials and these concerns will not be addressed adequately unless the following studies are undertaken by the NTP. The FDA recommends:

Nanoscale gold – Conduct (1) absorption, distribution, metabolism and elimination studies in rodents using oral and intravenous routes of administration (including blood-brain transfer), (2) acute (single and repeat dose) toxicity studies (28 days) in rodents, and (3) subchronic, dose-response toxicity studies in rodents (only if warranted). The studies should be conducted on nanoscale gold of one or two sizes (*e.g.* 10 nm - 60 nm) with and without surface coatings (*e.g.* polyethylene glycol or protein coated). The nanoscale material should be thoroughly characterized before use, and after recovery from tissues.

Nanoscale silver - Conduct (1) absorption, distribution, metabolism and elimination studies in rodents using oral and intravenous routes of administration (including blood-brain transfer), (2) acute (single and repeat dose) toxicity studies (28 days) in rodents, and (3) subchronic, dose-response toxicity studies in rodents (only if warranted). The studies should be conducted on nanoscale silver of one or two sizes (*e.g.* 10 - 60 nm). The nanoscale material should be thoroughly characterized before use, and after recovery from tissues.

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