This document was developed by NIEHS/NTP staff to facilitate internal and external review of a proposed research program prior to designing and conducting toxicology studies. The purpose of the research concept document is to outline the general elements of a research program that would address the specific public health concerns that prompted the nomination of the substance or issue for study. It may also encompass substance-specific studies that address larger public health issues or topics in toxicology. Additional information about the nomination, review, and selection of substances for study by the NTP is provided at ***Nominations to the NTP Testing Program*** (*http://ntp.niehs.nih.gov/go/nom*). A draft version of this research concept was reviewed by the NTP Board of Scientific Counselors at a public meeting on June 22, 2007 (*http://ntp.niehs.nih.gov/go/9741*), subsequently revised, and approved by the NTP Executive Committee.

NTP Research Concept: Nanoscale Silver

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Nomination Background and Rationale

The U.S. Food and Drug Administration nominated nanoscale silver (n-Ag) for study by the National Toxicology Program (NTP) 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 (http://ntp.niehs.nih.gov/go/29287). In addition, the U.S. EPA recently requested manufacturers of n-Ag containing devices to provide information regarding environmental fate and environmental impact.

The potential for human exposure may be through manufacturing, use of home sanitizing kits, or use of consumer products containing n-Ag (clothing, textiles). In addition, intentional exposure may occur through ingestion of colloidal silver or use of n-Ag in wound dressings. Silver has been used as an antimicrobial for many years; e.g. silver nitrate used for treatment of eyes of newborns, and silver sulfadiazine used in wound dressings. This activity of silver is believed to be due to the interaction of ionic silver (Ag+) with bacterial and fungal proteins. n-Ag is being included in products because of this well-known antibacterial activity of Ag+.

Silver occurs in the metallic state (Ag^0) and can also be oxidized to the ionic form (Ag+), and may occur as silver salts in the Ag(I), Ag(II) or Ag(III) states. Since nanoscale Ag^0 has a higher surface area than non-nanoscale Ag^0, nanoscale silver is being used as an alternate source of Ag+ since it is able to produce a more sustained release of Ag+.

There are several forms of silver to which individuals may be exposed. These include silver salts, e.g. silver nitrate, silver chloride; silver sulfadiazine; colloidal silver protein complexes (formed by mixing silver nitrate, sodium hydroxide and gelatin); metallic silver; and colloidal silver, which may contain both nanoscale Ag^0 and/or dissolved ionic Ag+.

Most toxicological information is available for silver salts, and is based on studies of silver nitrate, silver chloride, and silver acetate. Little information is available for well-characterized n-Ag. In general, silver (as silver salts) exhibits low acute toxicity. The most sensitive human response to silver is the "cosmetic" condition argyria, an irreversible blue-grey discoloration of the skin and mucous membranes associated with application or ingestion of silver-containing compounds, that results from precipitation of elemental silver in the skin as result of ultraviolet

light (UV) mediated photoreduction of ionic silver. Current guidelines for permissible levels of Ag in water (0.1 mg/L) are based on argyria in humans.

Published case reports note that ingestion of colloidal silver preparations over long periods results in argyria in humans. "Colloidal" silver may contain primarily nanoscale particles of Ag^0, whereas in other cases it may be primarily Ag+, depending upon how it is made. For the case reports of agyria induced by "colloidal silver", it is not known if these colloidal preparations contained primarily Ag^0 (and if so what particle size) or contained significant quantities of ionized Ag+.

The relationship between particle sizes, ionization to Ag+ and comparative absorption, distribution, metabolism and elimination (ADME) of Ag^0 and Ag+ *in vivo* is not known. While silver nitrate and other silver salts may be useful surrogates for the potential toxicity of Ag+ formed from ionization of Ag^0, there is insufficient data to evaluate whether the potential toxicity of n-Ag is due solely to the production of Ag+ or if there are effects of the nanoscale particles of Ag^0 itself. With regard to long-term exposure to well-characterized n-Ag, there are no adequate data on carcinogenicity, immunotoxicity, neurotoxicity, reproductive toxicity, developmental toxicity, or the potential role of particle size on the development of any adverse response.

Key Issues

For nanoscale materials the dose metric related to observed effects is a key issue. Particle number-based and surface area-based metrics increase with decreasing particle size and as such mass-based potency of nanoscale materials may differ from that of materials of larger size, but surface area-based potency may not. Some studies have shown that surface area-based metrics may be more appropriate for the comparison of potency of pulmonary toxicity of some metal oxides. While this may not be applicable to all nanoscale materials or all routes of exposure, it indicates that other dose metrics that scale with physicochemical properties, rather than the mass of nanoscale material, should be considered in the interpretation of dose-response data. Consequently, experimental approaches may require the comparative analysis of multiple forms of a given nanoscale material of similar composition but varying in particle size, coatings, shape, or other physicochemical parameters.

Proposed Approach

Hypothesis to evaluate are:

- The toxicity profile of nanoscale silver is the same as that of Ag+; i.e. that the biological responses are due to Ag+ formed from the ionization of Ag^0 to Ag+.
- The pharmacokinetics of nanoscale silver are the same as for silver salts such as silver nitrate, a highly ionized silver salt.
- Differences in potency of different sized particles of nanoscale silver are due to the relative differences in ionization to Ag+. This is due to the fact that smaller particles have higher surface area per unit mass and a corresponding higher proportion of Ag+ per unit mass.

Specific Aims

1. Characterize the relationship between nanoscale silver particle size and degree of ionization to Ag+.

We propose to determine the proportion of Ag^0 that is ionized to Ag+ *in vitro* and in biological media using Ag^0 of at least 3 sizes, spanning from <10 nm to > 100 nm. These data will inform the selection of appropriate particle sizes and the design of subsequent *in vivo* studies such that comparisons can be made on equivalent doses of Ag+.

2. Evaluate the effect of particle size and ionization state on the pharmacokinetic profile of nanoscale silver.

We propose to compare at least two sizes of Ag^0 (including <10 nm to > 100 nm) and one Ag+ species (e.g. silver nitrate as a highly ionized silver salt) and conduct time course and tissue disposition studies in rodents (rats and mice). We propose to evaluate tissue disposition after multiple routes (oral, dermal and intravenous) of administration. These studies will include quantitation of both Ag^0 and Ag+ in tissues using established methods for analyses and, if feasible, location within tissues of Ag^0 and Ag+. In addition, given the role of photoreduction of silver in the development of argyria, consideration will be given to the potential inclusion of specific UV-exposed groups to assess the impact of UV on the relative tissue distribution of Ag^0 and Ag+ in the skin, after subchronic exposure.

3. Evaluate the effect of particle size and ionization state on the toxicological profile of nanoscale silver *in vivo*.

We propose to compare two particle sizes of Ag^0, and one Ag+ species, using pharmacokinetic data to inform a study design that allows comparisons to be based on both equivalent mass dose and expected internal Ag+ dose. We propose to evaluate and compare the toxicological profile after subacute, subchronic and chronic exposure in rodents. Studies should include an evaluation of potential systemic toxicity and organ specific toxicity and the potential for toxicity to the immune and nervous systems. This is justified by known effects and distribution of silver salts. Consideration of inclusion of an *in utero*/perinatal exposure paradigm is justified based on the anticipated use pattern in consumer products and that exposure could occur during pregnancy.

Significance and Expected Outcome

While the extent of human exposure to nanoscale silver has not been quantified, the increasing use of nanoscale silver in consumer products increases the probability that a larger number of individuals will be exposed. Additionally, there are known exposures where individuals intentionally ingest nanoscale silver dietary supplements or receive treatment with medical devices containing nanoscale silver.

In addition, this project integrates with other studies being conducted as part of the NTP Nanotechnology Safety Initiative (http://ntp.niehs.nih.gov/go/nanotech). The intent of this initiative is to understand the potential adverse effects of nanoscale materials before widespread exposure has occurred, to identify key physicochemical properties that govern their safety and to

examine how they enter, travel through, and deposit in the body. The comparison of data on nanoscale silver to data of other nanoscale materials being evaluated will provide insight into these issues and thus provides further justification for this research program.

Studies of nanoscale silver were identified as research needs by the FDA, EPA and the CPSC and information from these studies will serve to increase the scientific base on which regulatory agencies make their interpretations of the potential adverse biological and toxicological events associated with exposure to nanoscale silver or products containing nanoscale silver.

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