NTP
National Toxicology Program

Research Concept: Nanoscale silver

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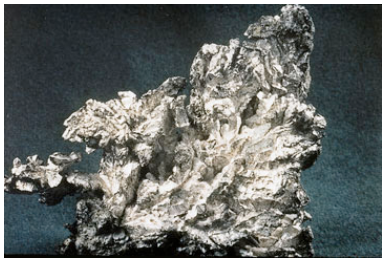
NIEHS/NTP

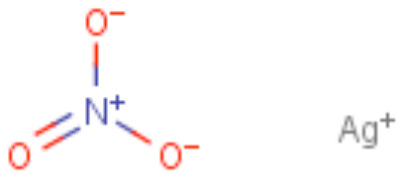
Nomination: Nanoscale materials

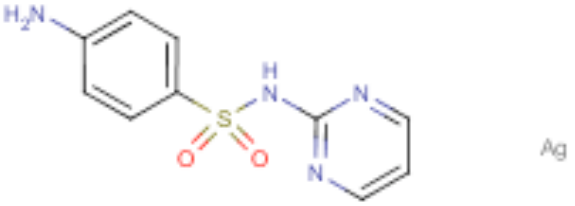
- Colloidal gold and **nanoscale silver**
- Nominated by the US Food and Drug Administration based on
  - Increasing widespread use in drug, food and cosmetic products
  - General lack of data on the toxicology and pharmacokinetics of these materials

Background: Forms of Silver



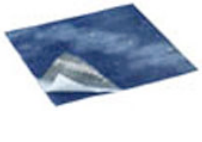
- Transition metal
- Metallic- Ag^0
- Ag(I), Ag (II), Ag(III)
  - Silver nitrate (highly ionized)
  - Silver drugs-Silver sulfadiazine
- Colloidal Silver
  - Colloid of nanoscale Ag^0 particles and ionic Ag^+
    - 10nm Ag^0 particle=30978 Ag atoms
  - Silver protein complexes





Properties and uses of nanoscale silver

- Add antibacterial/antifungal properties
  - Release of Ag^+ in aqueous environments
  - Interaction of ionic Ag^+ with bacterial/fungal proteins
- FDA-approved wound dressings
- Dietary supplements
  - Commercial suppliers of colloidal silver
  - Homemade electro-colloidal preparations
- Consumer products
  - Woodrow Wilson Center database
  - Nanoscale Ag (n-Ag)
    - 93 of 475 consumer products












http://www.nanotechproject.org

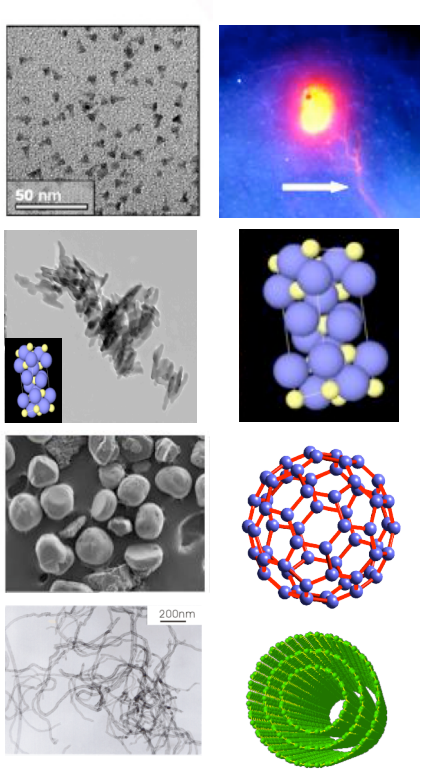
Rationale for NTP studying nanoscale silver

- Significance and Public Health Impact
  - Widespread uncertainty in how to assess health risk of nanomaterials
  - Identified research need by FDA, EPA, CPSC
    - May be used in their interpretation of the potential adverse biological and toxicological effects associated with exposure to nanoscale silver or products containing nanoscale silver
  - Anticipated that potential for exposure will increase as use increases
    - Actual exposure to nanoscale silver has not been quantified
- Project integrates with the NTP Nanotechnology Safety Initiative
  - Need to understand the effects of nanoscale materials in general before widespread exposure and/or effects have occurred
  - Identify key physicochemical properties that govern nanomaterial safety
  - Examine how nanomaterials enter, travel through, and deposit in the body

NTP Nanotechnology Safety Initiative

- Ongoing program of multiple classes of nanomaterials

- Studies ongoing/in development
  - Core shell structure
    - Quantum dots
  - Carbon fullerenes
  - Carbon nanotubes
  - Metal Oxides
    - Titanium dioxide
    - Ceric oxide
  - Dendrimers
  - Nanoscale metals
    - Nanoscale silver
    - Colloidal gold



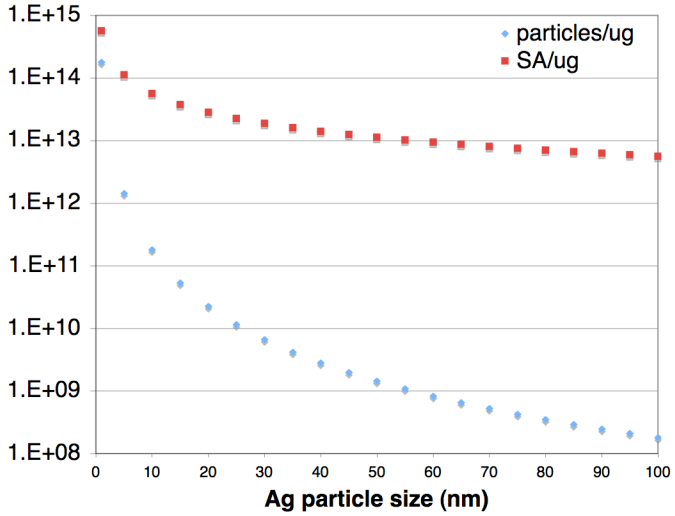
ntp.niehs.nih.gov/go/nanotech

Information on health effects for silver

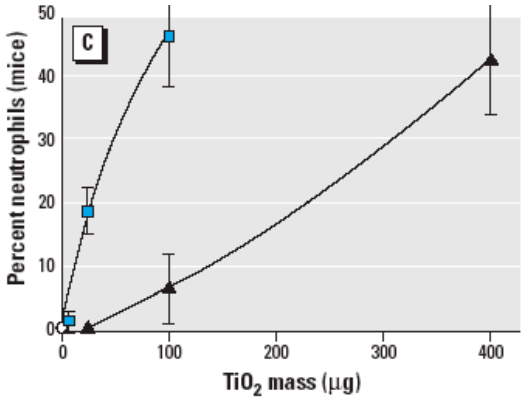
- Silver salts
  - Argyria
    - Deposition in multiple tissues; conjuctiva,liver spleen, skin, kidney, brain
    - Deposition of silver (requires accumulation of gram levels of Ag)
  - Hypoactivity in mice after oral exposure
  - Limited number of standard rodent acute/subchronic toxicology studies
  - Biochemical effects
    - metallothionein induction
    - lipid peroxidation
    - inhibition of glutathione peroxidase and gamma-aminolevulinic acid dehydratase

- Nanoscale silver
  - No publicly available *in vivo* toxicity studies of nanoscale silver of known particle size and/or knowledge of ionization status (Ag^0:Ag^+)
  - Human-case reports- high levels of exposure to colloidal silver
    - Argyria
    - Myoclonic status epilepticus
  - Transient elevated liver enzymes after use of wounds dressings containing nanoscale silver in burn victim
  - Anti-inflammatory effects in animals models of allergic contact dermatitis
  - *In vitro* cytotoxicity of nanoscale silver particles in a variety of cells lines

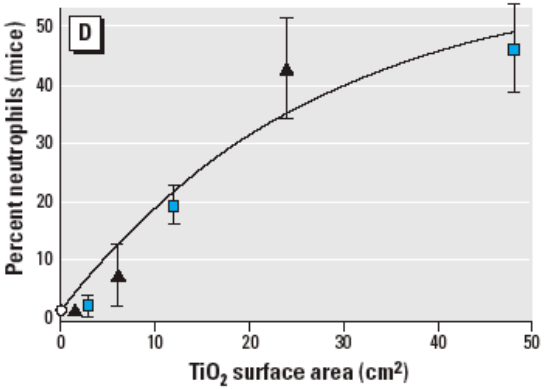
Key considerations for nanoscale materials: Dose metrics



Mass-based



Surface area-based

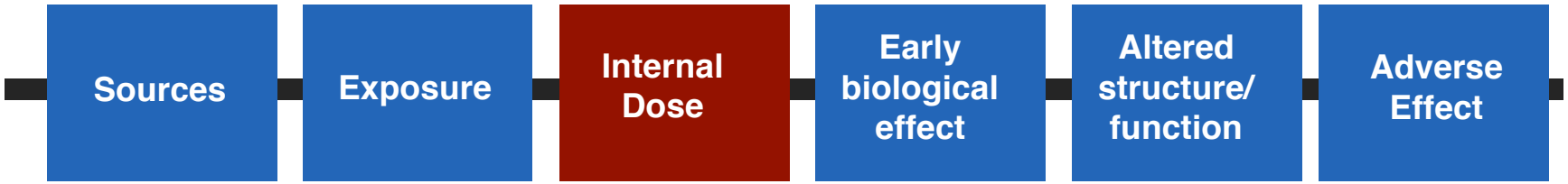


- Particle number-based and surface area-based metrics increase with decreasing particle size
- Mass-based potency may differ, but surface area-based potency may not
- Requires studying particles of similar composition but varying particle size, coatings, shape or other physicochemical parameter

Hypotheses to evaluate:

- The toxicity profile of nanoscale silver is the same as that of Ag^+
  - Biological responses are due to the ionization of Ag^0 to Ag^+
  - Compare to silver nitrate as a surrogate for Ag^+, since it is a highly ionized silver salt
- The pharmacokinetics of nanoscale silver are the same as for silver salts such as silver nitrate
- Differences in potency of different sized particles of nanoscale silver are due to the relative differences in ionization to Ag^+
  - Smaller particles have higher surface area/g and hence a higher proportion of Ag^+/g

$Ag^0 \rightarrow Ag^+ \rightarrow$ Biological responses



Aim 1

- Characterize relationship between nanoscale silver particle size and degree of ionization to Ag^+
  - Determine proportion of Ag^0 that is ionized to Ag^+
    - Use Ag^0 of at least 3 sizes spanning from <10 nm to > 100 nm
    - Evaluate Ag^0 and Ag^+ proportion in vitro and in biological media
  - Use data to inform selection of appropriate particle sizes and to design subsequent in vivo studies such that comparisons can be made on equivalent doses of Ag^+

Aim 2

- Evaluate the effect of particle size and ionization state on the pharmacokinetic profile of nanoscale silver
  - 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)
    - Conduct time course and tissue disposition studies in rodents (rats and mice)
    - Evaluate multiple routes (oral, dermal and intravenous) of administration
  - Include quantitation of both Ag^0 and Ag^+ in tissues using established methods for analyses.
    - If feasible, determine location within tissues of Ag^0 and Ag^+

Aim 3

- Evaluate the effect of particle size and ionization state on the toxicological profile of nanoscale silver in vivo
  - Compare two particle sizes of Ag^0, and one Ag^+ species
    - Use pharmacokinetic data to inform study design that allows comparisons to be based on both equivalent mass dose and expected internal Ag+ dose
  - Evaluate and compare 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
    - Justified by known effects and distribution of silver salts
    - Include in utero/perinatal exposure paradigm
      - Justified by anticipated use pattern in consumer products
      - Exposure could occur during pregnancy