

**Scientific, Technical, Research,
Engineering and Modeling Support
Final Report**

**State of the Science
Literature Review:
Everything Nanosilver
and More**

Scientific, Technical, Research, Engineering and Modeling Support Final Report

**Contract No. EP-C-05-057
Task Order No. 95**

State of the Science Literature Review: Everything Nanosilver and More

Prepared for

Katrina Varner, Task Order Manager
U.S. Environmental Protection Agency
National Exposure Research Laboratory
Environmental Sciences Division
Las Vegas, NV

Submitted by

Jessica Sanford, Task Order Leader, Battelle
Raghuraman Venkatapathy, Project Manager, Pegasus

Prepared by

Amro El-Badawy
David Feldhake
Raghuraman Venkatapathy

Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy. Mention of trade names and commercial products does not constitute endorsement or recommendation for use.

TABLE OF CONTENTS

List of Figures.....	iii
List of Tables	v
List of Abbreviations	vi
Executive Summary	xii
1. Introduction.....	1
2. Historical and Current Applications of Silver and Silver Nanomaterials.....	5
2.1 Elemental Silver Characteristics and Sources.....	5
2.2 Chemistry of elemental silver	5
2.3 Historical and Current Applications of Elemental Silver and Silver compounds	7
2.4 Nanosilver: History and Applications.....	10
2.5 Silver Regulations in the US.....	11
3. Uses of Silver Nanomaterials	14
3.1 Properties of nanosilver	14
3.1.1 Antibacterial properties.....	14
3.1.1.1 Antibacterial mode of action.....	15
3.1.2 Antifungal properties	16
3.1.3 Antiviral properties	17
3.1.4 Anti-inflammatory properties	17
3.1.5 Anti-glycoprotein film properties	17
3.1.6 Anti-biofilm properties	18
3.1.7 Surface plasmon resonance properties.....	18
3.1.8 Plasmonic heating properties	18
3.1.9 Metal-enhanced fluorescence properties.....	19
3.1.10 Properties of silver nanomaterials that promote its biosynthesis.....	19
3.2 Scientific Applications.....	20
3.3 Industrial Applications.....	21
3.3.1 Catalysis.....	21
3.3.2 Electronics.....	22
3.3.3 Other Industrial Applications.....	22
3.4 Applications in Consumer Products.....	22
3.5 Medical Applications	23
3.6 Proposed and Projected Applications	24
4. Synthesis and Properties of Silver Nanomaterials.....	27
4.1 Methods of Synthesis.....	27
4.1.1 Synthesis Categories	27
4.1.1.1 Top-Down versus Bottom-Up.....	28
4.1.1.2 Synthesis Reactants in Bottom-Up Techniques.....	30
4.1.2 General Discussion on Nanosilver Synthesis	32
4.2 Silver Nanocomposites and Bimetallic Nanoparticles.....	35

4.3 Environmental Perspective	35
4.4 Characteristics of the Silver Nanomaterials Products.....	37
4.5 Characterization Methods, Detection and Speciation.....	54
4.5.1 Methods for Measuring Ionic Silver in Nanosilver Suspensions.....	56
4.5.2 Methods for Isolating Ionic Silver from Nanosilver Suspensions.....	56
4.5.3 Novel Detection and Characterization Techniques for Environmental Samples.....	57
5. Potential Magnitude of Silver Nanomaterial Utilization and Environmental Exposure .	67
5.1 Inventory of Silver Nanomaterials: Industrial and consumer products	68
5.2 Routes of Release and Exposure, Ecological.....	72
5.3 Routes of Exposure, Human	77
5.3.1 Exposure via food	80
5.3.2 Exposure via consumer products	83
5.3.3 Exposure via Medical Applications.....	88
5.3.4 Exposure via occupation.....	92
5.4 Projected Quantities, Geographic and Demographic Distribution in the US	94
6. Toxicity and Health Effects.....	98
6.1 Silver Toxicity	98
6.2 Nanosilver Toxicity	100
6.2.1 Toxicity of Nanosilver to Organisms.....	102
6.2.2 Ecological or Multispecies Studies of Nanosilver Toxicity	105
6.2.3 Studies Concerning Human Health Including Mammalian Models	109
6.2.3.1 Respiratory Tract Toxicity	113
6.2.3.2 Neuronal Uptake.....	118
6.2.3.3 Dermal Toxicity.....	118
6.2.3.4 Gastrointestinal Tract Toxicity	120
6.2.3.5 Other Organ Toxicity.....	121
6.2.3.5.1 Kidney Toxicity	122
6.2.3.5.2 Liver Toxicity	123
6.2.3.5.3 Immune system Toxicity.....	123
6.2.3.5.4 Other blood effects.....	124
6.2.3.5.5 Reproductive system Toxicity	125
6.2.3.5.6 Genotoxicity, carcinogenicity	125
6.2.4 Cell Culture Nanosilver Toxicity.....	125
6.3 Conclusions on Nanosilver Toxicity.....	131
7. Life Cycle Analysis for Comprehensive Environmental Assessment	134
7.1 Nanosilver life cycle assessment	136
7.2 Nanosilver comprehensive environmental assessment.....	145
8. Data Gaps	147
9. Bibliography	150
Appendix A – List of US Companies Producing Nanosilver Containing Compounds.....	198
Appendix B – Woodrow Wilson Database Containing Nanosilver Products.....	207
Appendix C – List of Companies Producing Raw Nanosilver in the US.....	355

List of Figures

Figure 2.1: Nanomaterials dimensions on the metric scale (in nm).....	11
Figure 2.2: Analysis of FIFRA registered products containing nanosilver for the period 1950-2010.....	12
Figure 4.1: Top-down and Bottom-up synthesis approaches.....	30
Figure 4.2: Schematic for an organometallic approach of synthesis of silver nanoparticles.....	34
Figure 4.3: Schematic of a spinning disc processor for synthesis of silver nanoparticles.....	36
Figure 4.4: Characterization of silver nanoparticle size and morphology with scanning electron microscopy (SEM).....	38
Figure 4.5: Schematic diagram of the Electrospray-Scanning Mobility Particle Sizer (ES-SMPS) system.....	58
Figure 4.6: Cross section of a small part of the FIFFF channel.....	59
Figure 5.1: Forms of nanosilver incorporated in consumer products.....	69
Figure 5.2: Categories of nanosilver-containing products.....	70
Figure 5.3: Overview of silver flows triggered by biocidal plastics and textiles.....	75
Figure 5.4: Process simulated in the model developed by Blaser et al. (2008).....	76
Figure 5.5: Routes of exposure, uptake, distribution, and degradation of nanomaterials in the environment.....	78
Figure 5.6: Fresh Box, manufactured by FinePolymer, Inc. (South Korea), is a nanosilver antimicrobial food container.....	81
Figure 5.7: The Nano Tea Pot – Aroma manufactured by Top Nano Technology Co., Ltd (Taiwan).....	81
Figure 5.8: Samsung’s Silver Wash washing machine.....	85
Figure 5.9: Illustration showing production flow process and measurement locations in the Korean silver nanoparticle manufacturing facility.....	88
Figure 5.10: Anti-microbial burn dressing manufactured by Anson Nano-Biotechnology (Zhuhai) Co., Ltd., China.....	91
Figure 5.11: Conjunctival-corneal argyrosis in the craftsman occupationally exposed to silver ..	93
Figure 5.12: Number of companies, universities, laboratories and/or organization working in nanotechnology across the US.....	95
Figure 5.13: Nanosilver flows during high emission scenarios.....	96
Figure 6.1: Systemic argyria of the skin from ingestion of colloidal silver.....	99
Figure 6.2: HAADF image of an HIV-1 virus.....	104
Figure 6.3: A schematic of the human body with pathways of exposure to nanoparticles.....	110
Figure 6.4: Deposition of particles in the respiratory tract as a function of their size.....	114
Figure 6.5: A simplified depiction of potential factors that may influence the effects of engineered nanoparticles on the respiratory system.....	115
Figure 6.6: The proposed mechanism of nanosilver toxicity based on the experimental data ..	131
Figure 7.1: Comprehensive environmental assessment (CEA).....	136
Figure 7.2: Choices associated with a nanotechnology throughout its life cycle as proposed by Meyer et al. (2009).....	137
Figure 7.3: Choices associated with a nanotechnology throughout its life cycle as proposed by Gill (2007).....	138
Figure 7.4: The Nanomaterial Database maintained by Nanowerk.....	142

Figure 7.5: Quantified mass flows of silver triggered by the use of biocidal products and by other silver uses..... 144

Figure 7.6: Nanosilver flows from the nanomaterial containing products to various environmental compartments..... 145

List of Tables

Table 3.1: Emerging applications of nanosilver in medical products.....	26
Table 4.1: Description of Evidence for Silver Nanoparticle Synthesis for General Applications	39
Table 4.2: Description of Evidence for Silver Nanoparticles Synthesis for Specific Applications	45
Table 4.3: Description of Evidence for Silver Nanocomposites.....	49
Table 4.4: Description of Evidence for Bimetallic Silver Nanoparticles.....	51
Table 4.5: List of Acronyms used in Tables 4.1 to 4.4	52
Table 4.6: Possible Conventional Characterization and Detection Techniques for Nanosilver ..	62
Table 5.1: Main characteristics for human exposure to nanomaterials from food, consumer and medical products	78
Table 5.2: Ranking of potential human exposures to nanosilver	79
Table 5.3: Summary of applications of nanotechnology in the food production chain	82
Table 5.4: Product categories with examples of products containing nanosilver	86
Table 5.5: Medical devices containing nanosilver	90
Table 5.6: Emerging applications of nanosilver in medical products	91
Table 5.7: Predicted environmental concentrations in Rhine River.....	96
Table 7.1: Companies selling nanosilver as listed in the Nanowerk database.....	139

List of Abbreviations

-CN	Cyano group
-COOH	Carboxyl Group
-NH ₂	Amine Group
-SH	Thiol Group
AAS	Atomic Absorption Spectroscopy
ACGIH	American Conference of Governmental Industrial Hygienists
AFM	Atomic Force Microscopy
¹⁰⁷ Ag, ¹⁰⁹ Ag	Silver Isotopes
Ag	Elemental Silver
Ag ⁺ , Ag ²⁺ , Ag ³⁺	Ionic Silver
Ag ₂ O	Silver Oxide
AgBr	Silver Bromide
AgCl	Silver Chloride
AgF	Silver Fluoride
AgI	Silver Iodide
AgNO ₃	Silver Nitrate
AgCN	Silver Cyanide Complex
AgN ₃	Silver Azide
AgNP	Silver nanoparticle
AgOH	Silver Hydroxide
AgONC	Silver Fulminate
AgS	Silver Sulfide
ALP	Alkaline Phosphatase
ASTM	American Society for Testing and Materials
ATP	Adenosine Triphosphate
ATSDR	Agency for Toxic Substances and Disease Registry
Au	Gold
BBB	Blood Brain Barrier
B.C.	Before Christ
BET	Brunauer-Emmett-Teller Analysis

BPEI	Branched Polyethyleneimine
BSA	Bovine Serum Albumin
C ₂ H ₅ OH	Ethyl Alcohol
Ca	Calcium
CA	California
Cal/EPA	California Environmental Protection Agency
CE	Capillary Electrophoresis
CEA	Comprehensive Environmental Assessment
Cl ⁻	Chloride Ion
cm	Centimeter
CNT	Carbon Nanotube
CO	Carbon Monoxide
CTAB	Cetyltrimethylammonium Bromide
Cu	Copper
Cu(NO ₃) ₂	Cupric Nitrate
DI	Deionized
DLS	Dynamic Light Scattering
DMA	Differential Mobility Analyzer
DMF	N,N-Dimethyl Formamide
DNA	Deoxyribonucleic Acid
DOC	Dissolved Organic Carbon
DPR	Department of Pesticide Registration
DTSC	California Department of Toxic Substances Control
<i>E. coli</i>	<i>Escherichia coli</i>
EDX	Energy Dispersive X-ray Spectroscopy
EFSA	European Food Safety Authority
EMA	European Medicines Evaluation Agency
ES-SMPS	Electrospray Scanning Mobility Particle Sizer
ESEM	Environmental Scanning Electron Microscopy
Fe ₃ O ₄	Magnetite
FFF	Field Flow Fractionation

FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FIFFF	Flow Field Flow Fractionation
FTIR	Fourier Transformed Infrared Spectroscopy
GE	Gel Electrophoresis
GFAA	Graphite Furnace Atomic Absorption Spectroscopy
GI	Gastrointestinal
GLP	Good Laboratory Practice
H ₂	Hydrogen Gas
HAADF	High Angle Annular Dark Field
HDA	Hexadecylamine
HEK	Human Epidermal Keratinocytes
HIV	Human Immunodeficiency Virus
HNO ₃	Nitric Acid
HR	High Resolution
IC	Integrated Circuits
ICP	Inductively Coupled Plasma
IL	Interleukin
IR	Infrared
ISE	Ion Selective Electrodes
kg	Kilogram
L	Liter
LCA	Life Cycle Analysis
LCST	Lower Critical Solution Temperature
LDH	Lactate Dehydrogenase
M	Molar concentration
m ³	Cubic Meter
MA	Massachusetts
mg	Milligrams
g	Micrograms
M	Micromolar concentration
MS	Mass Spectrometer

MTR	Mass Transit Railway
N ₂ O	Nitrous Oxide
Na	Sodium
NaBH ₄	Sodium Borohydride
NaCl	Sodium Chloride
NaN ₃	Sodium Azide
NASA	National Aeronautics and Space Administration
NC	North Carolina
nm	Nanometer
NMR	Nuclear Magnetic Resonance
NO ₃ ⁻	Nitrate Ion
NOEC	No Observable Effect Concentration
NOM	Natural Organic Matter
OECD	Organisation for Economic Cooperation and Development
OES	Optical Emission Spectroscopy
OH ⁻	Hydroxide Ion
OPP	USEPA's Office of Pesticide Program
ORD	USEPA's Office of Research and Development
OW	USEPA's Office of Water
Pd	Palladium
PEC	Predicted Environmental Concentration
PEN	Project of Emerging Nanotechnologies
PNEC	Predicted No-Effect Concentration
PNIPAM	Poly(N-isopropylacrylamide)
POTW	Publicly Owned Treatment Works
ppb	Parts Per Billion
PVA	Polyvinyl Alcohol
PVP	Polyvinylpyrrolidone
QA/QC	Quality Assurance/Quality Control
QSAR	Quantitative Structure-Activity Relationship
RNA	Ribonucleic Acid

ROS	Reactive Oxygen Species
rpm	Revolutions Per Minute
Ru	Ruthenium
SDS	Sodium Dodecyl Sulfate
SEC	Size Exclusion Chromatography
SEM	Scanning Electron Microscope
SERS	Surface Enhanced Raman Spectroscopy
SiO ₂	Silicon Dioxide
SMPS	Scanning Mobility Particle Sizer
Sn	Tin
SNCI	Silver Nanotechnology Commercial Inventory
SNOMS	Single Nanoparticle Optical Microscopy and Spectroscopy
SNWG	Silver Nanotechnology Working Group
SPR	Surface Plasmon Resonance
SRHA	Suwannee River Humic Acids
STP	Sewage Treatment Plant
TEM	Transmission Electron Microscopy
TGA	Thermal Gravimetric Analysis
TiO ₂	Titanium Dioxide
TNF	Tumor Necrosis Factor
TSCA	Toxic Substances Control Act
TWT	Thermal Waste Treatment
UCPC	Ultrafine Condensation Particle Counter
US/USA	United States of America
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
USGS	United States Geological Society
UV	Ultraviolet light
UV-Vis	Ultraviolet-Visible light
WIP	Waste Incineration Plant
X-EDS	X-ray Energy Dispersive Spectrometry

XANES	X-ray Absorption Near Edge Structure
XPS	X-ray Photoelectron Spectroscopy
XRD	X-ray Diffraction
ZnO	Zinc Oxide

Executive Summary

Silver has been known to be a potent antibacterial, antifungal and antiviral agent, but in recent years, the use of silver as a biocide in solution, suspension, and especially in nano-particulate form has experienced a dramatic revival. Due to the properties of silver at the nano level, nanosilver is currently used in an increasing number of consumer and medical products. The remarkably strong antimicrobial activity is a major reason for the recent increase in the development of products that contain nanosilver.

Of the more than 1000 consumer products that claim to contain nanomaterials, more than a quarter of them contain nanosilver. Examples of consumer products that contain nanosilver include food packaging materials, food supplements, textiles, electronics, household appliances, cosmetics, medical devices, water disinfectants, and room sprays. While most of these nanosilver-containing products were in the past manufactured in North America, manufacture of nanosilver-containing products is shifting to the Far East, especially China, South Korea, Taiwan and Vietnam. Currently, tracking products that contain nanosilver is getting to be difficult because the products are almost always packaged under numerous brand names, and current labeling regulations do not require that the nanomaterial be listed as an ingredient.

Knowledge of silver nanomaterials synthesis methods is important from an environmental perspective. This information allows for the identification of characteristics and morphologies of the produced silver nanomaterials that are crucial for a more focused approach when evaluating their environmental fate, transport and toxicity. The main challenge in nanomaterials synthesis is the control of their physical properties such as obtaining uniform particle size distribution, identical shape, morphology, chemical composition and crystal structure. There are an extensive number of synthesis methods of silver nanoparticles that are readily available in the literature. All reported methods can be classified and categorized since they all follow common approaches and the differences are limited to the specific reactants used and the reaction conditions. Categories such as top-down versus bottom-up, green versus non-green and conventional versus non-conventional have been reported. Physical methods such as milling or attrition, repeated quenching and photolithography are usually involved in the top-down strategies while bottom-up

techniques start with silver salt precursor that is reduced in a chemical reaction. Synthesis methods can also be grouped under conventional and unconventional methods. Conventional synthesis methods include the use of citrate, borohydride, two phase (water-organic) systems, organic reducers, and inverse micelles in the synthesis process. Unconventional methods include laser ablation, radiocatalysis, vacuum evaporation of metal, and the Svedberg method of electrocondensation.

Increased manufacture and use of nanosilver in products will lead to an inevitable increase in the release of these particles into the environment at each and every step of its life starting from the cradle (raw materials) to its grave (disposal/reuse). The availability of methodologies for the detection and characterization of silver nanoparticles are thus essential in order to investigate their fate, transport and toxicity. Current literature is focused on either the manufacture or testing the toxicity of nanosilver. There is a lack of information on the characterization and detection especially in environmental samples. There is a need for developing methods to measure the nanosilver concentration, size, shape, surface charge, crystal structure, surface chemistry and surface transformations. Some important questions to answer: Does nanosilver leach from consumer products? If so, in what form? Is it aggregated or still in the nanoscale size? What are its surface properties and chemistry? Does nanosilver dissolve or convert to ionic silver with time or under different conditions such as pH? What is the speciation of silver? Is nanosilver toxic? What are the toxicity mechanisms? Under what conditions do the mechanisms occur? Do particles aggregate inside the testing media? Do particles aggregate inside the tested cells? In order to answer these questions, characterization tools are needed. Possible characterization and detection techniques for nanosilver include transmission electron microscopy, scanning electron microscopy, electrospray scanning mobility particle sizer, atomic force microscopy, dynamic light scattering, Brunauer-Emmett-Teller analysis, x-ray diffraction, x-ray photoelectron spectroscopy, thermal gravimetric analysis, nuclear magnetic resonance spectroscopy, x-ray absorption near edge structure, fourier transformed infrared spectroscopy, zeta size analysis, inductively coupled plasma mass spectroscopy, atomic absorption spectroscopy, and flow field flow fractionation, among others.

Nanomaterials have many potential benefits to society with their development and deployment in science, engineering and technology. Their benefits, however, need to be weighed with any potential cost to the environment and public health. The unknown health effects and risks associated with these materials have drawn considerable attention from researchers, consumers and regulators. As a result, scientists at the U.S. Environmental Protection Agency (USEPA) and elsewhere have recognized the need to develop risk assessment processes to study the potential health and environmental impacts of manufacturing nanomaterials as well as using these materials in other products. In addition to the toxicological concerns, there are other aspects that have to be considered during the risk assessment process. For example, the cost of transportation must be evaluated, including the amount of emissions that are released from trucks, trains and other vehicles that transport nanomaterials.

To address these issues, researchers have begun implementing more comprehensive assessment tools such as Life Cycle Assessment (LCA) and Comprehensive Environmental Assessment (CEA) to assess the cradle to grave cost/risk associated with any given product. A CEA combines LCA with the risk assessment paradigm, which includes hazard identification, dose-response assessment, risk characterization and exposure assessment. A CEA can establish the comparative impact of products or processes in terms of specified impact categories including the life cycle stages, environmental pathways, transport, transformation, exposure and effects using a well-defined and documented methodology. Typical impact categories include global warming/climate change, stratospheric ozone depletion, primary and secondary contaminants, exposure, human toxicity, ecotoxicity, photo-oxidant formation, acidification, eutrophication, land use, and resource depletion. The potential advantages of CEA-based evaluations for nanomaterials are that they can address both the health and environmental consequences associated with the inclusion of nanocomponents. The ultimate goal is to ensure that the potential benefits of nanocomponents are realized in a manner that is safe for both consumers and the environment without resulting in unintended consequences.

An LCA for nanomaterials generally has four main aspects: material selection, manufacturing, application, and disposal/recycle. The material selection aspect of nanosilver LCA involves both the composition (organic such as polymers, dendrimers, etc.; inorganic such as metals, metal

oxides, etc.; carbon such as carbon tubes or a combination of any of these) and geometry of the nanocomponents, which can be a variety of shapes (sphere, rod, etc.) and is dependent on the synthesis methods. The manufacturing aspect of nanosilver LCA involves synthesis techniques, while the application aspect of nanosilver LCA involves using the nanomaterials in either naturally dispersive or composite form for a range of applications. The disposal/recycle aspect of nanosilver LCA involves incineration, disposal in a landfill or removal during wastewater treatment, among others.

To perform a CEA on nanoparticles, it is important to have some knowledge of the methods for their synthesis. This information allows for the identification of the characteristics and morphologies of the silver nanomaterials that are crucial for a more focused approach when evaluating their environmental fate, transport and toxicity. These characteristics and morphologies of the particles are determined by the methods of synthesis and the reactants that are involved. The nanomaterials that are produced are known to aggregate unless the particle surface is capped with a stabilizing agent, or unless the particles are suspended in a dispersant to prevent their aggregation. Depending on the use of dispersants in the manufacturing process, or lack thereof, different particle morphologies (e.g., size, shape, texture, phase, etc.) and surface properties will emerge resulting in diverse characteristics that affect the fate, transport and toxicity of the produced silver nanoparticles. Generally, aggregates of nanoparticles pose a lesser risk to the environment than smaller nanoparticles.

Once information on the four aspects of an LCA have been determined (material selection, manufacturing, application, and disposal/recycle), and information on environmental pathways such as air, water, soil and food web, transport and transformation of primary and secondary contaminants, exposure through inhalation, ingestion and dermal absorption, and toxicity is collected, a CEA of the nanomaterials may be performed. The CEA determines the risk associated with using a particular nanomaterial in a particular product, which is a function of both exposure potential and toxicity. In some cases, risk may be low because the exposure potential is low or the toxicity is low, or both. On the other hand, risk may be relatively high even when exposure potential is low if the toxic potency is high, or *vice versa*. Calculating this risk may be stymied by the fact that a large number of data gaps exist when considering the

application of CEA to nanomaterials. Finding adequate data to model the potential fate and effects of unintended releases of nanomaterials into the environment may be difficult. Minimal data detailing the material inputs and environmental releases related to the manufacture, release, transport, and ultimate fate of nanomaterials exist in the literature. Studies have mainly focused on cradle-to-gate assessments (as opposed to the more extensive “cradle-to-grave” assessments that look at the whole life cycle of the product, including disposal in a landfill or recycling into raw materials for other products). Cradle-to-gate analyses investigate the production of either nanocomponents or nanomaterials up to the point these materials leave the “gate” or the manufacturing source. The usefulness of many nanomaterials has been demonstrated in laboratory studies, and is yet to be implemented in consumer products. As a result, much of the data must be estimated before a CEA can be performed.

Once nanosilver has been synthesized at a manufacturing facility, part of it may be used to produce a final product, part of it may be shipped to a second manufacturing facility where it is turned into a final product, and the remaining part may either be stored at the manufacturing facility, lost due to leaks in the manufacturing process or disposed. It is necessary to know the products that contain nanosilver, the amount each product contains, the process that is being used to manufacture the product, the location of the product, the demographics of the end users of the product, and the amount that is going to waste among other variables to perform a nanoparticle LCA. Information on the amount that goes directly to waste or the amount that gets released from a manufactured product due to interactions with its surrounding environment (e.g., nanosilver being released from socks during wash cycles) is necessary to determine the routes of release of nanoparticles from its products as well as to determine routes of exposure to humans and the ecosystem. The routes of release and exposure depend on the fate and transport of silver nanomaterials, as well as the factors that affect transport (aggregation, capping agents and environmental conditions such as pH, ionic strength, natural organic matter (NOM), etc.). In addition, exposure will depend on whether nanomaterials interact with various environments including soil, sediment, freshwater, groundwater, wastewater and marine environments.

There is evidence that silver, and in particular nanosilver, is toxic to aquatic and terrestrial organisms, a variety of mammalian cells *in vitro*, and may be detrimental to human health. While

undoubtedly silver and nanosilver have useful applications in the medical arena (for instance as coatings for medical devices or as wound care for severe burns victims), their use may need to be strictly controlled. Bacterial resistance to antibiotics is an ever increasing problem globally, and indiscriminate use of biocidal silver in numerous consumer products is not only unnecessary, but may further increase bacterial resistance to a dangerous level (Mühling *et al.*, 2009). There are preliminary indications that in nanoparticle form, the toxicity of ionic silver may be increased, or that the nanoparticles may exert their own toxicity. The disposal of biocidal silver products into wastewater raises a number of concerns as the resulting sewage sludge may be used on agricultural soils, disposed as solid waste in landfills or be incinerated. Biocidal silver may also disrupt the functioning of key soil microbial communities.

1. Introduction

Silver has been valued throughout history for many of its properties that are useful to humans. It is used as a precious commodity in currencies, ornaments, jewelry, electrical contacts and photography, among others. One of the most beneficial uses of silver has been as a potent antibacterial agent that is toxic to fungi, viruses and algae. Silver has long been used as a disinfectant; for example, the metal has been used in treating wounds and burns because of its broad-spectrum toxicity to bacteria as well as because of its reputation of limited toxicity to humans.

In nanotechnology, a nano particle is defined as a small object or particle that behaves as a whole unit in terms of its transport and properties. Nanotechnology takes advantage of the fact that when a solid material becomes very small, its specific surface area increases, which leads to an increase in the surface reactivity and quantum-related effects. The physical and chemical properties of nanomaterials can become very different from those of the same material in larger bulk form. Nanomaterials (such as nanotubes and nanorods) and nanoparticles are particles that have at least one dimension in the range of 1 to 100 nm. Nanoparticles are classified solely based on their size, and may or may not exhibit size-related properties that differ significantly from those observed in bulk materials (ASTM, 2006; Buzea *et al.*, 2007). Due to the properties of silver at the nanoscale, nanosilver is nowadays used in an increasing number of consumer and medical products. Nanomaterials are nanoparticles that have special physicochemical properties as a result of their small size (Buzea *et al.*, 2007).

One important use of silver nanoparticles is to give products a silver finish. Nanosilver's strong antimicrobial activity is a major reason for the development of nanosilver containing products. Of the more than 1000 consumer products that contain nanomaterials, roughly 25% are claimed to contain silver nanoparticles. Widely available consumer products that contain nanosilver include food contact materials (such as cups, bowls and cutting boards), odor-resistant textiles, electronics and household appliances, cosmetics and personal care products, medical devices, water disinfectants, room sprays, children's toys, infant products and 'health' supplements (Fauss, 2008).

Some of the applications of nanosilver have resulted in government concern and discussions among the public because, once released into the environment, the mobility, bioavailability and toxicity of nanosilver on any ecosystem is determined in part by its stability in the environment. An example of this is the addition of silver nanoparticles to socks in order to kill the bacteria associated with foot odor. Several studies have shown that silver can easily leach into wastewater during washing, thus, potentially disrupting helpful bacteria used in wastewater treatment facilities or endangering aquatic organisms in lakes and streams. Some brands of socks were shown to lose nearly all of their silver content within a few washings (Benn & Westerhoff, 2008). There is clear evidence that silver, and in particular nanosilver, is toxic to aquatic and terrestrial organisms, a variety of mammalian cells *in vitro*, and may be detrimental to human health. Surprisingly, there is little to no information on the behavior of silver nanoparticles in the environment.

The stability of silver nanoparticles in the environment may be a function of many factors including the type of capping agent (chemicals used in the synthesis of nanoparticles to prevent aggregation) that is used, and surrounding environmental conditions, such as the pH, ionic strength, nutrient levels, the presence of binding agents, etc. Because an extensive number of capping agents are being used to manufacture silver nanoparticles and because it is almost impossible to predict the behavior of silver nanoparticles in different environments, understanding the implications of silver metal in the environment may provide an important context for understanding the implications of nanosilver in the same environment. Nanosilver may dissociate to form silver ions in the presence of moisture so at least part of the risk from nanosilver will stem from release of these ions into the environment. The environmental risks from silver itself may be mitigated by a tendency of the silver ion to form strong complexes that are apparently of very low bioavailability and toxicity. In particular, the formation of complexes with sulfides may strongly reduce the bioavailability of silver ions under some circumstances (Luoma, 2008). It is not yet clear to what extent such speciation reactions will affect the toxicity of nanosilver. If organic/sulfide coatings or complexation in natural waters similarly reduce the bioavailability of nanosilver particles, the risks to natural waters will be reduced. It is also possible that nanoparticles shield silver ions from such interactions, delivering free silver ions to

the membranes of organisms or into cells. In that case, an accentuation of environmental risks would be expected beyond that associated with a similar mass of silver itself.

To conduct a risk assessment of nanosilver under different environmental conditions, it is important to characterize the nanoparticles, perform dose-metrics as well as quantify the physicochemical properties of the nanomaterial. Nanoparticles have novel properties compared to conventional chemicals. The characterization of these properties is important in order to enable realistic estimations of exposure to humans and the ecosystem. This information is also important to establish dose-response relationships for estimating the toxicity of these nanoparticles. The determination of nanoparticle dose necessitates the development of analytical tools to isolate and quantify these nanoparticles. Other analytical tools will be needed to quantify these nanomaterials in order to obtain an accurate estimate of the risk due to exposure to these particles.

Once a risk assessment of silver nanoparticles is performed, the regulatory policy challenge that emerges is how to match the antiquated air-water-land basis of existing laws with the inherently cross-media nature of the problem. Nanosilver can go from a manufacturing plant to a waste-treatment plant to sludge to crops to the human-food chain. It is considered primarily a water problem in the environment but primarily an air problem in the workplace. Like climate change, acid rain and genetically modified crops, nanosilver is a problem that fits poorly into the old boxes of the existing regulatory system. A cross-media approach is necessary as it allows a policy maker to consider which sources of pollution or exposure are most important and which can be most efficiently and effectively addressed. Current US government efforts to address nanosilver are using the few cross-media tools available. Specifically, policy makers in the US use the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and the Toxic Substances Control Act (TSCA) to regulate nanosilver in different ways. TSCA is broad, and potentially could cover most nanomaterials. FIFRA, by contrast, is limited to pesticides, which are defined to include antimicrobials. Since nanosilver is used primarily as an antimicrobial agent, most nanosilver products may fall under the regulation of FIFRA. The acts also differ in the degree of public protection and product oversight they offer. FIFRA is quite stringent and puts the burden of proof for safety on the manufacturer. TSCA has a number of loopholes and exemptions that

are perceived as lessening public protection and puts the burden of proof on the U.S. Environmental Protection Agency to show that a substance is harmful.

This review is primarily intended to summarize available information that can be used to perform a silver nanomaterial exposure assessment based on routes, quantities and effects of exposure. This information can be used to perform a comprehensive environmental assessment or an LCA of silver nanomaterials, which traces the path of silver nanomaterials from production to ultimate disposal. This review presents the current state of knowledge or beliefs concerning these topics and indicates what additional information is required to develop a thorough and effective risk assessment paradigm for use in silver nanomaterial risk management. The nature of this document requires that peer-reviewed literature and "grey" literature (e.g., posters, slide presentations, proceedings, web pages, and personal communications) be used from industry, consumer, academic and government sources of information. As more information becomes available in the literature, this document will incorporate that information, and will, therefore, be a living document that will adjust with knowledge and time.

2. Historical and Current Applications of Silver and Silver Nanomaterials

2.1 Elemental Silver Characteristics and Sources

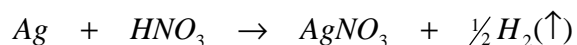
Elemental or metallic silver (Ag) is a malleable and ductile transition metal with a white metallic luster appearance (Brooks, 2010; Lenntech, 2010; Wikipedia, 2010). Of all metals, silver has the highest electrical conductivity (higher than copper that is currently used in many electrical applications) and thermal conductivity and has the lowest contact resistance (Brooks, 2010; Lenntech, 2010; USGS, 2010). Silver has high optical reflectivity compared to other metals (Edwards & Petersen, 1936). Silver is stable in pure air and water; the presence of ozone or hydrogen sulfide or sulfur in the air or water may result in silver tarnishing (Hammond, 2000) due to the formation of silver sulfide. The most common oxidation states of silver are 0 and +1, but other oxidation states (+2 and +3) are also known. Silver has many isotopes with ^{107}Ag being the most common (Smith & Carson, 1977). To date, 28 radioisotopes of silver have been characterized, with a majority of them having a half life of less than 3 minutes. Silver occurs naturally in its pure form, and as an alloy along with gold and other metals. In addition, it is also found in ores containing arsenic, sulfur, antimony and chlorine such as argentite, horn silver, chlorargyrite and pyrargyritein (Helmenstine, 2010; Smith & Carson, 1977; Wikipedia, 2010). The average concentration of silver in water is 0.5 ppb while its concentration in soil is approximately 10 ppb. Silver is mainly produced as by product of copper, gold, lead and zinc refining. Silver is generally extracted by amalgamation and displacement using metals such as mercury, or by smelting. The top silver producing countries are Peru, Mexico, China, Australia, Poland and Siberia. In the US, the state of Alaska leads the silver production through the Greens Creek Mine followed by Nevada through the Comstock Lode Mine (Silver Mining, 2010).

2.2 Chemistry of elemental silver

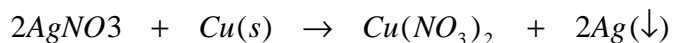
Silver is the sixty-third most abundant metal in Earth's crust, and exists as two isotopes, ^{107}Ag and ^{109}Ag , roughly occurring in the same proportion. The chemistry of silver was not well-known before 1980, although silver nitrate was used medicinally in the 1800s. Recent research has recognized the highly reactive nature of the silver ion and its ability to form numerous

inorganic and organic complexes (halide, sulfide, nitrate, oxide, and acetylide compounds, cyano-derivatives, olefin complexes, etc.). Ag(II) complexes are less stable than those of Ag(I) and Ag(III), but unlike many other silver compounds are brightly colored red or blue. Silver ion binds readily to proteins in the human body (including albumins and metallothioneins) and interacts with trace metals in metabolic pathways.

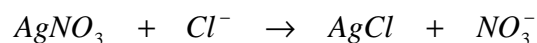
Silver metal readily dissolves in nitric acid (HNO₃) to form silver nitrate (AgNO₃). Silver nitrate is a transparent crystalline solid that is readily soluble in water, and is photosensitive. It is also used as the starting point for the synthesis of many other silver compounds.



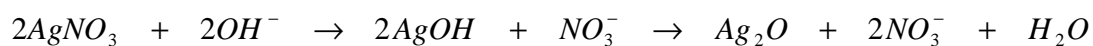
Silver nitrate can react with copper to form silver crystals and a blue-green solution of copper nitrate. Alkaline solutions of silver nitrate can also be used to reduce silver nitrate to silver metal in the presence of reducing sugars such as glucose. This reaction is used to silver glass mirrors and the interior of glass Christmas ornaments.



Silver or silver nitrate precipitates as silver chloride (AgCl) in the presence of chloride ions. Silver chloride and other silver halides are used in the manufacture of photographic emulsions.



Silver nitrate reacts with bases to form silver oxide (Ag₂O), which is used as a positive electrode in watch batteries.



Silver does not react with sulfuric acid; it reacts with sulfur or hydrogen sulfide to form silver sulfide, which is the tarnish that is commonly observed in silver jewelry, utensils or coins.



Silver metal reacts with nitric acid in the presence of ethyl alcohol (C₂H₅OH) to form silver fulminate (AgONC). Silver fulminate is a powerful touch-sensitive explosive used in percussion caps. Silver nitrate reacts with sodium azide (NaN₃) to form silver azide (AgN₃), which is also used as an explosive. Silver, in the presence of excess cyanide, forms cyanide complexes (AgCN) that are soluble in water; these complexes are used in silver electroplating.

2.3 Historical and Current Applications of Elemental Silver and Silver compounds

Besides elemental silver, other silver containing compounds that are found in the Earth's crust include silver halides (AgBr, AgCl, AgI, and silver fluorides), silver fulminate, silver nitrate and silver oxide (ATSDR, 1990; Greenwood & Earnshaw, 1997; Hammond, 2000; Romans, 1954) among others. These compounds vary in solubility from readily soluble to barely soluble in water. Throughout history, silver and its compounds have been used extensively for many applications as a result of their useful properties. It is believed that silver was known and used longer than what is recorded in history. Archeological evidence suggests that civilizations have been using silver since at least 3000 B.C. Ancient Egyptians and Persians used silver vessels to keep their water clean and safe. Romans and Greeks knew its powerful bactericidal effect and used it for healing wounds. During World War I, silver compounds were used to prevent wound infection before the emergence of antibiotics. In the American Old West, pioneers traveling along Oregon trails used to toss silver coins into their water storage barrels to keep their water fresh (Information and History, 2010; Russell & Russell, 1995; History of Silver, 2010; Wijnhoven *et al.*, 2009). During the 19th century, beyond home remedies, silver was applied in practical medicine such as eye treatment and the treatment of skin ulcers (Foot Defense, 2010). Other uses of silver include making currency coins, ornaments, jewelry, tableware and utensils. The US Food and Drug Administration approved silver solutions in the 1920s to be used as antibacterial agents (Wikipedia, 2010).

Silver and its compounds have an extensive number of applications in the 20th century including electrical conductors, electrical contacts, catalysis, photography, electronics, mirrors, drinking water filtration systems, swimming pool filtration systems, healthcare products and medical tools (Clement *et al.*, 1994; Luoma, 2008; Wikipedia, 2010). Since soluble silver compounds are toxic to some bacteria, viruses, algae and fungi, various applications have emerged based on the strong germicidal impacts of silver compounds. Silver is incorporated in textiles to inhibit the growth of bacteria and to keep odor at minimum (Clement *et al.*, 1994). In 1954, silver was registered in the US as a pesticide for use in disinfectants, sanitizers and fungicides. Various diseases ranging from mental illness to gonorrhea have been reported to be treated using silver compounds (Panyala, 1996). Silver was used in 2007 to make the first antibacterial glass used in hospitals to fight infections (AGC Glass, 2007). Silver is also used in catheters in order to make them more effective for reducing bacteriuria (a urinary tract infection) in adults at hospitals while having short term catheterization (Sanjay *et al.*, 2009). Not all silver compounds are known to have the same impact on infections; silver alloy catheters are significantly more effective in preventing urinary tract infections than are silver oxide catheters. NASA selected silver for purifying the drinking water in space shuttles (Information and History, 2010).

Other medical applications of silver include its use in the manufacture of bone prostheses, cardiac implants and replacement valves, needles used in ocular surgery, peritoneal catheters, and wound sutures. It is an antiseptic ingredient used in wound management. While silver has been used as an antiseptic for many years, new products that time-release silver in a sustained manner are starting to be available in the market. These products are showing promise in the treatment of skin wounds, skin ulcers, and burns. In these new products, which may contain elemental or nanosilver, silver ions are released from the dressings (ActicoatTM, ActisorbTM, etc.) in the presence of wound fluids, exudates, and the products are activated to keep the wounds clean. Activated silver ion is toxic to bacteria and yeasts. Silver is toxic to bacteria at low concentrations (10^{-5} to 10^{-7} Ag ions per cell). Although silver itself is not considered toxic, most of its salts are poisonous, due to the anions involved. Exposure to silver (metal and soluble compounds, as Ag) in air should not exceed 0.01 mg/m³ (8-hour time-weighted average for a 40-hour week) (LANL, 2010). Silver compounds can be absorbed into the circulatory system, with

the deposition of reduced silver in body tissues. This may result in argyria, which is characterized by a grayish pigmentation of the skin and mucous membranes. Silver absorbed through the skin is deposited in the liver and kidney and complexes with albumin and cellular proteins. A potential hazard of using silver in jewelry, medicinal products, coins, and antiseptics is allergies, which may result in red rashes, blisters, welts, hives, and itching or burning skin.

Sterling silver (i.e., 92.5% silver) is usually used for silverware and jewelry and some high-end musical instruments such as flutes. When alloyed with mercury, tin and other metals at room temperature, silver is used to make amalgams for use in dental filling. Silver is used in printed circuit boards and keyboards as an electrical contact and as wires in some high end audio hardware. Silver is also used as a catalyst in industrial processes such as catalyzing the conversion of ethylene to ethylene oxide or the production of formaldehyde from methanol. Applying a thin layer of silver on surfaces is also known to increase the galling resistance and reduce the wear of surfaces under heavy loads (ATSDR, 1990; Hammond, 2000; Wikipedia, 2010).

Silver nitrate is widely used in photography and in the synthesis of other silver compounds (see Section 2.2 for more information on the chemistry of silver) (Clement *et al.*, 1994; Wikipedia, 2010). Silver nitrate drops are used to prevent infections in infants' eyes, as an antiseptic, and in stained glass. Silver halides are used in gravimetric analytical methods and are extensively used in photography. Silver oxide is used as cathodes in batteries used for small devices. Silver azide and silver fulminate are powerful explosives. To produce rain, silver iodide is used in cloud seeding. Silver chloride can be made transparent and used in glass electrodes for pH and potentiometric measurements. It is also used as cement for glass.

The catalytic properties of silver make it ideal for use as a catalyst in oxidation reactions. Formaldehyde is produced from methanol and air in the presence of silver screens or crystallites that contain a minimum of 99.95% silver by weight. Silver-coated catalysts are probably the only catalysts currently available to convert ethylene to ethylene oxide. Ethylene oxide is ultimately used in the production of polyesters and other polymers that have multiple industrial

applications. Because silver readily absorbs free neutrons, it is commonly used to make control rods that regulate the fission chain reaction in pressurized water nuclear reactors.

2.4 Nanosilver: History and Applications

Silver nanomaterials are fine particles of metallic silver that have at least one dimension less than 100 nm (Figure 2.1). Nanosilver is not a new discovery; it has been known for over 100 years (USFDA, 2010). Previously, nanosilver or suspensions of nanosilver were referred to as colloidal silver. To produce colloidal silver, a positive electrical current is applied through pure silver bars suspended in water resulting in colloidal silver particles with a size range of 15-500 nm (Lindemann, 1997). Before the invention of penicillin in 1928, colloidal silver had been used to treat many infections and illnesses (Nano Health Solutions, 2010). By converting bulk silver into nanosized silver, its effectiveness for controlling bacteria and viruses was increased multifold, primarily because of the nanomaterials' extremely large surface area when compared to bulk silver, thus resulting in increased contact with bacteria and fungi. Nanosilver, when in contact with bacteria and fungus, adversely affects the cellular metabolism of the electron transfer systems, and the transport of substrate in the microbial cell membrane. Nanosilver also inhibits multiplication and growth of those bacteria and fungi which caused infection, odor, itchiness and sores (Nanotech Plc, 2010).

In 1951, Turkevich *et al.* reported a wet chemistry technique to synthesize nanosilver using silver nitrate as a silver ion source and sodium citrate as the reducing agent for the first time (Turkevich *et al.*, 1951). Recent advances in nanomaterials science in the last two decades have enabled scientists to engineer silver nanomaterials by controlling their size, shape and surface properties. This has been motivated by the unique chemical, physical and optical properties of nanosilver compared to the parent silver metal. The unique properties of nanosilver are mainly attributed to the high surface area to volume ratio, leading many industrial sectors to incorporate silver nanomaterials into their products. Nanosilver is being incorporated in plastics, fabrics, paper, paint, and surface coatings. More than 200 products containing nanosilver are now available for public use. Numerous other applications have been reported for silver nanoparticles in areas such as electronics, bio-sensing and surface enhanced Raman spectroscopy (SERS)

(Tolaymat *et al.*, 2010). More detailed information regarding the various applications of nanosilver is provided in Chapter 4.

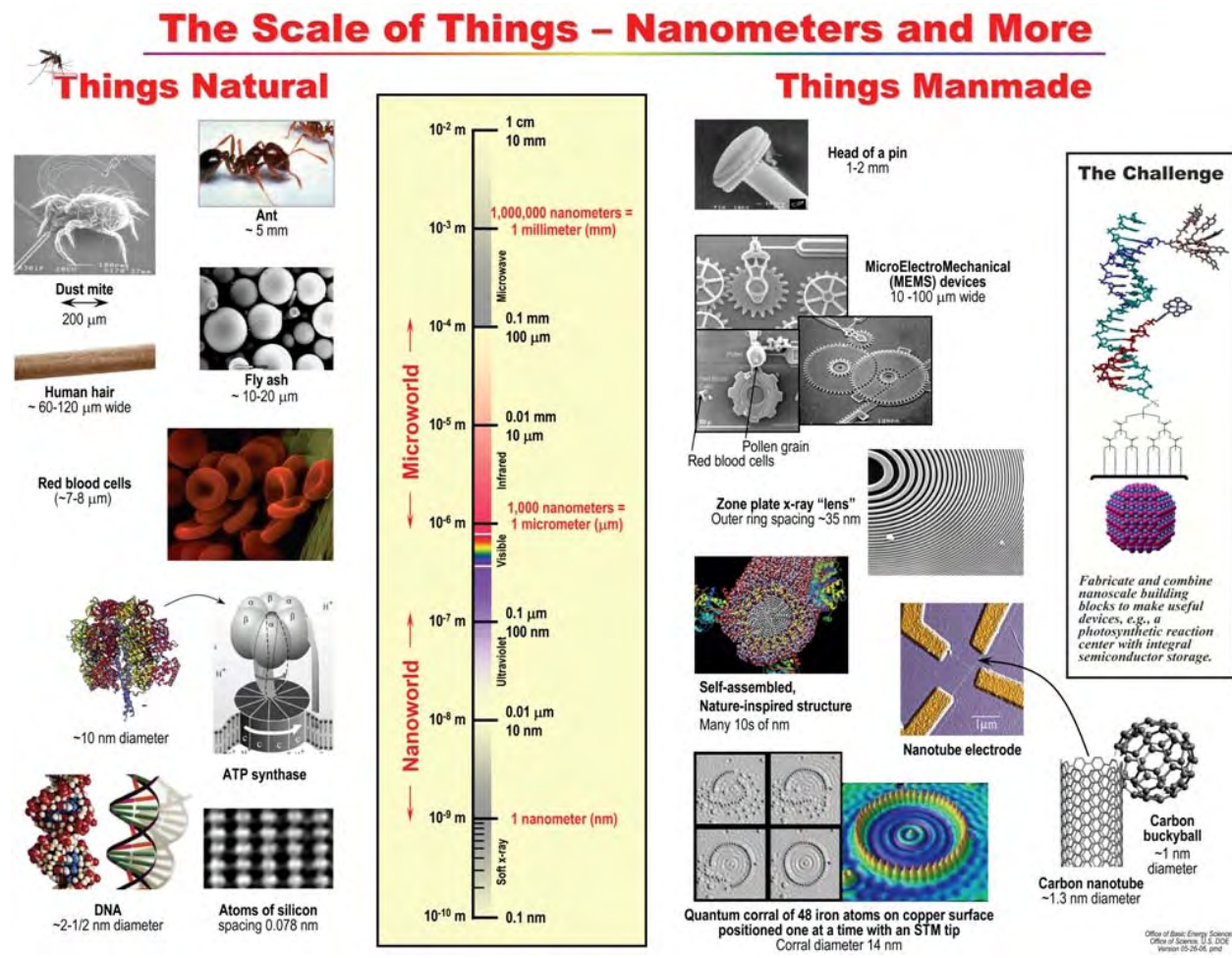


Figure 2.1: Nanomaterials dimensions on the metric scale (in nm) courtesy of the Office of Basic Energy Sciences, Office of Science, U.S. Department of Energy

2.5 Silver Regulations in the US

Silver was registered in the United States as a pesticide in 1954. Nanosilver products registered since 1950 are presented in Figure 2.2 (SNWG, 2009). The USEPA designated silver as a priority pollutant in natural waters in 1977. The studies that formed the basis for the USEPA regulation of silver were based on toxicity data from colloidal silver and not bulk silver (SNWG, 2009). A secondary maximum contaminant level was issued by USEPA's Office of Water (OW) for silver in 1991 based on the ability of silver to cause argyria. In 1991, the USEPA established

an oral reference dose of 0.005 mg/kg/day for silver. Between 1970 and 1990, all USEPA registered silver products were either colloidal nanosilver or nanosilver-composite products. The first product containing conventional silver was registered in 1994. Over 50% of USEPA's registered silver in recent years is based on nanosilver (Rosalind, 2009).

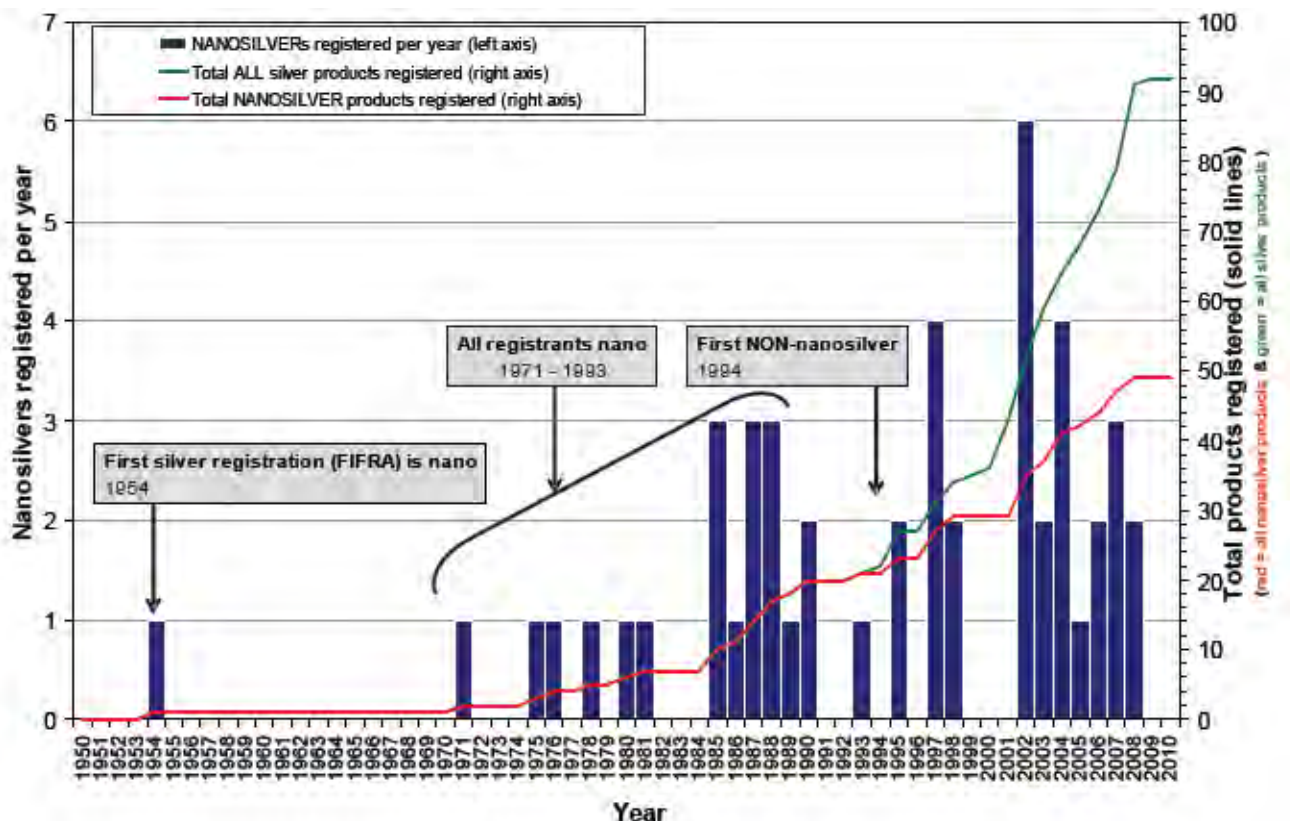


Figure 2.2: Analysis of FIFRA registered products containing nanosilver for the period 1950-2010 (SNWG, 2009). Reprinted with permission from the Silver Institute. Copyright © 2004 The Silver Institute

As a result of the expanding usage of nanosilver, USEPA has great concerns regarding its environmental fate, transport and toxicity. USEPA is currently conducting and/or funding fundamental research to help understand the potential human health and ecological implications from exposure to manufactured nanomaterials including nanosilver. The USEPA's Office of Research and Development (ORD) issued a nanomaterials research strategy in June 2009, in which silver was one of the seven materials selected to be investigated. The USEPA's Office of Pesticide Programs (OPP) plans to regulate certain consumer products containing nanosilver under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (Rosalind *et al.*, 2009).

Under FIFRA, products containing silver nanoparticles with the aim of killing microbes will be classified as pesticides. An example of a product that is classified as a pesticide under FIFRA would be washing machines that release silver nanoparticles to kill bacteria on clothes (Peabody, 2006). The regulations applicable to nanosilver containing products are yet to be issued. Under FIFRA, if a product is claimed to release nanosilver to kill bacteria, the manufacturer must submit product data to the USEPA, which is authorized to prohibit particular products that pose unreasonable health effects on the environment or humans (Peabody, 2006).

3. Uses of Silver Nanomaterials

Knowledge of the applications of silver nanomaterials in consumer products is crucial for an accurate prediction of release pathways, exposure, LCA, and risk assessment. During the last two decades, an extensive number of methods have been reported for the synthesis of silver nanoparticles with different particle sizes, shapes and surface properties. This advancement in their manufacturing techniques attracted the attention of many industries wanting to exploit the unique properties of silver nanomaterials for beneficial use. The applications of silver nanomaterials are scattered but they can be classified under three main categories: scientific, industrial, and consumer products.

3.1 Properties of nanosilver

Two primary factors cause nanomaterials to behave significantly differently than bulk materials: surface effects and quantum effects (Roduner, 2006). These factors affect the chemical reactivity of materials as well as their mechanical, optical, electric, and magnetic properties. Nanosilver has chemical and biological properties that are appealing to the consumer products, food technology, textiles/fabrics, and medical industries. Nanosilver also has unique optical and physical properties that are not present in bulk silver, and which are claimed to have great potential for medical applications.

3.1.1 Antibacterial properties

Nanosilver is an effective killing agent against a broad spectrum of Gram-negative and Gram-positive bacteria (Burrell *et al.*, 1999; Wijnhoven *et al.*, 2009; Yin *et al.*, 1999), including antibiotic-resistant strains (Percival *et al.*, 2007; Wright *et al.*, 1998). Gram-negative bacteria include genera such as *Acinetobacter*, *Escherichia*, *Pseudomonas*, *Salmonella*, and *Vibrio*. *Acinetobacter* species are associated with nosocomial infections, i.e., infections that are the result of treatment in a hospital or a healthcare service unit, but secondary to the patient's original condition. Gram-positive bacteria include many well-known genera such as *Bacillus*, *Clostridium*, *Enterococcus*, *Listeria*, *Staphylococcus*, and *Streptococcus*. Antibiotic-resistant bacteria include strains such as methicillin-resistant and vancomycin-resistant *Staphylococcus aureus*, and *Enterococcus faecium*.

Silver nanoparticles (diameter 5-32 nm, average diameter 22.5 nm) enhance the antibacterial activity of various antibiotics (Shahverdi *et al.*, 2007). The antibacterial activities of penicillin G, amoxicillin, erythromycin, clindamycin, and vancomycin against *Staphylococcus aureus* and *Escherichia coli* increase in the presence of silver nanoparticles (Wijnhoven *et al.*, 2009). Size-dependent (diameter 1-450 nm) antimicrobial activity of silver nanoparticles has been reported with Gram-negative bacteria (Baker *et al.*, 2005; Morones *et al.*, 2005; Panacek *et al.*, 2006) and Gram-positive bacteria (Panacek *et al.*, 2006). Small nanoparticles with a large surface area to volume ratio provide a more efficient means for antibacterial activity even at very low concentration.

In addition to size and concentration, shape-dependent antimicrobial activity of silver nanoparticles has been shown with Gram-negative bacteria (Pal *et al.*, 2007). Silver nanoparticles of different shapes (spherical, rod-shaped, truncated triangular nanoplates) have been developed by synthetic routes. Truncated triangular silver nanoplates display the strongest antibacterial activity (Wijnhoven *et al.*, 2009). The top basal plane of truncated triangular silver nanoplates is a high-atom-density surface, i.e., a {111} facet. Generally, spherical silver nanoparticles (generally with cubo-octohedral, multiple-twinned decahedral, or quasi-spherical morphology) have {100} facets along with a small percentage of {111} facets, whereas rod-shaped silver nanoparticles (e.g., pentagonal rods) have side surfaces with {100} facets and end with {111} facets (Wijnhoven *et al.*, 2009; Wiley *et al.* 2005). Silver reactivity is favored by {111} facets (Hatchett & White, 1996). Spherical silver nanoparticles with {111} facets attach directly to the bacterial surface of the cell membrane and are located inside bacteria (Morones *et al.* 2005). The strong anti-bacterial activity of truncated triangular silver nanoplates could be due to their large surface area to volume ratios and their crystallographic surface structures.

3.1.1.1 Antibacterial mode of action

Bacteria have different membrane structures, which are the bases of their general classification as Gram-positive or Gram-negative. Structural differences reside in the organization of the key component of the cell wall, peptidoglycan, which is located immediately outside the cytoplasmic membrane. The cell wall of Gram-positive bacteria contains a peptidoglycan layer that is ~30 nm

thick. Unlike the Gram-positive cell wall, the Gram-negative cell wall has only a thin peptidoglycan layer that is ~2-3 nm thick. In addition to the peptidoglycan layer, the Gram-negative cell wall also contains an additional outer membrane composed of phospholipids and lipopolysaccharides, which face into the external environment.

Although the antimicrobial effect of silver ions has been studied extensively, the effects of nanosilver on bacteria and the bactericidal mechanism are only partially understood. Based on studies that show that silver nanoparticles anchor to and penetrate the cell wall of Gram-negative bacteria (Morones *et al.*, 2005; Sondi & Salopek-Sondi, 2004), it is reasonable to suggest that the resultant structural change in the cell membrane could cause an increase in cell permeability, leading to an uncontrolled transport through the cytoplasmic membrane, and ultimately cell death. It has also been proposed that the antibacterial mechanism of silver nanoparticles is related to the formation of free radicals and subsequent free radical-induced membrane damage (Danilczuk *et al.*, 2006; Kim *et al.*, 2007). Hwang *et al.* (2008) performed a study of stress-specific bioluminescent bacteria, based on a synergistic toxic effect of the silver nanoparticles and the silver ions that they produce. The ions move into the cells and lead to the production of reactive oxygen species. Because of the membrane damage caused by the nanoparticles, the cells cannot effectively extrude the silver ions and limit their effect. Based on the greater tendency of silver ions to strongly interact with thiol groups of vital enzymes and phosphorus-containing bases (Hatchett & White, 1996) and on the presence of silver nanoparticles inside the cells (Morones *et al.*, 2005), it is likely that further damage could be caused by interactions with compounds such as DNA. This interaction may prevent cell division and DNA replication from occurring, and also ultimately lead to cell death. No DNA damage was found by Hwang *et al.* (2008). Other studies have suggested that silver nanoparticles may modulate the phosphotyrosine profile of putative bacterial peptides that could affect cellular signaling and, therefore, inhibit the growth of bacteria (Shrivastava *et al.*, 2007).

3.1.2 Antifungal properties

Nanosilver is an effective, fast-acting fungicide against a broad spectrum of common fungi including genera such as *Aspergillus*, *Candida*, and *Saccharomyces* (Wright *et al.*, 1999). The exact mechanisms of action of silver nanoparticles against fungi are still not clear, but

mechanisms similar to that of the antibacterial actions have been proposed for fungi (Wright *et al.*, 1999). Silver nanoparticles (diameter 13.5 ± 2.6 nm) are effective against yeast isolated from bovine mastitis (Kim *et al.*, 2007).

3.1.3 Antiviral properties

Silver nanoparticles (diameter 5-20 nm, average diameter ~10 nm) inhibit HIV-1 virus replication (Sun, Chen, *et al.*, 2005). Gold nanoparticles (average diameter ~10 nm) showed relatively low anti HIV-1 activity (6-20%) when compared to silver nanoparticles (98%). Size-dependent antiviral activity of silver nanoparticles has been shown with HIV-1 virus (Elechiguerra *et al.*, 2005). Interaction of silver nanoparticles with HIV-1 was exclusively within the range of 1-10 nm.

3.1.4 Anti-inflammatory properties

Nanosilver dressings as well as nanosilver-derived solutions proved to have anti-inflammatory activity (Nadworny *et al.*, 2010). In animal models, nanosilver alters the expression of matrix metallo-proteinases (proteolytic enzymes that are important in various inflammatory and repair processes) (Kirsner *et al.*, 2001), suppresses the expression of tumor necrosis factor (TNF)- α , interleukin (IL)-12, and IL-1 β , and induces apoptosis of inflammatory cells (Bhol & Schechter, 2005, 2007). Silver nanoparticles (diameter 14 ± 9.8 nm) modulate cytokines involved in wound healing (Tian *et al.*, 2007). The results indicate the possibility of achieving scar-less wound healing even though further studies using other animal models are required to confirm this.

3.1.5 Anti-glycoprotein film properties

Glycoproteins are proteins that contain oligosaccharide chains that are covalently attached to polypeptide side-chains. These proteins are important for normal immune system function such as white blood cell recognition, and often play a role in cell-cell interactions. Examples of glycoproteins in the immune system include molecules such as antibodies that interact directly with antigens. In the case of impregnation of medical-grade silicone with silver nanoparticles (diameter 10-100 nm) there is both a depot effect and a diffusion pressure available to equilibrate the silver concentration and to push silver through the glycoprotein conditioning film (Furno *et al.*, 2004). This unexpected finding has obvious clinical implications, because silver is known to

have a high avidity to protein and the presence of a glycoprotein film has been assumed to inactivate any silver ions released (Schierholz *et al.*, 1998). Surfaces of implanted devices immediately and rapidly become coated with patient-derived glycoproteins from tissue and blood plasma (Green *et al.*, 1999). Once protein adhesion has occurred, proliferation leads to the development of a biofilm which is insusceptible to most therapeutic agents.

3.1.6 Anti-biofilm properties

Nanosilver inhibits the formation of biofilms (Percival *et al.*, 2007). Biofilms are complex communities of surface-attached aggregates of microorganisms embedded in a self-secreted extracellular polysaccharide matrix. Biofilm forming bacteria act as efficient barriers against antimicrobial agents and the host immune system, resulting in a persistent colonization and/or infection at the site of the biofilm formation.

3.1.7 Surface plasmon resonance properties

Noble metal nanoparticles can be deposited onto a glass matrix and exhibit a very intense color, which is absent in bulk material as well as in individual atoms. Their origin is attributed to the collective oscillations or fluctuations in electron density with an interacting electromagnetic field. These resonances are denoted as surface plasmons. These oscillations are very sensitive to adsorption of molecules to the metal surface. The plasmonic coupling of metal nanoparticles with light enhances a broad range of useful optical phenomena which have application potential in ultra-sensitive biomolecular detection and lab-on-a-chip sensors (Moore & Goettmann, 2006). The effect of the size of silver nanoparticles on the surface plasmon resonance, i.e., plasmon band width and peak position, has been demonstrated (Thomas *et al.*, 2008). Decreasing nanoparticle size (diameter ≤ 10 nm) is associated with a red-shift and broadening of the plasmon-related absorption peak. The impact of silver nanoparticle shape on plasmon surface resonance has been less studied.

3.1.8 Plasmonic heating properties

Plasmonic photo activation of hollow polyelectrolyte-multilayer capsules incorporating silver nanoparticles and containing drug models has been demonstrated as proof-of-principle (Skirtach *et al.*, 2004). Silver nanoparticles were remotely activated using laser irradiation, causing not only absorption of photons but also heat transfer from the nanoparticles to the surrounding

polymer matrix. The local heating disrupts the polymer matrix and allows the encapsulated material/drug to leave the interior of the capsule. The concept of remote opening of polyelectrolyte-multilayer capsules incorporating silver nanoparticles (diameter > 20 nm) has been demonstrated in living cells (Skirtach *et al.*, 2006). The duration of laser treatment to open polyelectrolyte multilayer capsules is dependent on the size of silver nanoparticles (diameter 10-23 nm; Radziuk *et al.*, 2007).

3.1.9 Metal-enhanced fluorescence properties

Metallic nanostructures (size range 30-80 nm) alter the intrinsic spectral properties (i.e., emission intensity and photostability) of fluorophores. The proximity of silver nanostructures results in an increase in intensity of low-quantum-yield fluorophores. The effects of metallic surfaces include fluorophore quenching at short distance (~0-5 nm), spatial variation of the incident light field (~0-15 nm), and changes in the radioactive decay rate (~0-20 nm). Applications include immunoassays and DNA/RNA detection (Aslan *et al.*, 2005).

3.1.10 Properties of silver nanomaterials that promote its biosynthesis

Despite the antibacterial properties of nanosilver, the feasibility of biosynthesis of silver nanoparticles using bacteria has been demonstrated (Klaus *et al.*, 1999). Silver nanoparticles (sizes up to 200 nm) were synthesized using *Pseudomonas stutzeri* and found to be mostly located at the periplasmic area of the bacteria. Another silver-resistant bacteria used in the biosynthesis of silver nanoparticles (diameter 5-32 nm, average diameter 22.5 nm) is *Klebsiella pneumoniae* (Shahverdi, Fakhimi, *et al.*, 2007). Fungi have also been used to biosynthesize silver nanoparticles. Intracellular silver nanoparticles (diameter 25 ± 12 nm) were produced in *Verticillium* fungal cells (Mukherjee *et al.*, 2001) and extracellular silver nanoparticles (diameter 5-25 nm) using pathogenic filamentous fungi such as *Fusarium oxysporum* (Ahmad *et al.*, 2003) and *Aspergillus fumigatus* (Bhainsa & D'Souza, 2006). Extracellular silver nanoparticles (diameter 13-18 nm) have been biosynthesized using non-pathogenic fungus *Trichoderma asperellum* (Mukherjee *et al.*, 2008). In addition to microbial organisms, plant extracts can be used in the biosynthesis of metallic nanomaterial (Mohanpuria *et al.*, 2008). The widespread and increasing use of nanosilver within healthcare settings raises issues concerning bacterial and fungal silver resistance. Whether resistance is a threat in the clinical setting needs to be

elucidated (Chopra, 2007). Standardization for silver antimicrobial testing methods is lacking. This is partly due to the complex solubility issues affecting the bioavailability of silver.

3.2 Scientific Applications

The remarkable physical, chemical and optical properties of silver nanomaterials allows for their utilization in various scientific applications. These properties significantly depend on the size, shape and surface chemistry of the nanomaterials. Metallic nanoparticles, including nanosilver, exhibit surface plasmon resonance (SPR) upon irradiation with light giving rise to SPR peaks in the UV-Vis wavelength range (Luoma, 2008; Tolaymat *et al.*, 2010). The SPR is a result of the interactions between the incident light and the free electrons in the conduction band of the nanomaterials. The width and location of the SPR peaks are dependent on the size, shape and surface properties of the nanomaterials (Ju-Nam & Lead, 2008). Silver nanomaterials are widely used for surface enhanced Raman scattering (SERS). Raman scattering by molecules could be enhanced if the analyte molecules are adsorbed on rough metal surfaces. The enhancement factor can be as much as 10^{14} - 10^{15} which allows for enough sensitivity to detect single molecules (Doering & Nie, 2002). As a consequence of the SPR and SERS, silver nanomaterials are a promising tool for sensing applications, including detection of DNA sequences (Jacob *et al.*, 2008), laser desorption/ionization mass spectrometry of peptides (Hua *et al.*, 2007), colorimetric sensors for Histidine (Xiong *et al.*, 2008), determination of fibrinogens in human plasma (ZhiLiang *et al.*, 2007), real-time probing of membrane transport in living microbial cells (Xu *et al.*, 2004), enhanced IR absorption spectroscopy (Huo *et al.*, 2006), colorimetric sensors for measuring ammonia concentration (Dubas & Pimpan, 2008a), biolabeling and optical imaging of cancer (Wiley *et al.*, 2007), optical sensors for zeptomole (Nikolaj *et al.*, 2006), biosensors for detection of herbicides (Dubas & Pimpan, 2008b), and glucose sensors for medical diagnostics (Mishra *et al.*, 2007). SERS using nanosilver can be used for biological imaging, trace analysis of pesticides, anthrax, prostate-specific antigen glucose, nuclear waste, identification of bacteria, genetic diagnostics and detection of nitro-explosives (del Rocío *et al.*, 2006).

Silver nanomaterials are also known to be used for metal enhanced fluorescence applications. The intrinsic spectral properties of fluorophores can be altered by metallic nanostructures. The proximity of metallic nanosilver results in an increase in the intensity of low quantum yield

fluorophores. The effects include fluorophore quenching at short distances, spatial variation of the incident light field, and change in the radioactive decay rate (Wijnhoven *et al.*, 2009). These characteristics enable nanosilver to be used in applications such as immunoassays and DNA/RNA detection.

As previously mentioned, the characteristics of the silver nanomaterials are greatly influenced by their surface properties. Modifying the surface of silver nanoprisms by alkanethiol makes them potential candidates for streptavidin and anti-biotin sensing and may also aid in the diagnosis of Alzheimer's disease (Pastoriza-Santos & Liz-Marzán, 2008). Para-sulfonatocalix modified silver nanoparticles are used to probe histidine down to a concentration of 5×10^{-6} M (Xiong *et al.*, 2008). This is important since histidine is needed for the growth and the repair of tissue, as well as for maintenance of the myelin sheaths that act as the protector of nerve cells. It is manufactured in sufficient quantities in adults, but children may develop a shortage of this important amino acid (Xiong *et al.*, 2008).

3.3 Industrial Applications

3.3.1 Catalysis

The high surface area to volume ratio of silver nanomaterials provides high surface energy, which promotes surface reactivity such as adsorption and catalysis. This has resulted in the use of silver nanomaterials and silver nanocomposites to catalyze many reactions in industrial processes such as CO oxidation, benzene oxidation to phenol, photodegradation of gaseous acetaldehyde and the reduction of the p-nitrophenol to p-aminophenol (Tolaymat *et al.*, 2010). SiO₂ supported Ag catalysts (5 wt% Ag) exhibit good activity toward the decomposition of N₂O. Silver nanoparticles immobilized on silica spheres are used to catalyze the reduction of dyes by sodium borohydride (NaBH₄) (Nikolaj *et al.*, 2006). Ag nanoparticles synthesized in polyethylene glycol with simple bubbling of H₂ gas have been used to catalyze the three-component coupling reaction of aldehyde, alkyne, and amine with good to excellent yields in one reaction vessel (Yan *et al.*, 2006), thus saving time and materials.

3.3.2 Electronics

The high electrical and thermal conductivity of nanosilver along with the enhanced optical properties result in various applications in electronics. Nanosilver is used in electronic equipment, mainly in solder for circuit connections (DiRienzo, 2006). Silver nanowires are used as nanoconnectors and nanoelectrodes for designing and fabricating nanoelectronic devices (Kim *et al.*, 2007). Other applications include the preparation of active waveguides in optical devices (Roldan *et al.*, 2007), inks for printed circuit boards, optoelectronics, nanoelectronics (such as single-electron transistors, and electrical connectors), subwavelength optics, data storage devices, nonlinear optics, high density recording devices, intercalation materials for batteries, making micro-interconnects in integrated circuits (IC) and integral capacitors (Tolaymat *et al.*, 2010). Silver inks are used to replace wires and act as flat wires in printed circuit boards. In addition, silver inks are also used to repair circuit breaks in printed circuit boards, thus preventing their premature disposal in landfills (DiRienzo, 2006).

3.3.3 Other Industrial Applications

Nanosilver is being utilized in the paper industry. DocuGuard uses silver-based paper to protect hospital case notes and medical files against the proliferation of bacteria (DiRienzo, 2006). The company proposes future applications to include business stationery, envelopes, brochures and book-binding materials. Nanosilver is used in commercial water purification systems. The industry makes use of the antibacterial properties of nanosilver in the interior of automobiles such as steering wheels and in building materials such as sanitary tubing and coverings (Blaser *et al.*, 2008). Nanosilver is also used for wood preservation to resist mildew and mold. MTR Corporation in Hong Kong reports the use of silver nanoparticles in combination with titanium dioxide coating to enhance hygiene by spraying it onto surfaces in MTR train stations, inside train compartments, as well as MTR managed shopping malls, staff offices and recreational facilities (Senjen, 2007).

3.4 Applications in Consumer Products

Nanosilver is one of the most widely used nanomaterials that are incorporated in consumer products. Silver nanoparticles are used as antibacterial/antifungal agents in a diverse range of applications including air sanitizer sprays, socks, pillows, slippers, face masks, wet wipes,

detergent, soap, shampoo, toothpaste, air filters, coatings of refrigerators, vacuum cleaners, washing machines, food storage containers, cellular phones, and even in liquid condoms (a liquid containing spermicides that solidifies and becomes a protective condom when sprayed into the female genital area; the condom is designed to transform back to a liquid in the presence of semen, which releases the spermicide). The major reason for this prevalence is its strong antibacterial effect for a wide array of organisms (Tolaymat *et al.*, 2010). Samsung produced a version of washing machines (AG plus) that generate silver nanoparticles to disinfect clothes rather than using hot water and detergents (DiRienzo, 2006). Samsung and GSH are using nanosilver coating in their new refrigerators and air purifiers for the same purpose. Nanosilver can be found in personal-grooming kits, female-hygiene products, beauty soaps, cleansers and fabric softeners (Luoma, 2008). Nanosilver spraymist products are used to disinfect and deodorize surfaces in kitchens, bathrooms and baby clothes. It is used in cosmetics, lotions, creams, toothpastes, laundry detergents, soaps, surface cleaners, room sprays, toys, antimicrobial paints, home appliances, automotive upholstery, consumer electronics (e.g., cell phone covers), shoe insoles, brooms, food storage containers, tableware, slippers and shoe liners. Nanosilver is widely incorporated in textiles and fabrics such as outerwear, sportswear, underwear, socks, and bedding materials such as comforters, sheets and mattress covers (Luoma, 2008; Tolaymat *et al.*, 2010). More detailed information on consumer products containing nanosilver is available at the <http://www.nanoproject.org> website.

3.5 Medical Applications

Nanosilver has many medical applications including diagnosis, treatment, drug delivery, coating tools and medical devices. Nanosilver is used for coating medical tools and materials used in the areas of surgery, anesthesiology, cardiology and urology (Wijnhoven *et al.*, 2009). It is also incorporated in wound dressings, diabetic socks, scaffolds, sterilization materials in hospitals, medical textiles, medical catheters and contraceptive devices. Nanosilver is used in orthopedics in areas such as additives in bone cement, coating of implants for joint replacement and bone prostheses (Tolaymat *et al.*, 2010). Nanosilver is used in dentistry for making artificial teeth and in eye care for coating contact lenses (Senjen, 2007; Wijnhoven *et al.*, 2009). Advanced silver nanotechnologies are used to improve battery performance in next-generation active implantable medical devices. The use of nanosilver in combination with vanadium oxide in battery cell

components is one example of advanced silver nanotechnology that has resulted in improved cathode material homogeneity. Nanosilver is used for imaging of cell cancers (Boxall *et al.*, 2007). It is also used for treating dermatitis, ulcerative colitis and acne. By using wound dressings containing nanosilver, doctors found that not only did silver inhibit the growth of bacteria, the wounds actually healed more quickly (DiRienzo, 2006). Dressings have also been designed to release nanosilver slowly, depending on the presence of wound fluids. Nanosilver is used in diet supplements. One company's website recommends ingesting a teaspoon of silver colloid per day "to help maintain health," and one tablespoon four times per day to "help fortify the immune system" (Mesosilver® - Nanoparticle Colloidal Silver from Purest Colloids, Inc., Westhampton, NJ; <http://www.purestcolloids.com/mesosilver.htm>). Another website claims that "the number of people using colloidal silver as a dietary supplement on a daily basis is measured in the millions" (<http://www.silver-colloids.com/Tables/Experiment.PDF>). Silver nanoparticles are used as antibacterial/antifungal agents in a diverse range of applications including air sanitizer sprays, socks, pillows, slippers, face masks, wet wipes, detergent, soap, shampoo, toothpaste, air filters, coatings of refrigerators, vacuum cleaners, washing machines, food storage containers, cellular phones, and even in liquid condoms.

Silver has possible applications in the treatment of cancer (Asharani *et al.*, 2009). HIV-1 virus was reported to be inhibited from binding to the host cells through the use of silver nanoparticles (Elechiguerra *et al.*, 2005). Nanosilver is also used in drug delivery through plasmonic photoactivation of hollow polyelectrolyte-multilayer capsules incorporating silver nanoparticles and containing drug molecules (see Section 3.1.8).

3.6 Proposed and Projected Applications

All applications reported for bulk and ionic silver could be projected for silver nanoparticles, since nano-scale silver has more enhanced properties than the parent materials. Bulk silver is registered as a pesticide and is used in public hygiene. AmeriSwiss, a provider of public restroom equipment, is employing AgION-based silver ion antimicrobial products in a protective finish for door pulls and plates, which minimizes bacterial growth on the surface of the finished product. Bulk silver is being utilized in food processing and preservation. Commercial ice machines are using silver embedded hoses, clamps, pipe fittings, and in other places where

deposits (gunk) can build up and harbor bacteria. Meat processors are also using silver embedded tables, grinders, tools, refrigerators and hooks. Silver is also used in specialty packaging, occupational clothing worn by food processing workers, prevention of pathogen build-up in climate control systems and on the floors, walls, and ceilings of food processing and storage facilities. Silver is used to keep fruit, vegetables and cut flowers fresh while in transit. Silver is also used in wood preservation to withstand exposure to aggressive brown-rot fungus and resist molds. Home Water Purification Floatron, a small solar-powered ionization product, uses silver and copper ions to purify and soften water in swimming pools. Nanosilver is incorporated into nanocomposites and bimetallic nanoparticles; this will be an important gate for nanosilver applications. All of the previously mentioned applications can be projected for nanosilver, especially with advances in the nanomaterials industry (DiRienzo, 2006). Table 3.1 lists emerging applications of nanosilver in medical products.

Table 3.1: Emerging applications of nanosilver in medical products. Reprinted from *Nanotoxicology*, Vol. 3 (2), Wijnhoven, S.W.P., Peijnenburg, W.J.G.M., Herberts, C.A., Hagens, W.I., Oomen, A.G., Heugens, E.H.W., Roszek, B., Bisschops, J., Gosens, I., van de Meent, D., Dekkers, S., de Jong, W.H., van Zijverden, M., Sips, A.J.A.M., Geertsma, R.E., Nanosilver – a review of available data and knowledge gaps in human and environmental risk assessment, pp109-138, Copyright 2009 with permission from Informa Healthcare.

Medical domains	Examples	References
Anesthesiology	Coating of breathing mask Coating of endotracheal tube for mechanical ventilatory support	Patent -
Cardiology	Coating of driveline for ventricular assist devices Coating of central venous catheter for monitoring	- -
Dentistry	Additive in polymerizable dental materials Silver-loaded SiO ₂ nanocomposite resin filler	Patent Jia <i>et al.</i> , 2008
Diagnostics	Nanosilver pyramids for enhanced biodetection Ultrasensitive and ultrafast platform for clinical assays for diagnosis of myocardial infarction Fluorescence-based RNA sensing Magnetic core/shell Fe ₃ O ₄ /Au/Ag nanoparticles with tunable plasmonic properties	Walt, 2005 Aslan & Geddes, 2006. Aslan <i>et al.</i> , 2006 Xu <i>et al.</i> , 2007
Drug delivery	Remote laser light-induced opening of microcapsules	Skirtach <i>et al.</i> , 2006
Eye care	Coating of contact lens	Weisbarth <i>et al.</i> , 2007
Imaging	Silver dendrimer nanocomposite for cell labeling Fluorescent core-shell Ag@SiO ₂ nanoballs for cellular imaging Molecular imaging of cancer cells	Lesniak <i>et al.</i> , 2005 Aslan <i>et al.</i> , 2007 Tai <i>et al.</i> , 2007
Neurosurgery	Coating of catheter for cerebrospinal fluid drainage	Bayston <i>et al.</i> , 2007; Galiano <i>et al.</i> , 2007
Orthopedics	Additive in bone cement Implantable material using clay-layers with starch-stabilized silver nanoparticles Coating of intramedullary nail for long bone fractures Coating of implant for joint replacement Orthopedic stockings	Alt <i>et al.</i> , 2004 Podsiadlo <i>et al.</i> , 2005 Alt <i>et al.</i> , 2006 Chen <i>et al.</i> , 2006 Pohle <i>et al.</i> , 2007
Patient care	Superabsorbent hydrogel for incontinence material	Lee <i>et al.</i> , 2007
Pharmaceutics	Treatment of dermatitis Inhibition of HIV-1 replication Treatment of ulcerative colitis Treatment of acne	Bhol <i>et al.</i> , 2004; Bhol & Schechter, 2005 Elechiguerra <i>et al.</i> , 2005; Sun <i>et al.</i> , 2005 Bhol & Schechter, 2007 Patent
Surgery	Coating of hospital textile (surgical gowns, face mask)	Li <i>et al.</i> , 2006
Urology	Coating of surgical mesh for pelvic reconstruction	Cohen <i>et al.</i> , 2007
Wound care	Hydrogel for wound dressing	Yu <i>et al.</i> , 2007

4. Synthesis and Properties of Silver Nanomaterials

4.1 Methods of Synthesis

Knowledge of silver nanomaterials synthesis methods is important from an environmental perspective. This information allows for the identification of characteristics and morphologies of the produced silver nanomaterials that are crucial for a more focused approach when evaluating their environmental fate, transport and toxicity. Not all silver nanomaterials are the same and the characterization and morphology dictate physical properties such as solubility, diffusion behavior, and reactivity, which, in turn, dictate their environmental fate, transport, and toxicity. The immense leap in the applications of nanotechnology is mainly attributable to advances in the synthesis techniques of nanomaterials during the last two decades. The main challenge in nanomaterials synthesis is the control of their characteristics such as particle size distribution, shape, morphology, chemical composition and crystal structure. There are an extensive number of synthesis methods of silver nanoparticles that are readily available in the literature. All reported methods can be classified and categorized since they follow common approaches and the differences are limited to the specific reactants used and the reaction conditions. Categories such as top-down versus bottom-up, green versus non-green and conventional versus non-conventional have been reported.

4.1.1 Synthesis Categories

Top-down techniques rely on the generation of isolated atoms from the bulk materials using various distribution techniques. Physical methods such as milling or attrition, repeated quenching and photolithography are usually involved in the top-down strategies (Gao, 2004; Ju-Nam & Lead, 2008). Bottom-up techniques start with silver salt precursor (dissolved in solvent) that is reduced in a chemical reaction and the nanoparticles are formed through nucleation and growth (Tolaymat *et al.*, 2010). With the bottom-up strategies, the use of capping agents is crucial to control the particle size and shape, and to provide stability for the synthesized nanomaterials (Balan *et al.*, 2007). Figure 4.1 presents a schematic for the top-down versus bottom-up techniques.

Synthesis approaches can be classified as either green or non-green. Green approaches use environmentally friendly agents such as sugars and plant extracts to form and stabilize nanosilver (Sharma *et al.*, 2009). The weakness of the green approaches is the lesser amount of control a researcher or synthesizer has over the morphology of the produced nanosilver compared to the non-green methods.

Synthesis methods can also be grouped as conventional and unconventional methods.

Conventional synthesis methods include the use of citrate, borohydride, two phase (water-organic) systems, organic reducers, and inverse micelles in the synthesis process.

Unconventional methods include laser ablation methods, radiocatalytic methods, vacuum evaporation of metal and the Svedberg method of electrocondensation (Krutuyakov *et al.*, 2008).

The top-down or bottom-up approaches are commonly used to synthesize silver nanoparticles; typically, the bottom-up approaches involve wet chemistry techniques. It has to be mentioned that there is plenty of overlap between all the previously-mentioned categories. For instance, using plant extracts to synthesize nanosilver is a conventional/green/bottom-up synthesis method.

4.1.1.1 Top-Down versus Bottom-Up

Typical top-down fabrication techniques for the production of nanosilver are cutting, grinding and etching of a bulk piece of the material. Although small particle sizes ranging from 10 to 100 nm can be obtained, defects in the surface structure are likely to be present (Nikolaj *et al.*, 2006). The imperfection of the surface structure is one of the disadvantages that can significantly impact the properties of the manufactured nanoparticles. The bottom-up techniques overcome this deficiency by producing homogenous and stable nanosilver suspensions with the ability to tune their particle size and shape as well as functionalizing the nanosilver with capping agents that makes it suitable for specific applications. Top-down techniques are the methods of choice for the synthesis of highly complex structures. The scalability of production is an issue with the bottom-up techniques since not all methods are feasible for the massive production of enough quantities of nanomaterials for industrial use (Tolaymat *et al.*, 2010). Another concern with the wet chemical techniques is the accumulation of residual chemicals in the nanoparticles

suspension at the end of the synthesis processes. These impurities may have an impact on their applications in medicine, catalysis, microelectronics and sensing devices. The impurities usually include ionic silver since the reduction efficiency is not 100 % (El Badawy *et al.*, 2010). The presence of impurities disables the capability of determining the actual concentration of nanosilver in suspension. Most of the published scientific literature on synthesis of silver nanomaterials deals with the bottom-up (wet chemistry) techniques and thus more information is available for that method when compared to the top-down techniques (Tolaymat *et al.*, 2010). The reactants included in the wet chemistry techniques mostly contain a silver salt precursor, a reducing agent, a solvent and a capping agent. The morphology and surface chemistry of the synthesized silver nanoparticles are governed by the chemical nature of the capping agents, the molar ratio of that agent to the silver salt, the redox potential of the reducing agent, the stirring speed and the temperature of the synthesis reaction (Gulrajani *et al.*, 2008). Of all mentioned parameters, the concentration of the stabilizer has the highest influence. The solvent or the capping agents are occasionally used as reductants for the silver salt precursor (Tolaymat *et al.*, 2010).

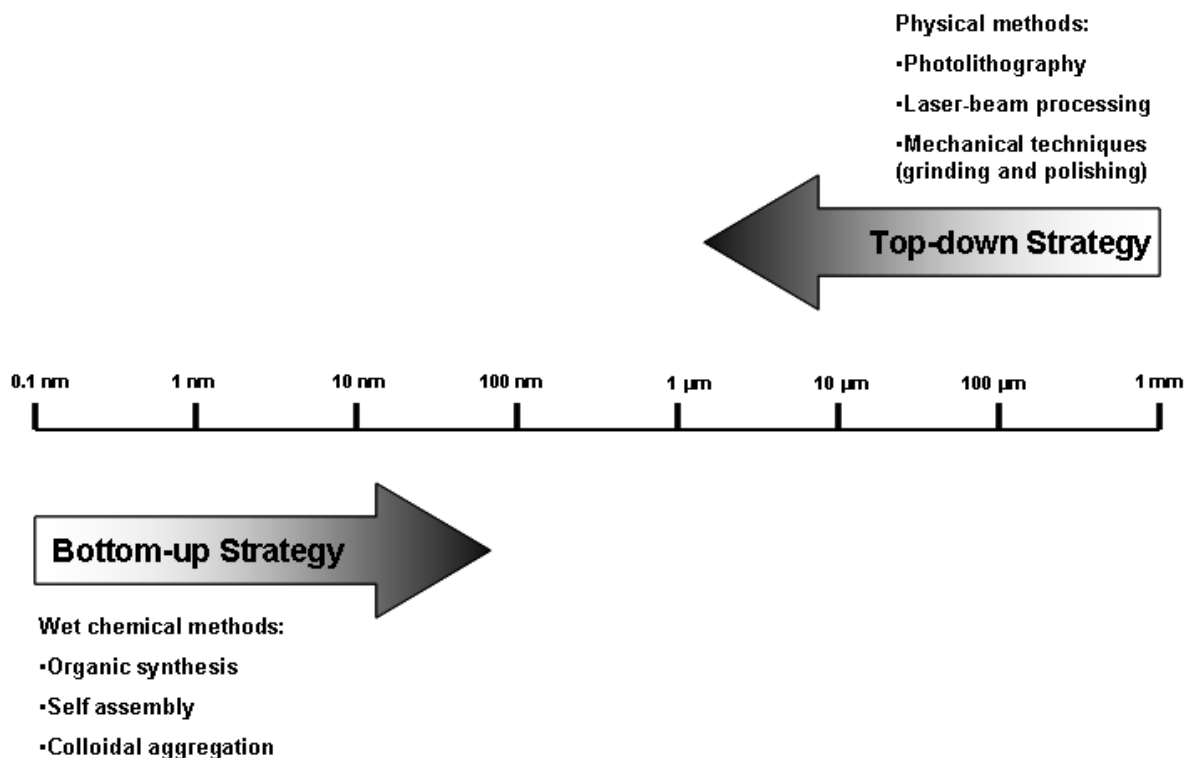


Figure 4.1: Top-down and Bottom-up synthesis approaches. Reprinted from Sci. Tot. Environ., Vol. 400 (1-3), Ju-Nam, Y. and Lead, J.R., Manufactured nanoparticles: An overview of their chemistry, interactions and potential environmental implications, pp396-414, Copyright 2008 with permission from Elsevier.

4.1.1.2 Synthesis Reactants in Bottom-Up Techniques

Silver nitrate (AgNO_3) is the most widely used silver ion precursor for the production of nanosilver. This is a result of its low cost and chemical stability compared to the other available silver salts. The use of silver nitrate makes it likely that nitrate (NO_3^-) will be the dominant anion associated with the silver nanomaterial synthesis processes. The reducing agents can refer to any chemical agents, plant extracts, biological agents or irradiation methods that provide free electrons to reduce silver ions and form silver nanoparticles. For the production of silver nanoparticles, various reducing agents are reported such as H_2 gas (Evanoff *et al.*, 2004), sodium borohydride (Lee & Meisel, 1982), hydrazine (Kim *et al.*, 2007), ethanol (Amendola *et al.*, 2007), ethylene glycols (Iyer *et al.*, 2007), Tollen's reagent (Fernandez *et al.*, 2008), ascorbic acid (Kashiwagi & Nakamoto, 2006) and aliphatic amines (Rao & Trivedi, 2006). Depending on the strength of the reducing agents, the particle size can be controlled. The strong and fast reducing agents cause the formation numerous silver seeds at the beginning of the synthesis

process, reducing the time of growth and preventing the formation of larger particles (Nikolaj *et al.*, 2006). Although many organic solvents are being used for the synthesis of nanosilver, water is the most frequently used solvent (Tolaymat *et al.*, 2010). An identified limitation to the use of water as a solvent is the difficulty to remove the stabilizer residues from the surface of the synthesized particles, leading to the use of organic solvents while synthesizing silver nanomaterials. This is observed when there is a need to produce relatively high particle concentrations coupled with predefined shapes and sizes (Dorjnamjin *et al.*, 2008; Yang *et al.*, 2005).

Solvents such as N, N-dimethyl formamide (DMF) and polyethylene glycol act as a reducing and capping agent. The capping agents are used to provide stability for the nanoparticles suspensions. By changing the ratio of the capping agent to the silver salt, different particle sizes and shapes are obtained. The molar concentration of the capping agent is usually significantly higher than that of the silver salt; this results in low nanoparticle concentrations. The capping agents include surfactants (such as sodium dodecyl sulfate [SDS]) or ligands and polymers that contain functional groups such as thiol (-SH), cyano (-CN), carboxyl (-COOH) and amine (-NH₂) groups that act as the stabilizer (Olenin *et al.*, 2008; Si & Mandal, 2007). Polymers are used extensively for the purpose of nanoparticles stabilization. The efficiency of a certain polymer is linked with the solvent properties. Good solvent permits the polymeric stabilizer attached to the particle surface to stretch away from the nanoparticles (Nikolaj *et al.*, 2006).

The selection of a capping agent may be driven by specific necessities. The catalytic properties of silver nanomaterials depend on the type of stabilizers used (e.g., Cetyltrimethylammonium bromide [CTAB] and SDS), which may decrease the adsorption of reactants to the silver surface (Jiang *et al.*, 2005). Citrate and polyvinylpyrrolidone are the most widely used stabilizers for nanosilver (Tolaymat *et al.*, 2010). Poly(N-isopropylacrylamide) (PNIPAM) is commonly used as a temperature-sensitive polymer that has a lower critical solution temperature (LCST). Below the LCST, PNIPAM is hydrophilic and soluble in aqueous solution, but upon raising the temperature above LCST, the polymer becomes hydrophobic and insoluble, and aggregates in solution. Because of this phenomenon, silver nanoparticles capped by (PNIPAM) allow for s

novel application that combine surface plasmon and thermal switching applications (Guo *et al.*, 2008).

4.1.2 General Discussion on Nanosilver Synthesis

Of the conventional methods for producing silver nanomaterials, the borohydride and citrate methods are the most common. The main reasons are the relatively high reactivity of sodium borohydride (compared to citrate and other reductants), moderate toxicity (compared to hydrazine and hydroxylamines) and greater lab safety when compared to hydrogen gas and other physical methods (i.e., dangerous steps involved in other synthesis methods such as pressurizing hydrogen at relatively high temperature are avoided while using sodium borohydride) (Krutyakov *et al.*, 2008). The size of the nanosilver produced is fairly small (1-15 nm). Sodium borohydride does not provide strong stability for the produced nanosilver and, therefore, stabilizing agents are needed to keep the nanoparticles in suspension. Citrate is a weaker reducing agent, and the reaction requires energy that is generally applied by heating the solution (Krutyakov *et al.*, 2008). The particle size produced by the citrate method is relatively larger, but the nanosilver suspensions are stable due to the presence of the citrate layer coating the nanoparticle surfaces.

Other conventional methods include biosynthesis, synthesis in two-phase systems, inverse micellar systems and polyol processes (Krutyakov *et al.*, 2008). Biosynthesis methods could be performed intra- or extracellularly. The extracellular methods are more advantageous because of the large scale processing and ease of control over the environment. Gurunathan *et al.* (2009) synthesized nanosilver extracellularly, through the reduction of silver ions by *Escherichia coli*. Many other organisms have been used for the synthesis of nanosilver such as *E. coli*, *Bacillus licheniformis*, *Aspergillus fumigatus* and *Klebsiella pneumoniae* (Gurunathan *et al.*, 2009). Synthesis in two-phase (water-organic) systems follows mainly the steps of the Brust-Schiffrin method that is generally used for making gold nanoparticles, but the process details are different for the two types of nanoparticles. The nanoparticles are prepared using reactants separated in two immiscible phases. The interphase between the two liquids controls the reaction between the metal salt precursor and the reducing agents. The process is also limited by the reactant transfer from aqueous to organic medium. Inverse micellar systems can be considered a set of nanoscale

chemical reactors formed by surfactant molecules. Two inverse emulsions are used in which one contains a silver salt dissolved in water and the surfactant contains the reducing agent. The diameter of the nanoparticles can easily be controlled by changing the molar ratio of water to surfactant.

The formation of nanoparticles occurs in four main stages: 1) coalescence of water cores of colliding micelles, 2) chemical reaction between the components 3) nucleation and 4) the intermicellar growth of nanoparticles nuclei (Krutyakov *et al.*, 2008). Krutyakov *et al.*, 2008 produced stable silver nanoparticles suspensions (5-6 nm) through the reduction of silver nitrate by cetyltrimethylammonium bromide (CTAB) and non ionogenic surfactants. Polyol processes for the synthesis of silver nanoparticles are also common. Polyvinyl alcohol (PVA) is used to reduce the ionic silver to form metallic nanosilver; PVA also acts as a stabilizer for the silver nanoparticles that are produced (Gautam *et al.*, 2007).

Organometallic methods for nanosilver synthesis are also reported (Fernández *et al.*, 2008) such as treating the complex $\text{NBu}_4[\text{Ag}(\text{C}_6\text{F}_5)_2]$ with AgClO_4 to form the nanoparticles precursor $[\text{Ag}(\text{C}_6\text{F}_5)]$, which is further converted to 10 nm silver nanoparticles by adding hexadecylamine (HDA) and refluxed for 5 h in toluene (Figure 4.2). Nanosilver embedded paint has been obtained in a single step by the naturally occurring free radicals generated *in situ* during the drying process of oils (Kumar *et al.*, 2008). These radicals reduce silver salts without the use of any external reducing or capping agents. This system does not require an external heating source since there is no heating step included. It is nontoxic and inexpensive. The nanosilver embedded paint shows excellent antimicrobial impact against Gram-positive human pathogens (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*).

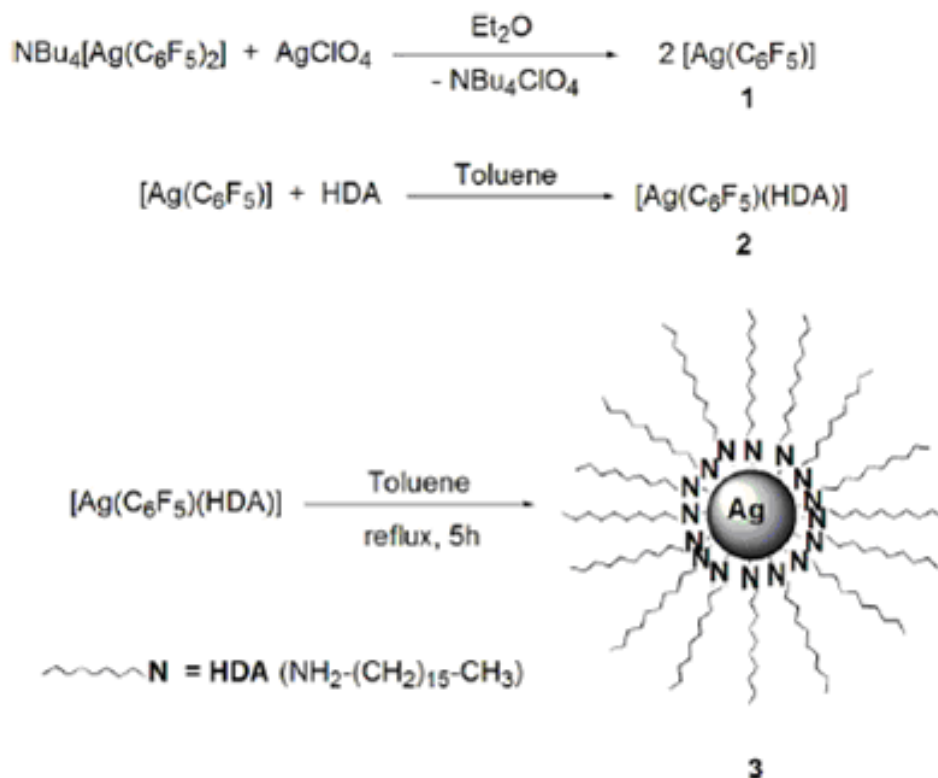


Figure 4.2: Schematic for an organometallic approach of synthesis of silver nanoparticles. Reprinted from *Nanotechnology*, Vol. 19:185602, Fernández, E.J., Barrasa, J.C., Laguna, A., Lopez de-Luzuriaga, J. M., Monge, M., Torres, C., The preparation of highly active antimicrobial silver nanoparticles by an organometallic approach. 6 pp. Copyright Institute of Physics (the “Institute”) and IOP Publishing 2009.

As a response to the concerns of potential risks associated with the use of the hazardous substances included in the synthesis processes, many researchers are utilizing a wide range of environmentally friendly solvents, reducing agents and stabilizing agents for the synthesis of nanosilver. The green synthesis methods follow wet chemistry procedures with the added benefit of using environmentally friendly reactants. For instance, spent mushroom substrate has been used to reduce ionic silver to nanosilver and further provides stabilization to nanosilver suspensions (Vigneshwaran, Kathe, *et al.*, 2007). Fatty acids such as cholic, stearic, palmitic and lauric acids are used as green agents for the formation and stabilization of silver nanoparticles (Rao & Trivedi, 2006). The USEPA strongly recommends using those techniques but there are limitations as a result of the difficulty in controlling the particle morphologies and the scalability issues.

Plant extracts (such as tea and coffee) and microorganisms such as bacteria, yeast, fungi and actinomycetes are also used for the reduction of silver ions to produce nanosilver (Tolaymat *et al.*, 2010). The characteristics of the produced nanoparticles can be manipulated by controlling parameters such as pH, temperature, substrate concentration and the exposure time to the substrate (Mohanpuria *et al.*, 2008).

4.2 Silver Nanocomposites and Bimetallic Nanoparticles

When silver nanomaterials are integrated into polymer matrices to form nanocomposites or when they are combined with other metal nanoparticles acting as a shell or as a core to form bimetallic nanoparticles, a combination of useful properties is achieved. Nino-Martinez *et al.* (2008) report an enhancement in the antibacterial activity of silver nanoparticles when it is incorporated into an Ag/TiO₂ nanocomposite. Jiang *et al.* (2005) report that the successful catalytic reduction of specific dyes is achieved using silver nanoparticles incorporated on silica spheres. Because of its unique characteristics when in the form of metallic nanoparticles, silver is often used as a substrate for magnifying surface enhanced Raman spectroscopy (SERS). Using Ag/Au (core/shell) as a substrate for SERS provides stronger spectra than data obtained from using each individual nanoparticle (Pande *et al.*, 2007). Some nanomaterials incorporated with nanosilver such as multiwalled carbon nanotubes and metallic nanoparticles (Sn, Ru and Pd) have been reported (Britz & Glatkowski, 2010). The integration of silver into nanocomposites and bimetallic nanoparticles is continual. With each combination, silver is being utilized to generate nanoparticles with new characteristics. The environmental impacts of nanocomposites and bimetallic nanoparticles should be examined on a case by case basis.

4.3 Environmental Perspective

The characteristics of the chemicals involved in the synthesis will affect the fate, transport and toxicity of these nanomaterials in the environment. Tan *et al.* (2007) report that the use of sodium citrate as a reducing agent generates negatively charged silver nanomaterials, which may behave differently than the positively charged nanosilver generated using branched polyethyleneimine (BPEI). The stability of the produced nanoparticles suspensions indicates the

potential for mobility of these nanoparticles in the environment. One of the issues associated with the synthesis of silver nanoparticles that has to be considered in their risk assessment is the scalability and reproducibility of the synthesis techniques. Scalability is one of the major factors that limit the commercialization of nanoparticles. Some of the synthesis processes under specific reaction conditions are not reproducible if they are scaled up. This might indicate that massive production is not feasible and, therefore, trace amounts of these materials will be released into the environment. One of the techniques that are reported to be scalable is the use of spinning disc processing with continuous flow (Iyer *et al.*, 2007; Figure 4.3). With this technique, silver nanoparticles with flexibly tuned characteristics can be produced. Industrial, large-scale synthesis of silver nanoparticles uses controlled thermolysis of silver alkyl carboxylates to produce nanosilver without the use of solvents (Kashiwagi *et al.*, 2006).

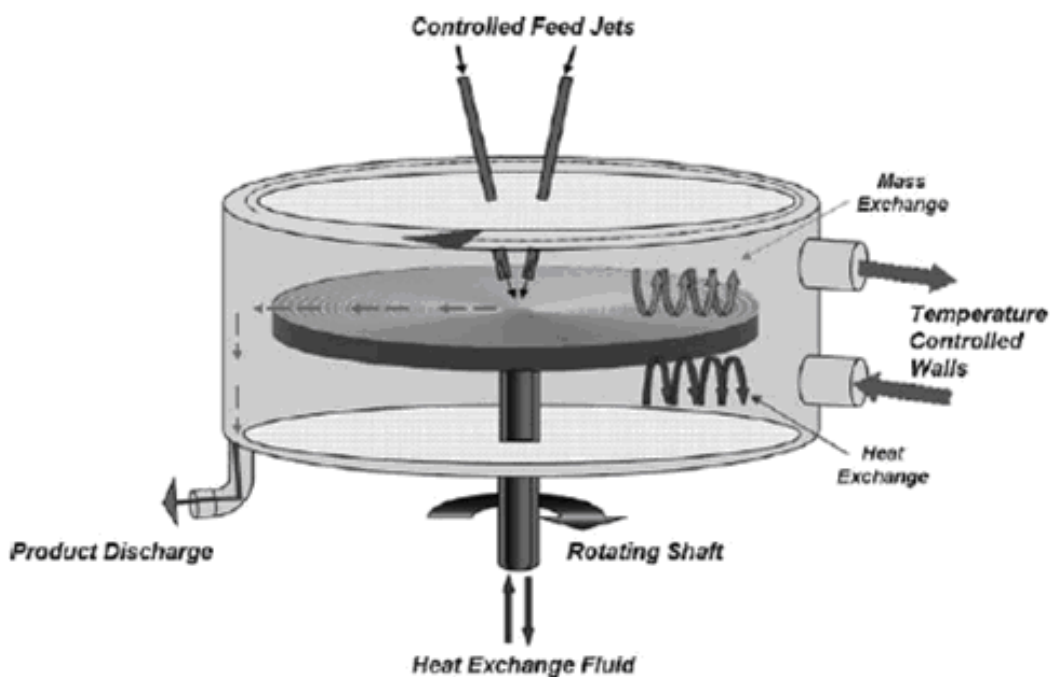


Figure 4.3: Schematic of a spinning disc processor for synthesis of silver nanoparticles. Reprinted from Lab Chip, Vol. 7, Iyer, K.S., Raston, C.L., Saunders, M., Continuous flow nanotechnology: manipulating the size, shape, agglomeration, defects and phases of silver nanoparticles, pp1800-1805, Copyright 2007 with permission from RSC Publishing.

4.4 Characteristics of the Silver Nanomaterials Products

A wide range of sizes are reported for the synthesized silver nanomaterials; the dominant particle size ranges from 1-20 nm (Tolaymat *et al.*, 2010). This is expected to be the target size, especially since the use of nanoparticles, in general, is driven by the unique properties that are observed at the nanoscale level. The optimum combination of these properties is obtained for particles in the range of 3 to 10 nm in size (Olenin *et al.*, 2008). The reasons behind the emergence of these new properties are the high surface area to volume ratio as well as the quantum confinement effect (the electrons are squeezed into a small area) caused by the extremely reduced size. Cataleya (2006) reports that the highest toxic effect for nanosilver on rat alveolar macrophages is obtained for 15 nm size when compared to larger nanoparticles with 30 and 55 nm. Navalandian *et al.* (2008) report that silver nanoparticles with 1-10 nm size demonstrates interaction with HIV by inhibiting the virus from binding to the host cells. Smaller particle size does not always translate to an enhancement in the particle properties. SERS applications, for example, show enhanced signals with larger particle sizes (Dong *et al.*, 2007). The thermodynamic properties of silver nanomaterials (e.g., melting point, molar heat of fusion) are directly proportional to particle diameter (Luo *et al.*, 2008). That is the reason nanosilver (<10 nm) is utilized in the semiconductor industry as well as printed electronics products for its lower melting point. Silver nanoparticles are synthesized in various shapes. They can be grouped as 1D objects (e.g., thin films), 2D objects (e.g., nanowires and nanorods) and 3D objects (e.g., spheres). Spherical particles are the most prevalent among nanosilver particles (Tolaymat *et al.*, 2010). The shape of silver nanomaterials might have an impact on some of its properties. Triangular silver nanoparticles are found to pose stronger biocidal action against Gram-negative bacterium *E. coli* than the spherical and rod shaped nanoparticles (Pal *et al.*, 2007). This is attributed to the arrangement of the atoms within the crystal structure reported.

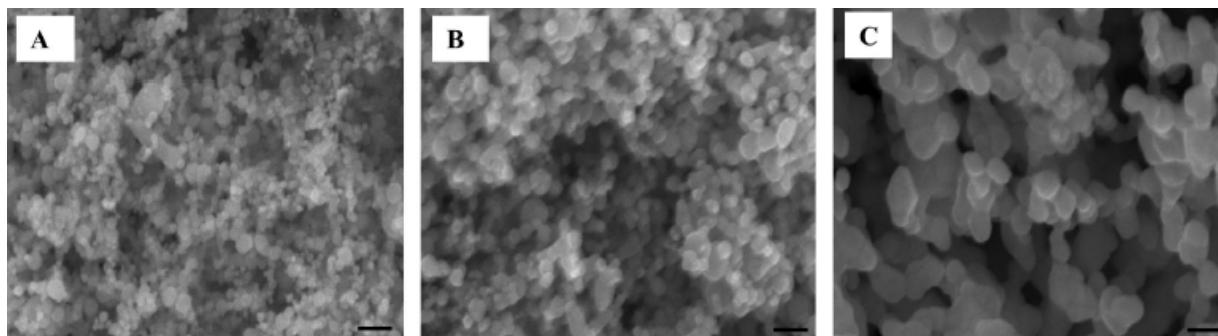


Figure 4.4: Characterization of silver nanoparticle size and morphology with scanning electron microscopy (SEM). Images of silver nanoparticles from Nanotechnologies, Inc., show the spherical morphologies that increase in average size between samples. (A) Ag-15nm, (B) Ag-30nm, and (C) Ag-55nm. Scale bars are 100 nm. Reprinted from *J. Phys. Chem. B*, Vol. 112 (43), Carlson, C., Hussain, S.M., Schrand, A.M., Braydich-Stolle, L.K., Hess, K.L., Jones, R.L., Schlager, J.J., Unique cellular interaction of silver nanoparticles: size dependent generation of reactive oxygen species, pp13608-13619, Copyright 2008 with permission from American Chemical Society.

Tolaymat *et al.* (2010) carried out a review on the bottom-up synthesis methods of nanosilver and the chemical agents used. Based on their review of approximately 200 scientific articles dealing with synthesis, it was concluded that the chemical agents used most frequently for the synthesis of nanosilver are sodium borohydride or sodium citrate as reducing agents and silver nitrate as the metal salt precursor dissolved in water as the solvent and citrate and PVP as the stabilizing agents. The most produced shape is spherical with a size of less than 20 nm. Table 4.1 provides a description of nanoparticles synthesized for general applications, while Table 4.2 provides a description of nanoparticles synthesized for specific applications. Table 4.3 provides a description of silver nanocomposite production, while Table 4.4 provides a description of bimetallic silver nanoparticle production. The four tables summarize the chemical agents used for the synthesis of nanosilver as well as the morphology of the produced nanoparticles. The tables also show which techniques are scalable and/or green and which of these techniques generate stable particles. Table 4.5 provides a list of acronyms used in Tables 4.1 – 4.4.

Table 4.1: Description of Evidence for Silver Nanoparticle Synthesis for General Applications (Adapted from Tolaymat *et al.*, 2010)

Ag Salt	Category *	Reducing Agent	Solvent	Stabilizing Agent	Size (nm)	Source	Notes
AgNO ₃	Sodium Borohydride reduction	NaBH ₄	Water	P(NIPAM-co-AA)	6-11	Dong <i>et al.</i> , 2008	s, sph
				P(NIPAM)	16-20	Guo <i>et al.</i> , 2008	s, sph
				PVA	Varies ^c	He & Kunitake, 2006	s, sph
				P(NIPAM)	Varies ^c	Morones & Frey, 2007	s, sph
				P(SS-co-M)	Varies ^c	Limsavarn <i>et al.</i> , 2007	s, sph
				OUDDPA	10	He <i>et al.</i> , 2007b	s, sph
				Trisodium Citrate	4.4 -5	Wang <i>et al.</i> , 1999b	s, sph
				Trisodium Citrate	NA	He & Zhu, 2008b	s, o
				NaBH ₄	12	Solomon <i>et al.</i> , 2007	s, sph
				Na-MPS or TOAB or CTAB or <i>n</i> -heptane	Varies ^c	Olenin <i>et al.</i> , 2008	s, sph
				Oleic acid	8	Seo <i>et al.</i> , 2006	s, sph
				MBA-AEPZ, MDA-AEPZ	Varies ^c	Sun <i>et al.</i> , 2008	s, sph
				PVP, Trisodium Citrate	< 3	Song <i>et al.</i> , 2008	o, s
				Carboxylates	Varies ^c	Wang <i>et al.</i> , 1999a	s, sph
				Water/ Chloroform	hexadecyl amine	Varies ^c	Kuila <i>et al.</i> , 2007
	Methanol	SBEHS	4	Setua <i>et al.</i> , 2007	s, sph		
	Ethanol	MBTZ	Varies ^c	Tan <i>et al.</i> , 2002	s		
		H-GSC	Varies ^c	Kuo <i>et al.</i> , 2004	s, sph		
	Ethanol/Toluene	Trisodium Citrate + Dodecylamine	7	Yang <i>et al.</i> , 2007a	s, sph		
	Toluene/BAm	dodecanoic acid (DDA)	7	Lee <i>et al.</i> , 2007	a, s, sph		
Sodium citrate reduction**	Trisodium citrate	Water	Trisodium citrate	varies	Lee & Meisel, 1982	S, sph	
	Trisodium Citrate & Nd:YAG laser	Water	Trisodium Citrate & Nd:YAG laser**	Varies ^c	Pyatenko <i>et al.</i> , 2007	s, sph	
	Trisodium Citrate & UV irradiation ^d	Water	Trisodium Citrate & UV irradiation ^d	NA	Jia <i>et al.</i> , 2006	s, sph	
Irradiation reduction	UV irradiation ^d	Water	Laponite	100-120	Yang & Huang, 2007	s	
		Ethanol	Poly-methylmethacrylate	5-8	Courrol <i>et al.</i> , 2007	s, sph	

Table 4.1 continued

Ag Salt	Category	Reducing Agent	Solvent	Stabilizing Agent	Size (nm)	Source	Notes
AgNO ₃	Irradiation reduction	UV irradiation ^d + BPEI	Water	UV irradiation ^d + BPEI	Varies ^c	Tan <i>et al.</i> , 2007	s, sph, o
		UV irradiation (Arg) + TSA	Water	NA	Varies ^c	Yang <i>et al.</i> , 2007c	a, sph
		γ- irradiation ^d	Water	PVP	Varies ^c	Li <i>et al.</i> , 2007b	a, o, s
			Acetic Water	oligochitosan GlcN	5-15	Long <i>et al.</i> , 2007	s, sph
			Chitosan	4-5	Chen <i>et al.</i> , 2007b	s, sph, o	
		Microwave irradiation ^d	Ethylene Glycol	PVP	Varies ^c	Navaladian <i>et al.</i> , 2008	o, s, sph
	E irradiation ^d	Water	PVA	Varies ^c	Bogle <i>et al.</i> , 2006	s, sph	
	Organisms and plant extract reduction	Enterobacteriaceae	Water	NA	52.5	Shahverdi <i>et al.</i> , 2007	g, sph
		Bacterium Bacillus licheniformis		Bacterium Bacillus licheniformis	50	Kalimuthu <i>et al.</i> , 2008	g, s, sph
		Enzyme nitrate reductase		Enzyme nitrate reductase	10-25	Kumar <i>et al.</i> , 2007	g, s, sph
		Fusarium semitectum		Fusarium semitectum	10-60	Basavaraja <i>et al.</i> , 2008	g, s, sph
		<i>Trichoderma asperellum</i>		<i>Trichoderma asperellum</i>	13-18	Mukherjee <i>et al.</i> , 2008	a, g, s, sph
		Fungus Aspergillus flavus		Fungus Aspergillus flavus	8.92	Vigneshwaran <i>et al.</i> , 2007a	a, g, s, sph
		Verticillium (Fungi)		Verticillium (Fungi)	25	Mohanpuria <i>et al.</i> , 2008	g
		Aspergillus fumigatus (Fungus)		Aspergillus fumigatus (Fungus)	5-25		
		Emblica Officinalis (Plant)		Emblica Officinalis (Plant)	10-20		
		Pelargonium graveolens (Plant)		Pelargonium graveolens (Plant)	16-40		
		Pseudomonas stutzeriv(Bacterium)		Pseudomonas stutzeriv(Bacterium)	Up to 200		
		Capsicum annum L. extract		Capsicum annum L. extract	10	Li <i>et al.</i> , 2007a	g, sph
		Spent mushroom substrate		Spent mushroom substrate	30.5	Vigneshwaran <i>et al.</i> , 2006a	a, g, s, sph
Geranium leaf extract		Geranium leaf extract		16-40	Shankar <i>et al.</i> , 2003	g, s, sph	

Table 4.1 continued

Ag Salt	Category	Reducing Agent	Solvent	Stabilizing Agent	Size (nm)	Source	Notes	
AgNO ₃	Organisms and plant extract reduction	Aloe vera Extract	Water	Aloe vera Extract	15.2	Chandran <i>et al.</i> , 2006	g, sph	
		heparin (polysaccharide)		heparin (polysaccharide)	20	Huang <i>et al.</i> , 2004	a,g, s, sph	
		green tea (Camellia sinensis) extract		green tea (Camellia sinensis) extract	NA	Vilchis-Nestor <i>et al.</i> , 2008	g, s, sph	
	Amines reduction	linear polyethylenimine	Water/Cyclohexane	linear polyethylenimine	Varies ^c	Sun & Luo, 2005	s, sph	
		Dodecylamine & Formaldehyde		Dodecylamine & Formaldehyde	4	Chen & Wang, 2008b	s, sph	
		Octadecylamine		Octadecylamine	SDS or CTAB	4.7	Wang <i>et al.</i> , 2008a	a, sph
		Alkylamines (<i>n</i> -Dodecylamine (C ₁₂ NH ₂), <i>n</i> -octadecylamine)		Alkylamines (<i>n</i> -Dodecylamine (C ₁₂ NH ₂), <i>n</i> -octadecylamine)	Alkylamines (<i>n</i> -Dodecylamine (C ₁₂ NH ₂), <i>n</i> -octadecylamine)	Varies ^c	Kashiwagi <i>et al.</i> , 2006	s, sph, o
		Alkylamine		Alkylamine	Alkylamine	2.9-5.3	Kashiwagi <i>et al.</i> , 2006	a,s, sph, o
		Poly(allylamine) (PAAm)		Water	Poly(allylamine) (PAAm)	4.4	Sardar <i>et al.</i> , 2007	s, sph
	DMF reduction	DMF	Water	PVP	5-12	Muthuswamy <i>et al.</i> , 2007	a, ,o s, sph	
				PMMA	10	Deng <i>et al.</i> , 2008	s, sph	
				---	20-75	He <i>et al.</i> , 2007a	o	
				PVP	Varies ^c	Yang <i>et al.</i> , 2007b		
	Ethylene Glycol reduction	EG	EG	PVP	Varies ^c	Bregado-Gutierrez <i>et al.</i> , 2008	s, sph	
					Varies ^c	Kim <i>et al.</i> , 2006	s, sph	
					Varies ^c	Wiley <i>et al.</i> , 2007	o, s	
	Hydrazine reduction	Hydrazine	Water	SDS	10-20	Choi <i>et al.</i> , 2005	s, sph	
		Hydrazine	Toluene	---	6	Kim <i>et al.</i> , 2007	o	
hydrazine hydrate		Water/Cyclohexane/ IAA	SDS microemulsion	6.5	Zhang <i>et al.</i> , 2008	s, sph		

Table 4.1 continued

Ag Salt	Category	Reducing Agent	Solvent	Stabilizing Agent	Size (nm)	Source	Notes
AgNO ₃	Ascorbic acid reduction	Ascorbic acid	Water	CTAB	35	Slawinski & Zamborini, 2007	o
				SDS	Varies ^c	Chaudhari <i>et al.</i> , 2007	o
				Daxad 19	Varies ^c	Sondi <i>et al.</i> , 2003	a, o, s
				Soluble starch	Varies ^c	Iyer <i>et al.</i> , 2007	a, g, s, sph
				P-4PV			
	Aldehyde reduction	Formaldehyde	Water	PVP	Varies ^c	Hsu & Wu, 2007	o, s
			Water/Ethanol	Thiosalicylic acid	8	Wu & Hsu, 2008a	o, s, sph
		Formaldehyde or TEAm or DEFAM	Benzene or Toluene	Stearic acid or Palmitic acid or lauric acid	25-40	Rao & Trivedi, 2006	s, sph
		Acetaldehyde	Water	---	67	Wang <i>et al.</i> , 2007	o, s
	Hydrogen gas reduction	H ₂ Gas	Water/Toluene	PVA/PVP/Starch	Varies ^c	Hasell <i>et al.</i> , 2007	s, sph
	Sugars reduction	D-glucose	Water	HTAB	Varies ^c	Yu & Yam, 2005	o
		Fructose, Glucose, Sucrose		Fructose, Glucose, Sucrose	Varies ^c	Panigrahi <i>et al.</i> , 2005	g, s, sph
	Other reducing agents	PVP	Water	PVP	Varies ^c	Hoppe <i>et al.</i> , 2006	s
		PVA		PVA	10-30	Fernandez <i>et al.</i> , 2008	s, sph
		Vitamin E		NA	3-14	Zhang & Lakowicz, 2006a	g, s, sph
		Vitamin B ₂ (Riboflavin)		Vitamin B ₂ (Riboflavin)	6.1	Nadagouda & Varma, 2008	g, s, sph
		Glycyl glycine		Glycyl glycine	NA	Yang <i>et al.</i> , 2006b	s
		NaBH(OAc) ₃		di-HCF ₆ /Sc CO ₂	3-5.2	Shervani <i>et al.</i> , 2007	s, sph
		Oligopeptide (NH ₂ -Leu-Aib-Trp-OMe)		Oligopeptide (NH ₂ -Leu-Aib-Trp-OMe)	13.6	Si & Mandal, 2007	s, sph
Genamin T020		NA		3-9.6	Faure <i>et al.</i> , 2003	o, sph	
WPU		NA		NA	Shen <i>et al.</i> , 2007	---	
Sodium carboxymethyl cellulose		Sodium carboxymethyl cellulose		15	Chen <i>et al.</i> , 2008c	g, s, sph	

Table 4.1 continued

Ag Salt	Category	Reducing Agent	Solvent	Stabilizing Agent	Size (nm)	Source	Notes
AgNO ₃	Other reducing agents	Soluble Starch	Water	Soluble Starch	10-34	Vigneshwaran <i>et al.</i> , 2006b	g, s, sph
		Poly(2,6-dimethyl-1,4-phenylene oxide)	Chloroform/methanol	Poly(2,6-dimethyl-1,4-phenylene oxide)	Varies ^c	Dorjnamjin <i>et al.</i> , 2008	s, sph
		Poly(<i>o</i> -methoxyaniline)	Water/Chloroform	Poly(<i>o</i> -methoxyaniline)	Varies ^c	Dawn <i>et al.</i> , 2007	s, sph
		Auto reduction of AgTh	Chloroform/Ethanol	NA	16.1	Mishra <i>et al.</i> , 2007	a, o
		N ¹ ,N ² -diphenylbenzamidine	Ethanol	N ¹ ,N ² -diphenylbenzamidine	10-30	Sharma <i>et al.</i> , 2007	s, sph
		benzyl mercaptan & Ultrasound irradiat. ^d		benzyl mercaptan & Ultrasound irradiat. ^d	1.7-10.4	Yang & Li, 2008	s, sph
		Superhydride	Ethanol/Toluene	DTC10	2.5-5	Tong <i>et al.</i> , 2006	s, sph
		Amphiphilic Polyester	Benzene	Amphiphilic Polyester	< 20	Voronov <i>et al.</i> , 2007	s, sph
		t-BuONa-activated NaH	THF	t-BuONa-activated NaH	3.3	Lee <i>et al.</i> , 2007	a, s, sph
		NEt ₃	NEt ₃	Carboxylate group	Varies ^c	Yamamoto <i>et al.</i> , 2006	s, sph
		Trioctylphosphine	Trioctylphosphine	Trioctylphosphine	6-10	Chen <i>et al.</i> , 2007c	s, sph
		Thermal decomposition of (AgCO ₂ (CF ₂) _n CF ₃)	N.A	Thermal decomposition of (AgCO ₂ (CF ₂) _n CF ₃)	5	Lee <i>et al.</i> , 2002	o, s
Ag ₂ SO ₄	Sodium borohydride reduction	NaBH ₄	Water	AOT & SDS	20-90	Mandal <i>et al.</i> , 2005	a, s
	Irradiation reduction	X ray irradiation ^d	Water	NA	28	Remita <i>et al.</i> , 2007	o, s, sph
	Other reducing agents	Polyoxometalates	Water	Polyoxometalates	40	Zhang <i>et al.</i> , 2007	g, s, sph
3-pentadecylphenol		Water/Chloroform	3-pentadecylphenol	11.8	Swami <i>et al.</i> , 2004	o, s, sph	
AgClO ₄	Ethylene glycol reduction	Ethylene Glycol	Water/EG	Ethylene Glycol	Varies ^c	Jacob <i>et al.</i> , 2007	s, sph
		dihydroxy benzenes	Water	dihydroxy benzenes	30	Jacob <i>et al.</i> , 2008	s, sph

Table 4.1 continued

Ag Salt	Category	Reducing Agent	Solvent	Stabilizing Agent	Size (nm)	Source	Notes
Ag ₂ O	Hydrogen gas reduction	H ₂ Gas	Water	N NA	50-200	Merga <i>et al.</i> , 2008	---
Ag acetate	Irradiation reduction	e- irradiation ^d	Water	NA	15-40	Li <i>et al.</i> , 2005	sph
Ag mysitrte	DMF reduction	DMF	DMF	NA	10	Khanna <i>et al.</i> , 2008	sph

* Categorized based on the type of reducing agent

Table Key: a = Scalable Technique, g = Green Synthesis, c = Varies by varying reaction conditions, d = Irradiation is not the reducing agent but it assist providing free electrons for reduction, s = Stable, sph = Particle shape is: Sphere, o = Other Chemicals or compounds are included in the synthesis process, NA = Not Applicable

Reprinted from Sci. Tot. Environ., Vol. 408 (5), Tolaymat, T., El Badawy, A., Genaidy, A., Scheckel, K., Luxton, T., Suidan, M., An evidence-based environmental perspective of manufactured silver nanoparticle in syntheses and applications: A systematic review and critical appraisal of peer-reviewed scientific papers, pp999-1006, Copyright 2010 with permission from Elsevier.

Table 4.2: Description of Evidence for Silver Nanoparticles Synthesis for Specific Applications (Adapted from Tolaymat *et al.*, 2010)

Ag Salt	Category	Reducing Agent	Solvent	Stabilizing Agent	Size (nm)	Source	Notes	Applications
AgNO ₃	Sodium borohydride reduction	NaBH ₄	Water	NA	5	Dubas & Pimpan, 2008b	s, sph	Biosensor for herbicides
				NA	8	He & Zhu, 2008b	o, s, sph	Bio-sensors
				NA	10,18,23	Radziuk <i>et al.</i> , 2007	sph	Remote Opening of Polyelectrolyte Microcapsules
				PVP, PAM	3-5	Murthy <i>et al.</i> , 2008	o, s	Antibacterial
				NA	Varies ^c	Nino-Martinez <i>et al.</i> , 2008	o, sph	Antibacterial Ag/TiO ₂
				NaBH ₄	1-8	Xiong <i>et al.</i> , 2008	o, s, sph	Colorimetric sensor to probe histidine
				NA	< 10	Liu <i>et al.</i> , 2005	o, sph	Nanocatalyst: CO oxidation
				Trisodium Citrate-PVP	Varies ^c	Junior <i>et al.</i> , 2003	S	Effect of Ag NPs on the photophysical properties of cationic dyes
				NA	5	Zhang & Lakowicz, 2006a	s, sph	DNA Detection
				Trisodium Citrate	18	Wiley <i>et al.</i> , 2007	s, sph	Enhanced IR Absorption Spectroscopy
				NaBH ₄	N.A	Pergolese <i>et al.</i> , 2004	s, sph	Adsorption of 1,2,3-Triazole for SERS
				Trisodium Citrate	< 60	Wen <i>et al.</i> , 2007	s, sph	Growth of Human Fibroblasts on Ag NPs
				NaBH ₄	15	Jiang <i>et al.</i> , 2007a	s, sph	Growth of selenium nanowires
				Trisodium Citrate	Varies ^c	Hasell <i>et al.</i> , 2007	o, s, sph	Effect of shape on antibacterial activity
NA	2	Lee <i>et al.</i> , 2007	s, sph	Interaction with HIV-1				

Table 4.2 continued

Ag Salt	Category	Reducing Agent	Solvent	Stabilizing Agent	Size (nm)	Source	Notes	Applications
AgNO ₃	Citrate reduction	Trisodium Citrate	Water	Trisodium Citrate	20-30	Thompson <i>et al.</i> , 2008	sph	Detection of DNA sequences
					160	Hua <i>et al.</i> , 2007	o, s, sph	Mass spectrometry of peptides
					24-30	Thomas <i>et al.</i> , 2007	o, sph	Antibacterial (hydrogel–silver nanocomposites)
					8	ZhiLiang <i>et al.</i> , 2007	s, sph	Determination of fibrinogen in human plasma
					60-80	Xu <i>et al.</i> , 2004	s, sph	Detection of Nitro-explosives
					48	Balaguera-Gelves, 2006	s, sph	Probing of Membrane Transport in Living Microbial Cells
					NA	Doering & Nie, 2002	s, sph	Surface Enhanced Raman (SERS)
					10	Wenseleers <i>et al.</i> , 2002	s, sph	Absorption of Dyes
					10	Kim <i>et al.</i> , 2004	s, sph	Decomposition of Benzyl phenyl sulfide (BPS) absorbed on the surfaces of Ag NPs
					NA	Xingcan <i>et al.</i> , 2003	s, sph	Interaction between serum albumins and Ag NPs
					NA	Gryczynski <i>et al.</i> , 2006	s, sph	Depolarized light scattering from Ag NPs
					NA	Wu <i>et al.</i> , 2005	s, sph	Adsorption for p-Hydroxybenzoic Acid on Ag Nanoparticles

Table 4.2 continued

Ag Salt	Category	Reducing Agent	Solvent	Stabilizing Agent	Size (nm)	Source	Notes	Applications
AgNO ₃	Citrate reduction	Ferric ammonium citrate (FAC)	Water	Ferric ammonium citrate (FAC)	Varies ^c	Rashid & Mandal, 2007	s	Catalysis
		Triammonium citrate (TAC)		Triammonium citrate (TAC)				
		Trisodium citrate dihydrate		Trisodium citrate dihydrate				
	Irradiation reduction	PMA & UV irradiation ^d		NA	8	Dubas & Pimpan, 2008b	s, sph	Ammonia Sensing
	Organisms and plant extracts reduction	Fusarium oxysporum	Water	Fusarium oxysporum	20-50	Carlson, 2006	sph	Antibacterial
		P. chrysosporium (white rot fungus)	Water	P. chrysosporium (white rot fungus)	50-200	Vigneshwaran <i>et al.</i> , 2007b	a, g, s, sph	Antibacterial
	DMF reduction	DMF	DMF	NA	80	Lakowicz & Sabanayagam, 2007	sph	DNA microarrays by metal-enhanced fluorescence
				β -cyclodextrin	Varies	Patakfalvi <i>et al.</i> , 2008	s, sph	Interaction with pollutant gases NO and SO ₂
				DMF				
		DMSO		DMSO	Varies	Patakfalvi <i>et al.</i> , 2008	s, sph	
	Ethylene Glycol reduction	EG	EG	PVP	60-100	McLellan <i>et al.</i> , 2006	s	Surface Enhanced Raman (SERS)
		PEG & H ₂		PEG & H ₂	8-10	Yan <i>et al.</i> , 2006	g, sph	Catalysis
	Hydrazine reduction	Hydrazine	Water	PVP	10-35	Gulrajani <i>et al.</i> , 2008	s, sph	Antibacterial
Other reducing agents	PAA	EG	PAA	30	Lee <i>et al.</i> , 2006	a, s, sph	Silver Inks for printed electronics	
	Co-sputtering of Ag and silica produce Ag NPs embedded in silica				2.5-5.2	Mishra <i>et al.</i> , 2007	sph	Medical diagnostics

Table 4.2 continued

Ag Salt	Category	Reducing Agent	Solvent	Stabilizing Agent	Size (nm)	Source	Notes	Applications
AgNO ₃	Other reducing agents	H ₂ , UHMWPE	NA	NA	NA	Morley <i>et al.</i> , 2007		Biomedical applications
		ATS	Ethanol	NA	5	Roldan <i>et al.</i> , 2007	o, sph	Applications in Waveguides
		Thermal decomposition	Water	NA	Varies ^c	Shim <i>et al.</i> , 2008	o, s, sph	Ink-jet printing
		HEPES Buffer		HEPES Buffer	5-20	Muthuswamy <i>et al.</i> , 2007	s, sph	Interaction with HIV-1
		Direct current of 100 μA	Water	NA	NA	Parfenov <i>et al.</i> , 2003		Enhanced Fluorescence from Fluorophores
AgClO ₄	Irradiation reduction	UV irradiation ^d	Water	NA	6	Henglein & Meizel, 1998	o, sph	Adsorption of Organosulfur Compounds on Ag NPs
	Other reducing agents	Thermal Decomposition	Diethyl Ether/Toluene	HDA	10	Fernandez <i>et al.</i> , 2008	s	Antibacterial
Ag ₂ O	Hydrogen gas reduction	H ₂ gas	Water	NA	30	Merga <i>et al.</i> , 2007	s, sph	Catalysis
Ag(acetate)	Hydrazine reduction	Phenylhydrazine	toluene	NA	< 10	Li <i>et al.</i> , 2005	s, sph	Printed electronics
	Other reducing agents	PAMAM / photoreduction	Water	NA	NA	Balogh <i>et al.</i> , 2001		Antibacterial silver-PAMAM dendrimer nanocomposite
Ag(ethx)	DMF reduction	DMF	DMF	Trisodium Citrate	Varies ^c	Patakfalvi <i>et al.</i> , 2007	o, s, sph	Catalysis
AgBF ₄	DMF reduction	DMF	Ethanol / DMF	NA	18-27	Hamouda <i>et al.</i> , 2007	o, sph	Olefin/paraffin separation in the oil industry
NA	Other reducing agents	Alkyd Paint	NA	NA	12-14	Kuo <i>et al.</i> , 2004	g, s, sph	Antimicrobial paints

Table Key: a = Scalable Technique, g = Green Synthesis, c = Varies by varying reaction conditions, d = Irradiation is not the reducing agent but it assist providing free electrons for reduction, s = Stable, sph = Particle shape is: Sphere, o = Other Chemicals or compounds are included in the synthesis process, NA = Not Applicable

Reprinted from Sci. Tot. Environ., Vol. 408 (5), Tolaymat, T., El Badawy, A., Genaidy, A., Scheckel, K., Luxton, T., Suidan, M., An evidence-based environmental perspective of manufactured silver nanoparticle in syntheses and applications: A systematic review and critical appraisal of peer-reviewed scientific papers, pp999-1006, Copyright 2010 with permission from Elsevier.

Table 4.3: Description of Evidence for Silver Nanocomposites (Adapted from Tolaymat *et al.*, 2010)

Ag Salt	Category	Reducing Agent	Solvent	Template	Size (nm)	Source	Notes	Nanocomposite
AgNO ₃	Sodium borohydride reduction	NaBH ₄	Cyclohexane	AOT-TMH Isostearic acid	2-4	Anand <i>et al.</i> , 2006	s, sph	Ag- AOT-TMH
				MCAP, APS	20	Jiang <i>et al.</i> , 2007b	sph	Ag/polypyrrole
				Graphite Oxide		Cassagneau & Fendler, 1999	s, sph	Ag-GO
	Citrate reduction	Trisodium Citrate	Water	o-Toluidine	20	Reddy <i>et al.</i> , 2008	s, sph	POT-Ag
	Irradiation reduction	HMEM, UV irradi.	NA	SDS	3	Lu <i>et al.</i> , 2007	s, sph	PS-PAA-Ag
		Ultrasonic Irrad ^d	DMF	PAN- <i>b</i> -PEG- <i>b</i> -PAN, PEA	12-20	Lei & Fan, 2006	s, sph	---
		UV Irrad. ^d	Water	Rubber Latex	4-10	Abu Bakar <i>et al.</i> , 2007	s, sph	---
	DMF reduction	DMF	DMF	MoS ₂ / citrate	Varies ^c	Patakfalvi <i>et al.</i> , 2008	s, sph	Ag/C, Ag/ MoS ₂
		DMA		PSS	Varies ^c	Huang & Wen, 2007	o, sph	PDMA-PSS-Ag
	Hydrazine reduction	Hydrazine	Water/Ethanol	PVP, MWCNT	18	Dai <i>et al.</i> , 2007	sph	Ag/MWCNT
	Aldehyde reduction	Formaldehyde gas or Irrad. ^d	Water	TSPP	Varies ^c	Liu <i>et al.</i> , 2008	sph	---
		Formaldehyde	Ethanol	TSD, TEOS, NH ₃ .H ₂ O	2-8	Chen <i>et al.</i> , 2007d	o, sph	Ag/SiO ₂
	Sugar reduction	Glucose	Water	[C14mim]BF ₄	25	Wu & Hsu, 2008	sph	Ag/C
	Other reducing agents	Calcination 500 °C	Water/Ethanol	Titanium(IV) isopropoxide	Varies ^c	Hamal & Klabunde, 2007		Ag/(C, S)-TiO ₂
		Heat, (NH ₄) ₂ S ₂ O ₈	Water	MBAm, acrylamide	4-7	Saravanan <i>et al.</i> , 2007	s, sph	Ag/polyacrylamide
Thermolysis, H ₂ gas		NA	Monolithic silica aerogel	Varies ^c	Ameen <i>et al.</i> , 2007	s	---	

Table 4.3 continued

Ag Salt	Category	Reducing Agent	Solvent	Template	Size (nm)	Source	Notes	Nanocomposite
AgNO ₃	Other reducing agents	NH ₄ VO ₃	Water	NA	Varies ^c	Li <i>et al.</i> , 2008	sph	---
		Thermolysis	MIBK	PMMA, HFA	Varies ^c	Deshmukh & Composto, 2007		Ag-HFA-PMMA
Ag(acetate)	Sodium borohydride reduction	NaBH ₄	Water	PAH & PAA	Varies ^c	Logar <i>et al.</i> , 2007	sph	---
	Amine reduction	Oleylamine	Toluene		6-12	Zhang <i>et al.</i> , 2006b	s, sph	Ag-Fe ₃ O ₄
AgBF ₄	Other reducing agents	Poly(n-hexylsilane)	Cyclohexane	Poly(n-hexylsilane)	8.4	Shankar <i>et al.</i> , 2003	s, sph	---
AgCF ₃ SO ₃	Irradiation reduction	UV Irrad. ^d	THF	Pvf-co-ctf-gp-omec	4-8	Koh <i>et al.</i> , 2008	sph	---
				Pvf-co-ctf-gp-omec	5	Lee <i>et al.</i> , 2008	sph	---
AgSbF ₆	Other reducing agents	DMPA, UV irradi.	---	EEC	NA	Sangermano <i>et al.</i> , 2007	s, sph	Ag/Epoxy

Table Key: a = Scalable Technique, g = Green Synthesis, c = Varies by varying reaction conditions, d = Irradiation is not the reducing agent but it assist providing free electrons for reduction, s = Stable, sph = Particle shape is: Sphere, o = Other Chemicals or compounds are included in the synthesis process, NA = Not Applicable

Reprinted from Sci. Tot. Environ., Vol. 408 (5), Tolaymat, T., El Badawy, A., Genaidy, A., Scheckel, K., Luxton, T., Suidan, M., An evidence-based environmental perspective of manufactured silver nanoparticle in syntheses and applications: A systematic review and critical appraisal of peer-reviewed scientific papers, pp999-1006, Copyright 2010 with permission from Elsevier.

Table 4.4: Description of Evidence for Bimetallic Silver Nanoparticles (Adapted from Tolaymat *et al.*, 2010)

Ag Salt	Category	Reducing Agent	Solvent	Stabilizing Agent	Size (nm)	Source	Notes	Bimetal (core/shell)
AgNO ₃	Sodium borohydride reduction	NaBH ₄	Water/toluene	Sodium Citrate-Dodecylamine	Varies ^c	Yang <i>et al.</i> , 2005	o, s, sph	Ag/Au
			Methanol	1,10-phenanthroline	10	Jiang <i>et al.</i> , 2007b	o, s, sph	Sn/Ag
		NaBH ₄ & Ascorbic acid	Water	Trisodium Citrate & TDDM	36.3	Bakshi <i>et al.</i> , 2007	o, s, sph	Au/Ag
	Organisms and plant extracts reduction	Neem (<i>Azadirachta indica</i>) leaf broth	Water	Neem (<i>Azadirachta indica</i>) leaf broth	NA	Shankar <i>et al.</i> , 2003	o, s, sph	Au/Ag
	Sugars reduction	Fructose	Water	Fructose	10	Panigrahi <i>et al.</i> , 2005	o, sph	Au/Ag
	Other reducing agents	Beta-cyclodextrin (β -CD)	Water	Beta-cyclodextrin (β -CD)	13 & 11	Pande <i>et al.</i> , 2007	o, s, sph	Au/Ag, Ag/Au
Ag ₂ SO ₄	Hydrazine reduction	Hydrazine	NA	N.A	Varies ^c	Damle <i>et al.</i> , 2002	o, sph	Ag/Pd
AgClO ₄	Other reducing agents	Ethanol	Ethanol/Water	PVP	Varies ^c	Toshima <i>et al.</i> , 2005	o, s, sph	Ag/Pt, Ag/Rh
Ag acetate	Amine reduction	Dodecylamine, OLEA	Dodecylamine	NA	6	Wang <i>et al.</i> , 2008b	o, sph	Ag/TiO ₂

Table Key: a = Scalable Technique, g = Green Synthesis, c = Varies by varying reaction conditions, d = Irradiation is not the reducing agent but it assist providing free electrons for reduction, s = Stable, sph = Particle shape is: Sphere, o = Other Chemicals or compounds are included in the synthesis process, NA = Not Applicable

Reprinted from Sci. Tot. Environ., Vol. 408 (5), Tolaymat, T., El Badawy, A., Genaidy, A., Scheckel, K., Luxton, T., Suidan, M., An evidence-based environmental perspective of manufactured silver nanoparticle in syntheses and applications: A systematic review and critical appraisal of peer-reviewed scientific papers, pp999-1006, Copyright 2010 with permission from Elsevier.

Table 4.5: List of Acronyms used in Tables 4.1 to 4.4 (Adapted from Tolaymat *et al.*, 2010)

Acronym	Definition	Acronym	Definition	Acronym	Definition
P(MMA)	Polymethyl Methacrylate	THF	Tetrahydrofuran	tiopronin	<i>N</i> -(2-Mercaptopropionyl)glycine
P(NIPAM-co-AA)	poly[(<i>N</i> -isopropylacrylamide)-co-(acrylic acid)]	E irradi.	Electron Irradiation	PAH	polyallylamine
CTAB	Cetyltrimethylammonium bromide	PVP	poly(vinyl pyrrolidone)	PAA	polyacrylic acid
EG	Ethylene Glycol	PVA	Poly-vinyl Alcohol	TEOS	Tetraethylorthosilicate
SBEHS	Sodium bis(2-ethylhexyl)sulfosuccinate	SDS	Sodium Dodecyl Sulfate	PMMA	poly(methyl methacrylate)
TSPP	5,10,15,20-tetra-4-oxy(2-stearic acid) phenyl porphyrin	DMF	<i>N,N</i> -dimethyl formamide	HTAB	<i>n</i> -Hexadecyltrimethylammonium bromide
P(SS-co-M)	poly(styrene sulfonate-co-maleic)	TEAm	Triethanolamine	MIBK	methyl-isobutylketone
Na-MPS	Sodium 3-mercaptopropanesulfonate	DEFAM	Dimethylformamide	DMA	2,5-dimehtoyaniline
TOAB	Tetraoctylammonium bromide	IAA	Isoamylalcohol	PSS	poly(styrene sulfonic acid)
di-HCF ₆ di-HCF ₆	(sodium bis(1 <i>H</i> ,1 <i>H</i> ,7 <i>H</i> ,dodecafluoroheptyl)sulfosuccinate)	AgTH	silver thiolate	TSD	<i>N</i> -[3-(Trimethoxysilyl)dropyl]ethylene diamine
MBA-AEPZ	poly (N,N0-methylene bisacrylamide N-aminoethyl piperazine)	MBTZ	2-mercaptobenzothiazole	Genamin T020	mixture of fatty ethoxylated amines
MDA-AEPZ	poly(N,N0-dodecyl diacrylamide N-aminoethyl piperazine)	BAm	<i>n</i> -butylamine	H-GSC	Hydrophile-Grafted Silicone Copolymers
AOT	Aerosol OT (sodium <i>bis</i> -2-ethylhexyl-sulfosuccinate)	P-4PV	poly(4-vinylpyridine)	HFA	1,1,1,5,5,5-hexafluoroacetylacetone
Daxad 19	sodium salt of naphthalene sulfonate formaldehyde condensate	Arg	arginine	OUDDPPA	O/-di(10-undecene)dithiophosphonic acid)
ATS	Aminosilane N-[3-(trimethoxysilyl)propyl]diethylenetriamine]	TSA	Tungstosilicate acid	UHMWPE	ultra-high molecular weight polyethylene
TDDM	Trimethylene- 1,3-bis (dodecyldimethylammonium bromide)	PAA	poly(acrylic acid)	PTA	phosphotungstic acid [PTA, H3(PW12O40)
HMEM	2-[<i>p</i> -(2-Hydroxy-2-methylpropiophenone)]-ethyleneglycol methacrylate	PAM	poly(acrylamide)	MBAm	<i>N,N</i> '-methylene-bisacrylamide
AOT-TMH	Sodium bis(3,5,5-trimethyl-1-hexyl)sulfosuccinate	DMSO	Dimethyl sulfoxide	POT	poly(o-toluidine)

Table 4.5: continued

Acronym	Definition	Acronym	Definition	Acronym	Definition
([C14mim]BF ₄)	imidazolium ionic liquid 1-n-tetradecyl-3-methylimidazolium tetrafluoroborate	PEG	polyethylene glycol	DMPA	2,2-dimethoxy-2-phenyl acetophenone
EEC	3,4-epoxycyclohexylmethyl-3,4-epoxycyclohexanecarboxylate	BSA	Bovine Serum Albumin	MCAP	Mercaptocarboxylic acid Pyrrole
Pvf-co-ctf-gp-omec	poly(vinylidene fluoride-co-chlorotrifluoroethylene)-graftpoly (oxyethylene methacrylate)	PAMAM	poly(amidoamine)	MWCNT	Multiwalled carbon nanotube
APS	ammonium peroxydisulfate	PMA	poly(methacrylic acid)	GO	Graphite Oxide
WPU	Segmented copolymer of waterborne polyurethane	OLEA	Oleic acid		
PAN- <i>b</i> -PEG- <i>b</i> -PAN, PEA	Polyacrylonitrile-block-poly(ethylene glycol)-block-Polyacrylonitrile	NEt ₃	triethylamine		

Reprinted from Sci. Tot. Environ., Vol. 408 (5), Tolaymat, T., El Badawy, A., Genaidy, A., Scheckel, K., Luxton, T., Suidan, M., An evidence-based environmental perspective of manufactured silver nanoparticle in syntheses and applications: A systematic review and critical appraisal of peer-reviewed scientific papers, pp999-1006, Copyright 2010 with permission from Elsevier.

4.5 Characterization Methods, Detection and Speciation

The increasing use of manufactured silver nanoparticles makes their release into the environment inevitable. Various environmental streams such as water systems, ground water, wastewater and landfills might be influenced. Methodologies for the detection and characterization of silver nanoparticles are thus essential in order to investigate their fate, transport and toxicity. Current literature is focused on the manufacture or toxicity testing of nanosilver. There is a lack of information on characterization and detection, especially in environmental samples. There is a need for developing methods to measure nanosilver concentration, size, shape, surface charge, crystal structure, surface chemistry and surface transformations. Some important questions to answer: Does nanosilver leach from consumer products? If so, in what form? Is it aggregated or still in the nanoscale size? What are its surface properties and chemistry? Does nanosilver dissolve to form ionic silver with time or under different conditions such as pH? What is the speciation of silver? Is nanosilver toxic? What are the toxicity mechanisms? Under what conditions do the mechanisms occur? Do particles aggregate inside the testing media? Do particles aggregate inside the tested cells? In order to answer these questions, characterization tools are needed.

There are plenty of techniques to characterize pure silver nanomaterials. The majority of these techniques will, of course, work for pure nanomaterials suspensions, but there are still challenges to fully characterize these pure suspensions. One of the major challenges that inhibit characterization is that it is difficult to determine the speciation of silver compounds in the nanosilver suspensions. The mechanisms of formation and the interactions between the capping agents and the surface of silver nanoparticles are not fully understood. The characterization and detection of nanomaterials become more complicated when dealing with real environmental samples such as wastewater or landfill leachate, which contains a variety of different impurities, colloids and organic materials. The sample matrix will make it almost impossible to determine aggregation state or speciation. Based on an extensive search of the literature, there are no methods available to answer any of the questions mentioned in the previous paragraph for environmental samples. It may be necessary to use a combination of many different known techniques to detect and estimate nanomaterials in the environment. This section will highlight

conventional techniques that are currently available for characterization and detection in hopes that researchers can make modifications to existing methods or combine two or more methods to help characterize the nanosilver in environmental samples. Table 4.6 (at the end of the chapter) provides a summary of these techniques and the limitations of each technique.

At the end of the synthesis processes, nanomaterials suspensions usually contain residual chemicals and reaction byproducts. The ratio of the capping agent to silver ions is usually high in order to achieve stabilization, leaving an excess of capping agents in the synthesized nanoparticle suspensions. Another major impurity that is present is residual ionic silver that is not reduced to metallic silver. El Badawy *et al.* (2010) report that the conversion efficiency for ionic silver to the metallic form is not always 100%. The presence of ionic silver in the nanoparticles suspension has two major impacts. First, it will prohibit the ability to determine the concentration of nanosilver in suspension. The reason is that the conventional methods for determining the silver concentration in a sample rely on digesting silver in concentrated acid and then analyzing the total amount of silver using Inductively Coupled Plasma (ICP) or Atomic Absorption (AA) Spectroscopy. The concentration of the nanosilver can not be determined since it completely dissolves to ions during the digestion step. Another possibility for determining the nanosilver concentration in pure nanosilver suspensions is using UV-Vis spectroscopy. Nanosilver has a distinct SPR peak in the UV-Vis region (Amendola *et al.*, 2007). In order to construct a calibration curve to determine the concentration of silver nanoparticles in unknown samples, a stock solution of known concentration of the specific nanoparticles suspension is prepared. Some of the nanosilver in the stock solution will break down to form silver ions, which again fails to address the problem of having a method to determine the nanosilver concentration in a suspension containing silver ions. Second, the toxicity of nanosilver cannot be accurately determined because silver ions are also toxic. During toxicity tests, it will not be clear which compound is the source of toxicity. The contradictory results obtained by Buzea *et al.* (2007) from toxicity of nanosilver compared to silver ions supports that point. This leads to the conclusion that methods for quantifying ionic silver in nanosilver suspensions are needed or otherwise purification methods for removal of ionic silver and other chemical impurities are crucial.

4.5.1 Methods for Measuring Ionic Silver in Nanosilver Suspensions

Conventional methods exist for determining ionic silver concentration in solution without acid digestion and analysis of total metals. These methods might be utilized with refinement to measure ionic silver concentration in environmental samples containing nanosilver. A mass balance can then be obtained and the difference between the total metal concentration as determined by ICP or AA analysis and the silver ion concentration is the nanosilver concentration. Titration methods using sodium chloride or sodium thiocyanate have been commonly used for the detection of silver ion concentration (Kraemer & Stamm, 1924). These are colorimetric methods that depend on color change when the endpoint is reached. Since the nanosilver suspensions are colored, the endpoint cannot be easily determined leading to incorrect quantification of ionic silver. The use of ion selective electrodes (ISE) for measuring free silver ions in solutions and in nanosilver suspensions is also reported (Benn & Westerhoff, 2008). The limitations of using this technique are possible matrix interference and the need for ionic strength adjustment for the tested suspensions in order to match the ionic strength of the calibration standards. Dileen *et al.* (2008) developed a method for measuring silver ions in solution at concentration as low as 0.2 g/L in surface water using square-wave stripping voltammetry at a carbon paste electrode. Sample matrix was found to be an important factor affecting the measurement results. Because of these matrix effects, the peak shape of the voltammograms varied and multiple stripping peaks for silver were observed. These techniques were not tested previously for measuring the ionic silver in nanosilver suspensions or environmental samples containing both the ionic and the metallic forms, but they might be a good start.

4.5.2 Methods for Isolating Ionic Silver from Nanosilver Suspensions

Using ion exchange resins is a conventional method for isolating silver ions present in the wastewater of film processing plants. This water is rich in ionic silver. Cerjan-Stefanovi *et al.* (1991) used Ionenaustauscher I, II and IV resins to isolate ionic silver and then eluted it using highly concentrated solutions such as nitric acid or sodium hydroxide. To date, there are no reported studies for testing this technique for isolating ionic silver from nanosilver suspensions. Another method that was reported to clean nanoparticle suspensions is centrifugation and washing. The samples are centrifuged under specific conditions (e.g., 15000 rpm for 20 min, [Benn & Westerhoff, 2008]). The supernatant is removed and replaced with clean water and this

process is repeated until clean nanosilver suspensions are obtained. Theoretically, the supernatant is free of nanosilver and other impurities and by analyzing the supernatant using ICP or AA, the ionic silver can be determined. This procedure is not proven to be accurate and is time consuming. It is also not scalable for large quantities of purified nanosilver suspensions. Dialysis can also be used to purify nanosilver suspensions or environmental samples. In this method, dialysis tubing with a specific size is used to separate particles from ions. It could be a suitable purification technique but is time consuming, and the accuracy level is not controlled (Sweeny *et al.*, 2006). None of the techniques mentioned in this paragraph are proven, and researchers should investigate and improve these techniques for isolating nanosilver from silver ions.

One of the promising techniques for purification of nanoparticle suspensions is the use of ultrafiltration membranes. Hollow fiber tangential flow filtration was successfully used for the purification of gold nanoparticles from both organic and inorganic impurities (Sweeny *et al.*, 2006). It is an efficient, rapid and scalable alternative to traditional methods of nanoparticle purification such as ultracentrifugation, ion exchange resins and dialysis. In this technique, the membrane pore size determines the retention or transmission of solution components. The permeate contains the rejected impurities and the retentate contains the nanoparticles. The nanoparticle suspension is continuously recirculated through the membrane while it is exchanged with clean buffers (such as water) until the impurities are completely removed. This technique is more efficient and scalable compared to the other separation techniques. Dialysis is effective in removing excess salts but leaves free ligands in the solution. Size exclusion chromatography is useful for removing both salts and free ligands; but, the nanoparticles tend to irreversibly precipitate on the chromatography supports, leading to decreased yields at the end of the process (Sweeny *et al.*, 2006). Sweeney *et al.* (2006) developed a purification method using diafiltration to overcome the limitation of the other purification techniques, which yields highly pure nanoparticles compared to other methods.

4.5.3 Novel Detection and Characterization Techniques for Environmental Samples

Using an electrospray atomizer coupled to a scanning mobility particle sizer (ES-SMPS) (Figure 4.5) is a novel method investigated by Elzey *et al.* (2009) for determining whether commercially

manufactured silver nanoparticles form agglomerates, behave as isolated particles, or dissolve as ions in neutral or low pH (6.5-0.5) aqueous suspensions. By drawing a liquid nanosilver suspension through a capillary tip, the electrospray generates an aerosol of nanoparticles and the droplets are sprayed into a stream of dry air. The exiting nanoparticles flow through a diffusion dryer and the remaining liquid diffused away from the nanoparticles is captured by silica beads. The nanoparticle aerosols are then charged using a radioactive source and enter the SMPS for size determination. The particles flow down the high voltage column of the nano differential mobility analyzer (DMA) where their size is sorted based on their electrical mobility. The nano-DMA allows only particles within a narrow mobility diameter to exit, which implies that the stream consists only of monodisperse particles that enter the ultrafine condensation particle counter (UCPC) for the determination of particle concentration. The combination of the nano-DMA and the UCPC allows the SMPS to provide particle size distribution of a nanosilver suspension. This is a promising technique; however, the analysis of environmental samples is still challenging because other colloidal particles may be present.

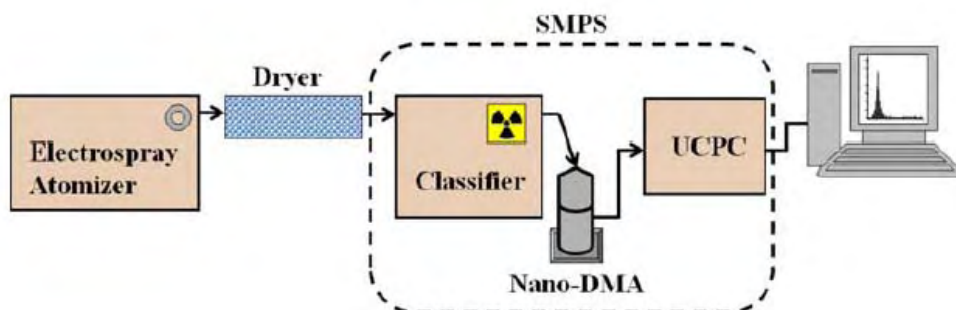


Figure 4.5: Schematic diagram of the Electro Spray-Scanning Mobility Particle Sizer (ES-SMPS) system Reprinted from J. Nanoparticle Res., doi:10.1007/s11051-009-9783-y, Elzey, S., Grassian, V.H., Agglomeration, isolation and dissolution of commercially manufactured silver nanoparticles in aqueous environments, Copyright 2009 with permission from Springer.

Flow Field Flow Fractionation (FIFFF) is probably the most promising technique for nanosilver characterization in environmental samples. Ranville (2009) suggested that the development of FIFFF-ICP-MS based tools will facilitate the future studies of nanoparticle behavior in environmental systems. The difference in diffusion coefficients of the colloids in a sample is the basis for size separation using FIFFF technique (Stolpe *et al.*, 2005). The sample is eluted through a thin channel by a laminar hydrodynamic flow. A cross flow is applied perpendicular to

the channel flow forcing the sample colloids towards the lower wall of the FIFFF channel which is covered by an ultrafiltration membrane with specific cutoff size. The ability of a colloid to diffuse against the cross flow determines the average position of the colloid. Smaller colloids are transported with faster flow elements in the channel flow (Figure 4.6). The hydrodynamic diameter of the colloids can be determined from the retention time and the channel dimensions. Using ICP-MS as an online detector for FIFFF allows its use for various applications especially with the low detection limits, high sensitivity and the ability of ICP-MS to detect large number of elements (Lyven *et al.*, 2003). FIFFF has also been coupled with other detectors such as UV, light scattering, graphite furnace atomic absorption spectroscopy (GFAA), transmission electronic microscopy (TEM), scanning electron microscopy (SEM) and atomic force microscopy (AFM).

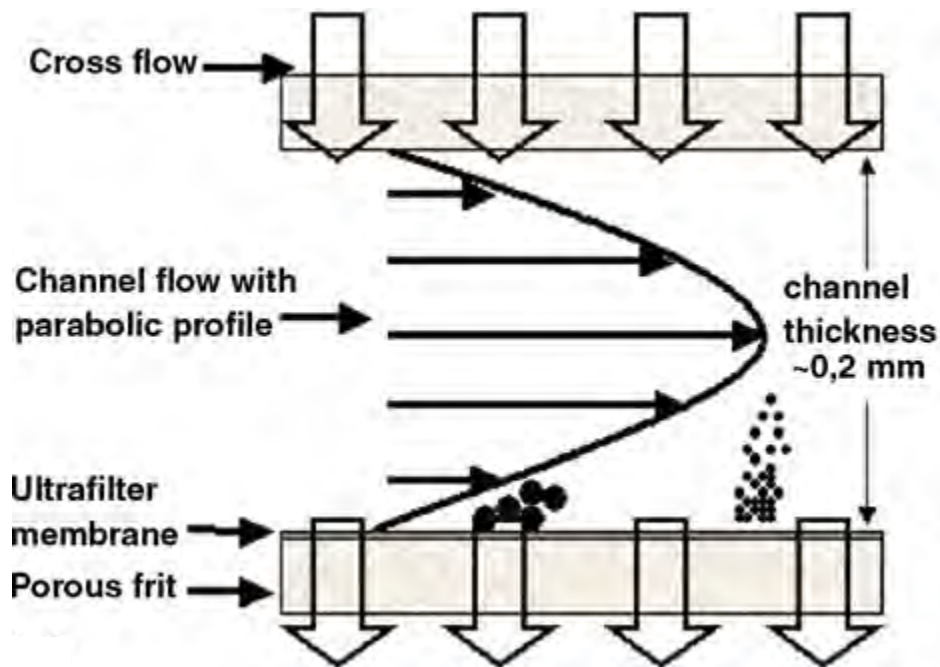


Figure 4.6: Cross section of a small part of the FIFFF channel. Reprinted from *Anal. Chim. Acta*, Vol. 535 (1-2), Stolpe, B., Hassellöv, M., Andersson, K., Turner, D.R. High resolution ICPMS as an on-line detector for flow field-flow fractionation; multi-element determination of colloidal size distributions in a natural water sample, pp109-121, Copyright 2005 with permission from Elsevier.

FIFFF ICP-MS has been used for the elemental characterization of colloidal materials (1-50 nm hydrodynamic diameters) in natural fresh water samples (Lyven *et al.*, 2003; Stolpe *et al.*, 2005). FIFFF-AFM was used to quantify the structure of the smallest size fraction (< 5 nm) of aquatic

colloids in surface water samples. Baalousha *et al.* (2006) used FIFFF-ICP-MS and TEM coupled to X-ray energy dispersive spectrometry (X-EDS) in series to characterize the physical properties, surface chemical composition and colloid-trace elements association for the colloidal materials present in surface water samples. Gimbert *et al.* (2007) used FIFFF to determine the particle size distribution of ZnO nanoparticles spiked in soil suspensions.

Although the literature lacks information on using FIFFF for nanosilver detection and characterization in environmental samples, it is promising and more attention should be paid to developing methodologies for nanosilver detection using this technique coupled with suitable detectors. The main challenge for this technique is sample preparation and preconcentration that increases the potential for particle aggregation.

A number of approaches have been proposed for the detection and characterization of nanoparticles in aquatic samples, including microscopy-based techniques, light scattering methods and several based on chromatography. The most promising of these involve the use of separation techniques such as field flow fractionation (FFF; Baalousha *et al.*, 2005a, 2005b, 2006a, 2006b; Chen & Beckett, 2001; Siepmann *et al.*, 2004; von der Kammer *et al.*, 2005a, 2005b), liquid chromatography (Arangoa *et al.*, 2000; Jiminez *et al.*, 2003; Saridara & Mitra, 2005; Sivamohan *et al.*, 1999; Song *et al.*, 2003, 2004; Wilcoxon *et al.*, 2000, 2001, 2005), size exclusion chromatography (SEC; Bolea *et al.*, 2006; Bootz *et al.*, 2005; Huve *et al.*, 1994; Krueger *et al.*, 2005; Liu & Wei, 2004; Wang *et al.*, 2006; Wei & Liu, 1999), gel electrophoresis (GE; Bruchert & Bettmer, 2005), and capillary electrophoresis (CE; Feick & Velegol, 2000; Lin *et al.*, 2007; Schmitt-Kopplin & Junkers, 2003; Schnabel *et al.*, 1997). Where these have been combined with element-specific detectors, such as ICP-MS (Giusti *et al.*, 2005; Hassellöv *et al.*, 1999; Helfrich *et al.*, 2006); Metreveli *et al.*, 2005; von der Kammer *et al.*, 2004), an additional degree of selectivity is gained, thereby, increasing the quality of the data obtained. To our knowledge, only FFFICP-MS has been successfully applied to samples in environmental media (e.g., Gimbert *et al.*, 2007; Stolpe *et al.*, 2005), the others having only been used on standards and/or simple solutions.

Tiede *et al.* (2009) evaluated the methods listed in this section, and came to the following conclusions as to the limitations of detection and characterization techniques in environmental samples: (a) complexity and time demands of FFF; membrane interactions and membrane cut-off effects, (b) limited size separation range of SEC columns, (c) intricate post-separation sample handling of gels, and (d) GE complex interpretation of migration times, i.e., distinguishing size-based from non-size-based interactions (CE and GE).

Table 4.6: Possible Conventional Characterization and Detection Techniques for Nanosilver

Technique	Function	Limitations
Particle size and morphology		
Transmission Electron Microscopy (TEM) & Scanning Electron Microscopy (SEM)	<p>TEM and SEM offer nanometer resolution for measuring nanoparticle size.</p> <p>High resolution TEM (HRTEM) provides more information regarding the lattice fringes and crystal structure.</p> <p>HRTEM sometimes provides information on the thickness of the capping agent layer.</p> <p>TEM, with appropriate detectors and software, also gives a range of other data such as fractal dimensions, elemental composition and chemistry (bonding and redox activity).</p> <p>Proper sample preparation is needed.</p>	<p>The sample has to be dehydrated before analysis. This might cause major structural changes for the nanoparticles.</p> <p>Drying may cause shrinkage for the capping agent molecule which affects the size measurement. The PVP molecule coating the nanosilver was reported to be shrunken after drying.</p> <p>For environmental samples containing nanosilver both techniques cannot be used since these techniques do not distinguish between different particles unless the analysis was performed after a separation step or there was an EDX attached to the instrument to differentiate various elements and it will still be difficult.</p> <p>In order to ensure no bias in the size analysis, several images representing diverse regions of the TEM grid have to be acquired and several hundreds to thousands of nanoparticles are taken. This helps to avoid artificial size separation or skewing as a result of drying effects or aggregation.</p>
Electrospray atomizer coupled to a scanning mobility particle sizer (ES-SMPS)	<p>ES-SMPS can be used to determine particle size distribution of AgNPs in suspensions.</p> <p>Samples are analyzed in aqueous form. This give the ES-SMPS advantage over TEM and SEM which needs a drying step that might impact the particle size.</p> <p>Samples can be centrifuged and the supernatant is further analyzed for the ionic silver using ICP or AA.</p>	<p>The presence of other types of colloidal particles might prohibit the applicability of this technique to determine the size distribution in environmental samples.</p>
BET (Brunauer-Emmett-Teller) analysis	<p>Provides the ability to measure the surface area of nanosilver.</p>	<p>Not applicable for environmental samples which contain various impurities.</p> <p>Samples have to be dry. Thus dehydration must be considered, as major structural changes can occur due to this process.</p>

Table 4.6: continued

Technique	Function	Limitations
Atomic Force Microscopy (AFM)	<p>The AFM is an instrument capable of measuring the topography of a given sample.</p> <p>A nanosized tip attached on a cantilever is traced over the sample and a 3D image of the sample topography is generated on a computer.</p> <p>AFM is used for investigating the size and shape of silver nanoparticles in three dimensions.</p> <p>AFM provides higher resolution than SEM.</p> <p>Dehydration is not a concern with AFM.</p> <p>Visualization can be achieved by using AFM under humid or fully hydrated conditions.</p>	<p>Sample preparation is a crucial issue in order to obtain the required results.</p> <p>The choice of the right tip is also a limitation to obtain good images.</p> <p>AFM is slower than SEM and the scanning area is smaller than SEM.</p> <p>Works for silver nanoparticle suspensions and most probably would not work for environmental samples.</p>
Environmental Scanning Electron Microscopy (ESEM)	<p>Allow imaging and determining the size of the samples under ambient conditions without the need for drying.</p>	<p>ESEM has difficulties analyzing the small nanoparticle sizes.</p>
Dynamic Light Scattering (DLS)	<p>DLS is a frequently used technique for the characterization of manufactured silver nanomaterials.</p> <p>Provides information on the hydrodynamic diameter of nanosilver in suspensions.</p> <p>Used frequently for the aggregation studies of manufactured nanomaterials under different environmental conditions.</p> <p>DLS is capable of measuring particles in the size ranges from a few nanometers to few micrometers</p>	<p>Not suitable for analysis of environmental samples.</p> <p>Larger particles or aggregates in the sample will ‘mask’ the presence of nanoparticles and cause difficulty in interpretation.</p> <p>Even if the nanoparticles can be detected, there is no method to ensure that the detected particles are nanosilver and not other colloids.</p> <p>Relatively high concentrations of nanosilver have to be present in order to measure the particle size.</p>
Flow Cytometry	<p>Provides quantitative information regarding the cellular interactions with nanoparticles.</p> <p>Provide information regarding the physical properties of the nanoparticles.</p> <p>Measures both light scattering and fluorescence from particles.</p>	<p>The reliance of this technique on light scattering may limit its applicability for detection of nanoparticles in environmental samples.</p>

Table 4.6: continued

Technique	Function	Limitations
X-ray Diffraction (XRD)	Determines the crystal structure of nanosilver and the elemental composition.	Sample has to be dried. It is a qualitative technique. For environmental samples detection limit might prohibit the ability to detect the presence of nanosilver with the relatively low concentrations expected to be present in the environment.
Centrifugation	Differential centrifugation can be used to separate particles in a suspension based on size. Centrifugation can be also used as a primary step for the determination of nanosilver concentration in a suspension using ICP or AA. Particles are removed by centrifugation and the supernatant is analyzed using ICP or AA. The concentration of nanosilver is determined from the mass balance between the total silver and the ionic silver concentration.	Not applicable for environmental samples as a result of the presence of other colloidal particles. There is no guarantee for complete removal of nanosilver from suspension after centrifugation. This limits the applicability for using this technique as a primary step for nanosilver concentration in aqueous suspensions.
Surface and Coating Layer Characterization Techniques		
X-Ray Photoelectron Spectroscopy (XPS)	XPS is a useful surface analysis technique for analyzing solid state samples. It gives information on oxidation states and elemental composition on a sample surface. XPS can be used to determine the concentration of ligands bound to the surface of a nanoparticle as well as determine how much free inorganic impurities are associated with the sample.	Silver can be detected in environmental samples but the oxidation state might be undistinguishable.

Table 4.6: continued

Technique	Function	Limitations
Thermal gravimetric analysis (TGA)	<p>TGA is a method for monitoring mass changes of a heated sample.</p> <p>It can be used to determine the percentage of volatile organic species associated with a sample or the amount of organic capping agent coating the nanoparticles surface.</p> <p>The temperature at which mass loss occurs as well as the mass loss profile can be correlated with the identity of the ligand and the strength of ligand binding.</p>	Not applicable for environmental samples, which usually contain lots of organics that will interfere with the organic coating on the nanosilver.
Nuclear magnetic resonance (NMR)	<p>NMR can be used primarily to confirm the composition of the ligand shell coating the nanoparticles and to identify any impurities in the suspension.</p> <p>Used to study the mechanism of the nanosilver formation and testing the type of intermediate compounds formed.</p> <p>Can be used to test the behavior of the polymers and organic coating at different solution chemistries</p>	It is a challenge to use with environmental samples, but more research should focus on this powerful technique, especially with advances of NMR techniques such as 2D NMR and solid state NMR.
X-ray Absorption Near Edge Structure (XANES)	<p>Of the various applications of XANES, it can be used to determine surface transformation of nanosilver in the presence of various anionic, cationic and organic compounds.</p> <p>Useful for aging studies.</p>	<p>Not easily accessible instrument.</p> <p>Powerful technique but research is needed to develop methodologies to handle the complexity of environmental samples</p>
Fourier Transformed Infrared Spectroscopy (FTIR)	Can be used to identify organic impurities in the nanoparticle suspensions and the functional groups on the nanosilver surface.	Not applicable for environmental samples
Zeta Potential Measurement	<p>Provide information on the surface charge of nanosilver.</p> <p>It is also used for the determination of point of zero charge (or isoelectric point), which is the pH at which the surface is neutralized (no charge).</p>	<p>Require relatively high concentration of nanosilver in the sample.</p> <p>Not applicable for environmental samples full of other colloidal particles. Thus the charge obtained will be a result of a combination of colloidal particles not only nanosilver.</p>

Table 4.6: continued

Technique	Function	Limitations
Measurement of nanosilver concentration		
Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) & Atomic Absorption spectroscopy (AA) and Graphite Furnace AA (GFAA)	These are major techniques used to quantify the total concentration of silver. The selection of AA or ICP versus GFAA depends on the concentration of silver in the sample. For extremely low concentrations, the graphite furnace is used.	Since the sample is digested using concentrated acids before analysis, the total silver in the sample is obtained. There is no ability to distinguish between ionic silver and nanosilver using these techniques unless the residual ions are removed.
UV-visible Spectroscopy	Excellent technique for measuring nanosilver concentration in pure nanosilver suspensions with relatively low detection limits ($\mu\text{g/L}$ range). SPR contains information on size, aggregation and surface chemistry since the peak shifts in response to change in these parameters.	The nanosilver has to be stable. SPR does not exist for aggregated form of nanosilver. Not applicable for environmental samples because of the possible interferences from the different compounds and probably other nanoparticles present in the sample.

5. Potential Magnitude of Silver Nanomaterial Utilization and Environmental Exposure

The environmental fate and effects of silver has received considerable attention since the early 1990s, in part, due to concerns about the potentially low silver effect levels that were determined on the basis of toxicity tests in laboratory water. It was expected that the environmental effect levels that were applicable to field conditions might differ markedly from levels measured in lab waters as a result of the effect of site-specific water quality on metal speciation and bioavailability. Between 1991 and 2006, a group of photographic manufacturers (referred to as the Silver Coalition and later as The Silver Council, and first managed by the National Association of Photographic Manufacturers (NAPM) during the early 1990s, then the Photographic Imaging and Manufacturing Association (PIMA) in 1997, and most recently by the International Imaging Industry Association (I3A) in 2001), working in cooperation with regulatory agencies, provided support for a coordinated \$10 million silver research and development (R&D) effort (called the Silver Research program, SRP) that was designed to gain an improved understanding of the environmental fate and effects of silver. The SRP consisted of investigations of the fate and effects of silver in various environmental media, including soil, water, and sediment. Chemistry investigations were undertaken to review existing analytical techniques and to develop new and improved methods to characterize and predict silver speciation in environmental media. Physiology and ecotoxicology studies were undertaken to better understand the mechanisms of toxicity and how effect levels varied with the characteristics of a particular medium. Finally, modeling studies were performed with the aim of developing improved methods for quantitatively evaluating silver speciation and the potential for effects. Paquin *et al.* (2007) published an overview of the SRP, which had resulted in more than 200 peer-reviewed publications and 300 conference presentations, in a report entitled ‘Overview of the Silver Research and Development Program (1991-2006): Advancing the Science of the fate and Effects of Silver in the Environment’.

Unlike silver, there is not much information on the fate and effects of nanosilver in the environment. This report attempts to list potential methods for synthesizing and using silver nanomaterials in commercial products, potential sources and routes of exposure, and toxicity

information generated to date. The following subsections describe potential routes of exposure from manufacturing consumer products that contain nanosilver.

5.1 Inventory of Silver Nanomaterials: Industrial and consumer products

The project on emerging nanotechnologies that was established in April 2005 as a partnership between the Woodrow Wilson International Center for Scholars and the Pew Charitable Trusts has published a fair amount of information on the inventory of consumer products containing nanomaterials including nanosilver. There is still a lack of information in regards to the characteristics of the particles (shape, size and surface chemistry), synthesis methods, production quantities, production losses, production consumption and the geographic distribution of these nanosilver-containing products in the US. These data are required in order to perform risk assessment and LCA. A survey was constructed to collect data to fill the gaps highlighted above. The survey was sent to a total of 122 companies, 53 of which are in the US producing various types of consumer products containing nanosilver, 32 are international companies that produce consumer products that contain nanosilver, and the remaining 37 US companies produce raw silver nanomaterials. A list of these companies in addition to the questionnaire is included in Appendix A. There was a very low response to the survey (0.8% of the companies answered the survey). The websites of the companies producing consumer products containing nanosilver (both domestic and international) were visited in the hope that information on production rates, source(s) of raw materials, amount used in each product, etc. could be found. Unfortunately, the amount of information available on the company websites was very limited. The most common information found on the websites is the concentration of nanosilver in the product. Since no new information was obtained, this section is a review of inventory information available in the literature.

In 2008, Fauss compiled the Silver Nanotechnology Commercial Inventory (SNCI) during an internship with the Emerging Nanotechnologies project (Fauss, 2008). A total of 65 companies (from 11 different countries) producing 240 products were listed (Appendix B): 214 were general commercial products and 26 were precursor products. The product information was obtained through the websites, product listings, email and/or phone conversations with customer service representatives. Out of 214 consumer products

containing nanosilver, 45% reported the nanoparticle size used in the product ranged from 0.3-250 nm. The average particle size of nanosilver used was 24 nm. Fauss (2008) reported the various forms of nanosilver incorporated in the surveyed consumer products and the results are presented in Figure 5.1. Products that are coated during the manufacturing process are listed under “coating”.

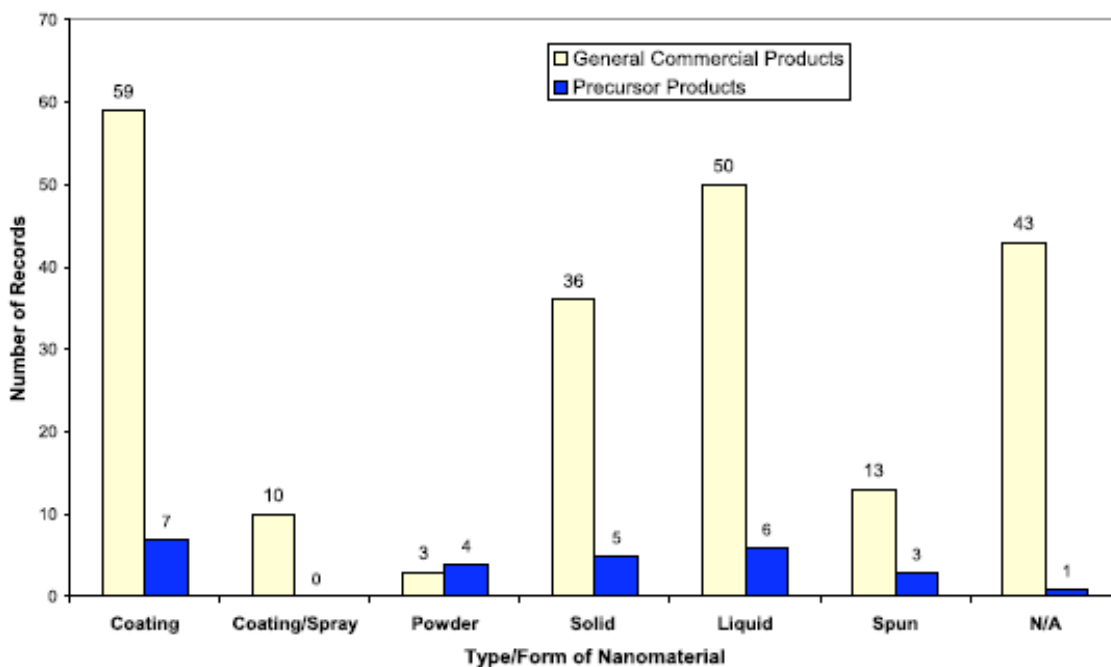


Figure 5.1: Forms of nanosilver incorporated in consumer products. (Fauss, 2008)

The products were categorized according to their applications. As presented in Figure 5.2, nanosilver is most notably incorporated into health and fitness products, including sporting goods, clothing, cosmetics, and personal care products.

Wijnhoven *et al.* (2009) reviewed the inventory of consumer products containing nanosilver. They, too, concluded that there is a lack of data on the concentrations of nanosilver in these products, as well as the size and form in which it is present. The authors specified the missing information as a knowledge gap. In this review, tables including the products, manufacturers, countries of manufacturing, form of nanosilver in the products and particle size were constructed (Appendix C).

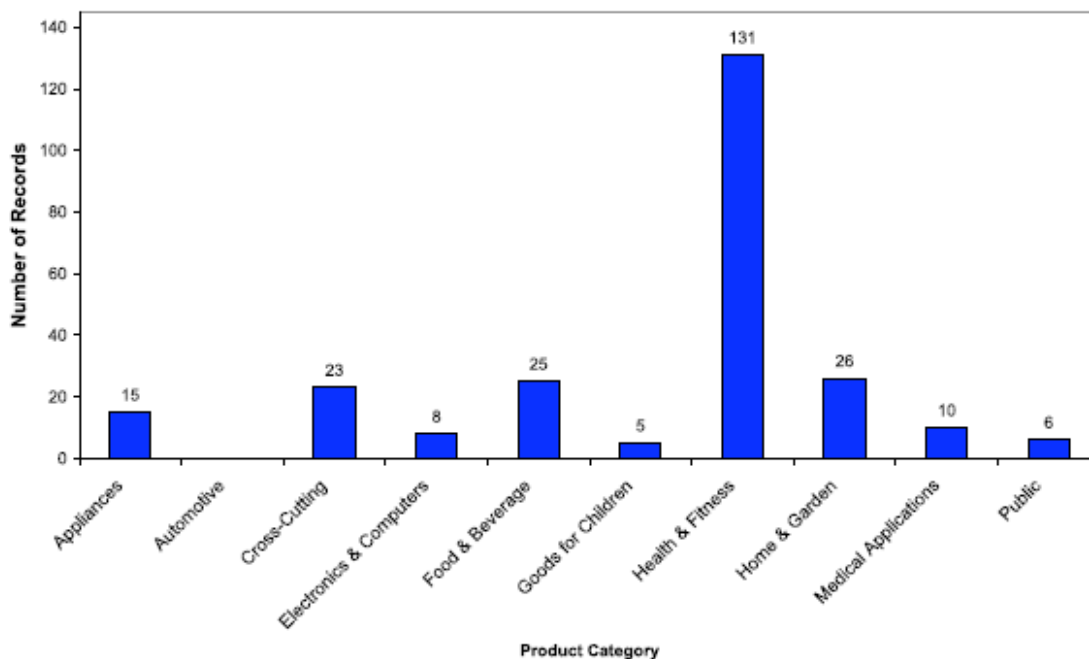


Figure 5.2: Categories of nanosilver-containing products. (Fauss, 2008)

There is information on manufacturers of either raw nanosilver or products containing nanosilver on the Internet and/or in the literature. However, there is a knowledge gap in the characteristics and quantities of nanosilver that is produced and used in consumer products. More attention needs to be paid to find this information and to enlist the companies in providing this information in order to assess the potential risks associated with nanosilver.

The California Department of Toxic Substances Control (“DTSC”) has announced its intention to request information and data for “reactive nanometal oxides” and other nanomaterials. The original announcement, on April 14, 2009, identified nanoscale aluminum oxide, silicon dioxide, titanium dioxide, and zinc oxide as examples of possible nanometal oxides to be reviewed. A recent update added nanoscale silver, nano zerovalent iron, and cerium oxide. DTSC’s formal, mandatory data call-in is the second call-in for nanomaterials under California’s Chemical Information Call-In law, A.B. 289, California Health and Safety Code, Chapter 699, sections 57018-57020.

In January 2009, DTSC issued its first call-in for carbon nanotubes (CNTs); the call-in ended in January, 2010. Through the proposed data call-in, DTSC intends to expand its current knowledge of “analytical test methods, fate and transport in the environment, and other

relevant information” for the nanomaterials selected. Information being requested in the call-in includes the following (DTSC, 2010):

“Value chain for a company? For example, in what products are carbon nanotubes used by others? In what quantities? Who are the major customers?

What sampling, detection and measurement methods are being used to monitor (detect and measure) the presence of chemical in the workplace and the environment? Provide a full description of all required sampling, detection, measurement and verification methodologies. Provide full QA/QC protocol.

What is the knowledge about the current and projected presence of chemical in the environment that results from manufacturing, distribution, use, and end-of-life disposal?

What is the knowledge about the safety of chemical in terms of occupational safety, public health and the environment?

What methods are being used to protect workers in the research, development and manufacturing environment?

When released, does chemical constitute a hazardous waste under California Health & Safety Code provisions? Are discarded off-spec materials a hazardous waste? Once discarded, are the carbon nanotubes being produced a hazardous waste? What are the waste handling practices for carbon nanotubes?”

DTSC issued the request to “manufacturers,” defined to include businesses located in California that produce these materials or import them for sale in California. After DTSC issued the request, companies subject to the call-in had one year to respond to DTSC with the required information, including generating any data that DTSC requires (DTSC, 2010).

DTSC and California Environmental Protection Agency’s (Cal/EPA) Department of Pesticide Registration (DPR) began collaborating on nanosilver in 2009. In May 2010, the two agencies entered into an agreement to work together on this nanomaterial (DTSC, 2010). The two agencies have received two pesticide registrations involving nanosilver use as of the date of this report, and four additional registrations are pending. DPR and DTSC anticipate an increase in applications to register pesticides and anti-bacterial containing nanosilver in California. Through this collaborative partnership, the two agencies aim to share registration, research and other information about nanosilver (DTSC, 2010).

As part of the chemical information call-in under California's Chemical Information Call-In law, A.B. 289, to assist DTSC on carbon nanotube call-in process, DTSC sought research and analytical support from the Sustainable Technology & Policy Program at the University of California, Los Angeles (UCLA). Pursuant to State Standard Agreement No. 08-T3631, UCLA will review and evaluate responses received by DTSC in response to the formal information request under A.B. 289 relating to carbon nanotubes. UCLA will also review the process used by DTSC for this carbon nanotube call-in and will develop a written evaluation, including recommendations for future chemical call-ins under A.B. 289 and other information collection authorities available to DTSC. This work is a continued partnership on nanomaterials research between DTSC and UCLA that has been in place for over three years (DTSC, 2010).

5.2 Routes of Release and Exposure, Ecological

Once released into the environment, the mobility, bioavailability and toxicity of silver nanoparticles in any ecosystem is largely determined by colloidal stability. Colloidal stability is a function of many factors including the type of capping agent, the characteristics of the surrounding environment such as the pH, ionic strength, presence/absence of humic acids and other ligands, and the background electrolyte composition (Chen, 2006; Cosgrove, 2005; Tielemans *et al.*, 2006). An extensive number of capping agents have been investigated to enhance the ability of nanoparticles to stay suspended in solution. Capping agents are chemicals that are used in the synthesis of silver nanoparticles to prevent their aggregation through electrostatic repulsion, steric repulsion or both. In the case of silver, the most prevalent capping agents are citrate, sodium borohydride (NaBH₄) and polyvinylpyrrolidone (PVP) (Tan *et al.*, 2007; Tolaymat *et al.*, 2010). The mechanism and functional groups involved in colloid stabilization differ with capping agents, which may lead to varying particle size and stability. Colloidal interactions, mobility and toxicity may differ.

Although numerous studies have investigated the effect of the colloidal surface properties on the stability of various nanoparticles under various environmental conditions, there is little information available in regards to silver nanoparticles. In a study by Jiang *et al.* (2009), the size of TiO₂ nanoparticles increased 50 fold upon increasing the solution ionic strength from

1 to 100 mM NaCl. The same study also showed that varying the solution pH resulted in a significant change in the particle surface charge. In another study, guar gum adsorbed on the surface enhanced the mobility of nano zero valent iron in sandy porous media regardless of the pH and ionic strength (Tiraferrri & Sethi, 2009). Saleh *et al.* (2008) reported that uncoated nano zero-valent iron particles were immobile in water-saturated sand columns while triblock copolymer-coated particles were highly mobile. Coating quantum dot nanocrystals with polyethylene glycol suppressed agglomeration and stabilized the suspension regardless of the ionic strength (Jiang *et al.*, 2009). Research has also shown that increases in cation valence and surface adsorption of ionic species significantly impacts suspension stability.

Gaiser *et al.* (2009) compared different sizes of nanosilver and cerium oxide nano particles for their potential for uptake by aquatic species, human exposure via ingestion of contaminated food sources and to assess their resultant toxicity. The results of their study demonstrated the potential for uptake of nano and larger particles by fish via the gastrointestinal tract, and by human intestinal epithelial cells, suggesting that ingestion is a viable route of uptake into different organism types.

Cumberland *et al.* (2009) studied the particle size distributions of silver nanoparticles under environmentally relevant conditions. As part of the study, monodisperse 15 nm citrate-stabilized silver nanoparticles were synthesized, characterized and then fractionated by flow field-flow fractionation (FIFFF) at environmentally relevant conditions (pH 5 or 8, presence of natural organic macromolecules (NOM) and presence of sodium or calcium). At low ionic strength, nanosilver particle size increased as pH increased from 5 to 8. However, changing the ionic strength from 10^{-3} to 10^{-2} M Na increased instability of the nanosilver. In the presence of humic substance, a reduction in nanoparticle size was seen, most likely due to a reduction in the diffuse layer. The presence of Ca^{2+} ions, at the higher ionic strengths caused complete loss of the solution nanosilver, with or without humic acids, most likely due to aggregation. The presence of humic acids improved stability of silver nanoparticles under these conditions by forming a surface coating resulting in both steric and charge stabilization. Cumberland *et al.* (2009) theorize that silver nanoparticles could have long residence times in aquatic systems in the presence of humic substances, potentially resulting in increased bioavailability.

El Badawy *et al.* (2010) studied the impact of capping agent and environmental conditions (pH, ionic strength and background electrolytes) on the surface charge and aggregation potential of five nanosilver suspensions. The nanosilver suspensions examined were: uncoated nanosilver (Hydrogen reduced AgNPs), electrostatically stabilized (Citrate-AgNPs and NaBH₄-AgNPs), sterically stabilized (PVP-AgNPs) and electrosterically stabilized (Branched polyethyleneimine (BPEI) stabilized AgNPs). The uncoated and the electrostatically stabilized nanosilver tended to aggregate at higher ionic strength and/or acidic pH. The authors reported that the ionic strength, pH and electrolyte type had no impact on the aggregation of the sterically stabilized nanosilver.

Mühling *et al.* (2009) tested whether silver nanoparticles released into estuarine environments result in increased antibiotic resistance within the natural bacterial population in estuarine sediments. A 50-day microcosm exposure experiment was carried out to investigate the effects of nanosilver (50 nm average diameter) on the antibiotic resistance of bacteria in sediments from an estuary in southwest England. Sediment samples were screened at the end of the exposure period for the presence of bacteria resistant to eight different antibiotics. The antibiotics used in the study were erythromycin, oxytetracycline, sulfadiazine, trimethoprim, lincomycin, ceftazidime, amoxicillin and vancomycin. Multivariate statistical analyses showed that there was no increase in antibiotic resistance amongst the bacterial population in the sediment due to dosing of the microcosms with silver nanoparticles. This study indicated that, under the tested conditions, nanosilver released into the coastal marine environment did not increase antibiotic resistance among naturally occurring bacteria in estuarine sediments.

Blaser *et al.* (2008) estimated the cumulative aquatic exposure and risk due to nanosilver being released from plastics and textiles using the flow diagram presented in Figure 5.3. The authors presented the analysis in four stages; (i) silver mass flow analysis and estimation of emissions, (ii) assessment of the fate of silver in a river system and estimation of predicted environmental concentrations (PECs), (iii) critical evaluation of available toxicity data for environmentally relevant forms of silver and estimation of predicted no-effect concentrations (PNECs), and (iv) risk characterization. The authors also estimated silver use in the year 2010, focusing on the Rhine river as a case study. The process simulated in the Rhine River model is shown in Figure 5.4. In 2010, biocidal plastics and textiles were predicted by the model to account for up to 15% of the total silver released into water in the European Union.

The majority of silver released into wastewater was incorporated into sewage sludge and were spread on agricultural fields. The amount of silver reaching natural waters depended on the fraction of wastewater that was being effectively treated. The authors found that the modeled PECs in the Rhine River were in satisfactory agreement with monitoring data from other river systems. This agreement indicated that the silver mass fluxes entering the aquatic system were reasonable estimates and that the emission scenarios provide a useful basis for the exposure assessment of freshwater ecosystems.

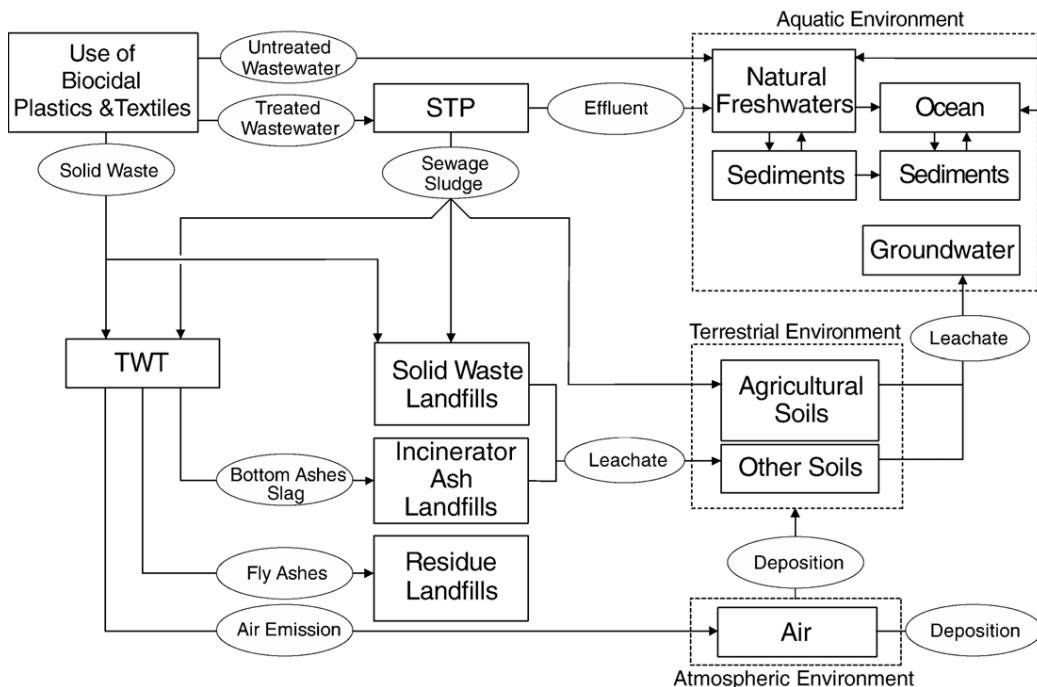


Figure 5.3: Overview of silver flows triggered by biocidal plastics and textiles. Arrows represent silver flows; dashed lines represent different environmental spheres. In the Figure, TWT represents thermal waste treatment and STP represents sewage treatment plant. Reprinted from *Sci. Tot. Environ.*, Vol. 390, Blaser, S.A., Scheringer, M., MacLeod, M., Hungerbuhler, K., Estimation of cumulative aquatic exposure and risk due to silver: contribution of nano-functionalized plastics and textiles, pp396-409, Copyright 2008 with permission from Elsevier.

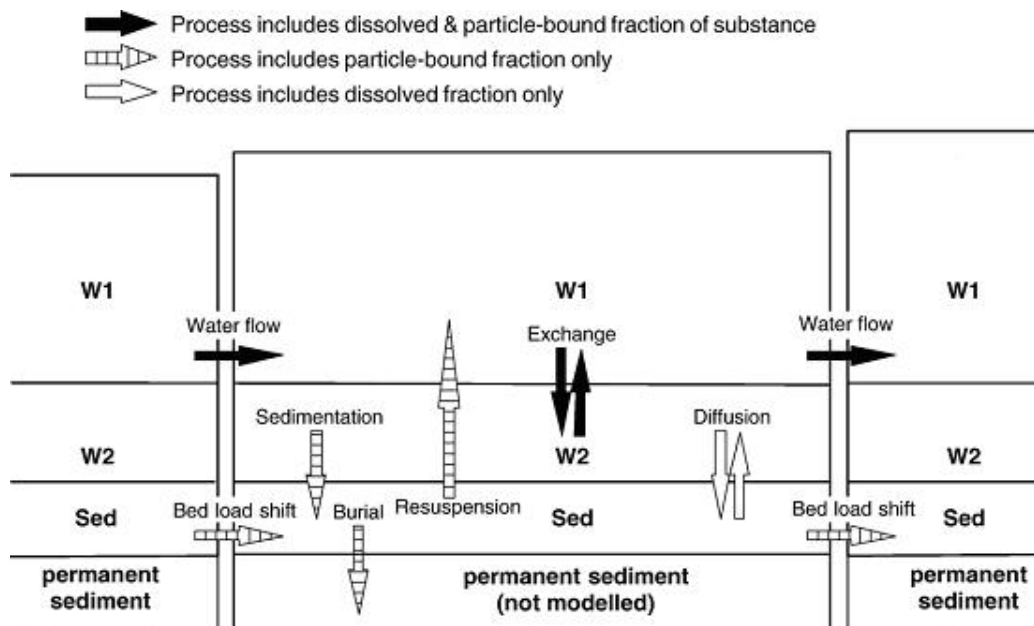


Figure 5.4: Process simulated in the model developed by Blaser et al. (2008). In the Figure, W1 represents the moving water, W2 represents stagnant water and ‘Sed’ represents the top layer of the sediment. Reprinted from *Sci. Tot. Environ.*, Vol. 390, Blaser, S.A., Scheringer, M., MacLeod, M., Hungerbuhler, K., Estimation of cumulative aquatic exposure and risk due to silver: contribution of nano-functionalized plastics and textiles, pp396-409, Copyright 2008 with permission from Elsevier.

Nanoparticles released from various nanotechnology-enhanced consumer products will inevitably enter sewers and wastewater treatment plants (WWTPs). A project funded by the Water Environment Research Foundation (WERF) evaluated how silver nanoparticles would affect wastewater treatment systems and anaerobic digestion. Under this project, researchers set up several lab-scale wastewater treatment modular units using activated sludge processes designed to remove organic matter and nutrients in wastewater. The results demonstrated that nitrifying bacteria were especially susceptible to inhibition by silver nanoparticles. At a concentration of 0.4 mg/L total Ag, a mixture of positively charged silver ions and silver nanoparticles (50:50 in mass ratio, average size = 15-21 nm) inhibited the growth of nitrifying bacteria from the modified Ludzack-Ettinger bioreactor by 11.5 percent. In an experiment on shock loading of 100% silver nanoparticles (lasting for 12 hours), a peak concentration of 0.75 mg/L total Ag in the activated sludge basin (more than 95% associated with biomass) was detected, and about 50% nitrifying bacterial growth inhibition (or nitrification inhibition) accompanied with a slight accumulation of nitrite concentration in wastewater effluent was observed. Studies of anaerobic digestion, a commonly used solid stabilization process in wastewater treatment plants, indicated that silver nanoparticles at

concentrations of 19 mg/L (19,000 ppb) or above in biomass might inhibit anaerobic microbial activities. Most of the silver particles were in the activated sludge. After considering concentration factor and safety factor, the researchers suggested a threshold concentration of 0.1 mg/L total silver including nanosilver in wastewater influent. A report on the WERF study is expected to be published on April 1, 2011 (Hu *et al.*, 2011). This study suggests that accumulation of silver in activated sludge could have a detrimental effect on wastewater treatment, if the concentration reaches threshold levels.

5.3 Routes of Exposure, Human

Humans are exposed to nanosilver primarily through the ingestion of drinking water and food, and through dermal contact with various consumer products and/or medical applications that contain nanosilver. Nanosilver is incorporated in everyday products such as water filters and washing machines; the presence of nanosilver in these products easily leads to leaching, which discharges into the aqueous environment. Once the nanomaterials reach the environment, there are a myriad of ways through which these materials are transported to media such as other water bodies, plants and sediments, which then get recycled back to humans. Table 5.1 lists the main characteristics for human exposure to nanomaterials from food, consumer and medical products. Figure 5.5 illustrates potential routes of exposure, uptake, distribution, and degradation of nanomaterials in the environment.

Table 5.1: Main characteristics for human exposure to nanomaterials from food, consumer and medical products. Reprinted from Nanotoxicology, Vol. 3 (2), Wijnhoven, S.W.P., Peijnenburg, W.J.G.M., Herberts, C.A., Hagens, W.I., Oomen, A.G., Heugens, E.H.W., Roszek, B., Bisschops, J., Gosens, I., van de Meent, D., Dekkers, S., de Jong, W.H., van Zijverden, M., Sips, A.J.A.M., Geertsma, R.E., Nanosilver – a review of available data and knowledge gaps in human and environmental risk assessment, pp109-138, Copyright 2009 with permission from Informa Healthcare.

Characteristic	Comments
Type of nanomaterials	Free nanoparticles or integrated nanostructures into larger materials
Exposure route	Inhalation, dermal or oral exposure. Intravascular, intrathecal, intravesical, urethral, ophthalmic, intramedullary, intraperitoneal exposure
Physical form of product	Spray, powder, liquid, emulsion or solid (coating)
Application of the consumer product	Applications with direct human exposure (e.g., sunscreen products, medical applications) or indirect human exposure (e.g., food storage bags, computers). Applications with direct emissions to an environmental compartment (e.g., tooth paste) or without direct emissions to the environment (e.g., computers).
Type, use of the consumer product	Widely used or rarely used product. Frequency and amount of product used
Concentration of nanomaterial in product	Unknown

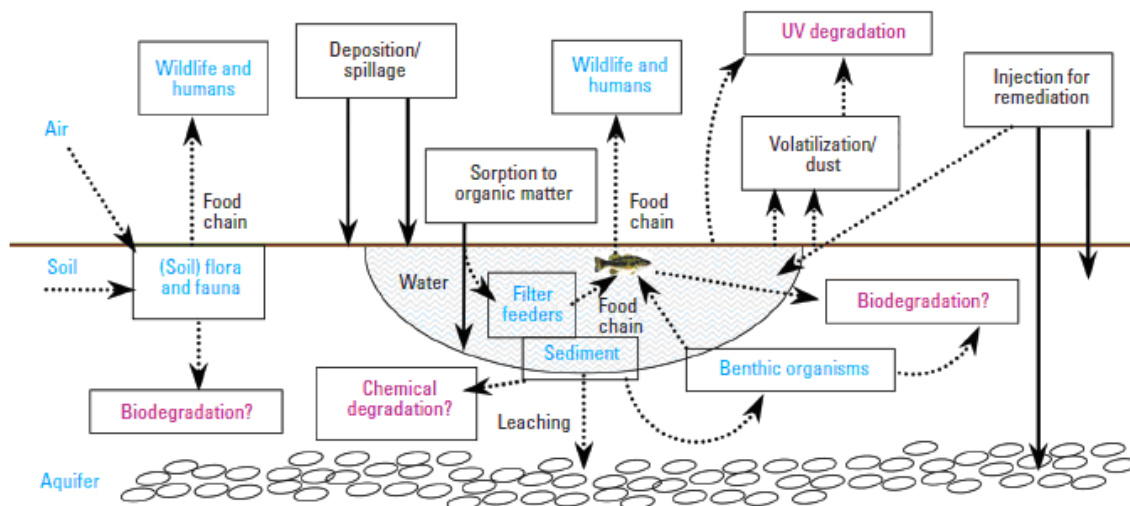


Figure 5.5: Routes of exposure, uptake, distribution, and degradation of nanomaterials in the environment. Solid lines indicate routes that have been demonstrated in the laboratory or field or that are currently in use (remediation). Magenta lettering indicates possible degradation routes, and blue lettering indicates possible sinks and sources of nanomaterials. Reprinted from *Environ Health Perspect.*, Vol. 113 (7), Oberdörster, G., Oberdörster, E., Oberdörster, J., *Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles*, pp823-839, Copyright 2005 with permission from National Institute of Health.

The growing application of nanosilver in food products, medical applications, cleaning sprays and other consumer products with increasing use and disposal to the environment indicates that human exposure to nanosilver is expected to increase in the future. The exposure to these nanoparticles depends on the way they were incorporated into the product (free nanoparticles or nanomaterials integrated into larger scale structures) in combination with the application of the product (with either direct or indirect human exposure). For example, products containing free nanoparticles with direct human exposure (e.g., food supplements or sunscreen products) are considered to have a high potential exposure, while products in which nanomaterials are integrated into larger scale materials with indirect human exposure (e.g., food storage bags or computers) are considered to have a low potential exposure. The route of exposure seems to be important as well. Inhalation exposure via sprays or oral exposure of food supplements are considered to have the highest risk. For medical applications, especially for coated catheters and orthopedic implants, more specific exposure routes are possible, depending on the location of application. Most of the time, this is local exposure; however, intravascular catheterization can lead to intravenous and, thus,

systemic exposure. Table 5.2 ranks the potential human exposure to nanosilver for common products/applications that contain the nanomaterial. The fact that a product category is ranked with either a high or low potential exposure in the previously-mentioned table should not be seen as evidence for absolute high exposures or the lack thereof, but as an indication of potentially high exposures (Dekkers *et al.*, 2007b). A point of interest for the future could be cumulative exposure; currently no information on this is available. To determine the risk for exposure, more information is needed on the concentrations of nanosilver in the product, the size and the form in which it is present (aggregates, agglomerates) as well as the probability of release of nanosilver from the products.

Table 5.2: Ranking of potential human exposures to nanosilver. Reprinted from Nanotoxicology, Vol. 3 (2), Wijnhoven, S.W.P., Peijnenburg, W.J.G.M., Herberts, C.A., Hagens, W.I., Oomen, A.G., Heugens, E.H.W., Roszek, B., Bisschops, J., Gosens, I., van de Meent, D., Dekkers, S., de Jong, W.H., van Zijverden, M., Sips, A.J.A.M., Geertsma, R.E., Nanosilver – a review of available data and knowledge gaps in human and environmental risk assessment, pp109-138, Copyright 2009 with permission from Informa Healthcare.

Category	Subcategory	Exposure route	Potential exposure*
Food and beverages	Cleaning	Inhalation/dermal	High
	Cooking utensils, coatings	Dermal	Low
	Storage	Dermal	Low
	Supplements	Oral	High
Personal care and cosmetics	Skin care	Dermal	High
	Oral hygiene	Oral	High
	Cleaning	Dermal	High
	Hair care	Dermal	Low? High? High?
	Baby care	Dermal	
	Over the counter products	Dermal?	
Textile and shoes	Clothing	Dermal	?
	Other textiles	Dermal	?
	Toys	Dermal/oral	?
Electronics	Personal care	Dermal	Low
	Household appliances	Dermal	Low
	Computer hardware	Dermal	Low
	Mobile devices	Dermal	Low
Household products/home improvement	Cleaning	Inhalation/dermal	High High?? Low
	Coating	Dermal	Low
	Furnishing	Dermal	
	Furnishing/coating	Dermal	
Filtration, purification, neutralization, sanitization	Filtration	Inhalation	?
	Cleaning	Inhalation/dermal	High

Table 5.2: continued

Category	Subcategory	Exposure route	Potential exposure*
Medical products	Breathing mask	Inhalation	High
	Endotracheal tube	Inhalation	?
	Gastrointestinal tube	Oral	?
	Catheters	Intravascular/intrathecal/intravesical/urethral Ophthalmic	?
	Contact lens	Ophthalmic	?
	Incontinence material	Dermal	?
	Orthopedic implants	Intramedullary	?
	Orthopedic stockings	Dermal	?
	Pharmaceuticals	Oral/dermal	?
	Sling for reconstructive pelvic surgery	Intraperitoneal	?
	Surgical mask/textile	Inhalation/dermal	?
	Wound dressings	Dermal	High

* ‘High’ indicates either a high probability of exposure or a possibility of high exposure or both. ‘Low’ indicates a low probability of exposure, or a possibility of low exposure, or both. ‘?’ indicates that there is no sufficient information available.

5.3.1 Exposure via food

Nanotechnologies are being used throughout all phases of food production including cultivation (for example, through the application of pesticides or providing nutrients to plants), processing and packaging (Bouwmeester *et al.*, 2007). Nanotechnologies are also being used to enhance the nutritional aspects of food by means of nanoscale additives and nutrients and nanosized delivery of drugs. The way nanotechnology is used within the food production leads to a first estimate of potential consumer exposure and, thus, can be used as a ranking of risks. Nanotechnology used for food production without introducing/adding nanoscale products or compounds in the food can be considered as low risk for the consumer (for example, using storage containers that contain minute amounts of nanosilver; Figure 5.6). Direct consumer exposure may be expected when nanoparticles are included into food directly or when the nanomaterials act directly on the food (for example, using water filters or using a nanosilver coated teapot to brew tea; Figure 5.7). Table 5.3 lists a summary of applications of nanosilver in the food production chain.



Figure 5.6: Fresh Box, manufactured by FinePolymer, Inc. (South Korea), is a nanosilver antimicrobial food container. The manufacturer claims that the container “shows excellent antimicrobial properties against various bacteria and fungus due to the effect of finely dispersed nanosilver particles and hence it make a food fresh longer compared with conventional food containers.” (http://goodgary.en.ec21.com/Nanosilver_Food_Container--1600253_1600254.html)



Figure 5.7: The Nano Tea Pot – Aroma manufactured by Top Nano Technology Co., Ltd (Taiwan). The manufacturer claims that the teapot is manufactured using patented precious metal techniques and that it releases tea flavor in 30 seconds.

Table 5.3: Summary of applications of nanotechnology in the food production chain. Reprinted from RIKILT/RIVM Report 2007.014, Bouwmeester, H., Dekkers, S., Noordam, M., Hagens, W., Bulder, A., De Heer, C., Ten Voorde, S., Wijnhoven, S., Sips, A., Health impact of nanotechnologies in food production, Copyright 2007 with permission from RIKILT – Institute of Food Safety.

Chain phase	Applications	Nanotechnology	Function
Agricultural production	Nanosensors	Nanospray on food commodities Hand-held devices Incorporated in packaging materials	Binds and colors microorganisms Detection of contaminants, etc. Detection of food deterioration
	Pesticides	Nanoemulsions, encapsulates Triggered release nanoencapsulates	Increased efficacy, water solubility and crop adherence Triggered (local) release
	Water purification Soil cleaning	Filters with nanopores Nanoparticles	Pathogen contaminant removal Removal or catalyzation of oxidation of contaminants
Production and processing of food	Food production Refrigerators, storage containers, food production equipment	Nanoceramic devices Incorporated nanosized particles, mostly silver, occasionally zinc oxide	Large reactive surface area Anti-bacterial coating of storage and food handling devices
Conservation	Food products Packaging materials	Nanosized silver sprays Incorporated sensors Incorporated nanoparticles Incorporated active nanoparticles	Anti-bacterial action Detection of food deterioration, monitoring storage conditions Increasing barrier properties, strength of materials Oxygen scavenging, prevention of growth of pathogens
'Functional food' consumption	Supplements	Colloidal metal nanoparticles Delivery system 'nanoclusters' Nanosized clustered food drinks (nutrients)	Claimed to enhance desirable uptake Protecting and (targeted) delivery of content Claimed enhanced uptake

Food/nutritional supplements that contain nanosilver are known to have statements such as 'Purifying and conservation of unknown targets', 'Supporting the immune system' and 'Helpful against severe illness' (Wijnhoven *et al.*, 2009). Since these statements have not been evaluated by, for instance, the European Medicines Evaluation Agency (EMA), the European Food Safety Authority (EFSA) or the US Food and Drug Administration (FDA), the products are not medical products and are not intended to diagnose, treat, cure or prevent any disease. Appendix C lists some of the food-related products that contain nanosilver.

It is difficult to estimate the exposure of humans to products that contain coatings of nanosilver to prevent bacterial growth. The expected human exposure remains low as long as

the inert nanosilver particles are bound in the packaging materials or in the coatings on surfaces of packaging materials and food preparation devices. When nanosilver particles are bound to other materials, exposure to nanosilver is only expected to occur when there is a risk of wear-off or migration of nanosilver particles in the free or aggregated form into the food (SCENIHR, 2006). Since potential benefits of using nanosilver in consumer products are due to the release of silver in some form (mostly as silver ions), exposure to nanoparticles in consumer products cannot be prevented. The potential benefit of using nanosilver in paint is to prevent the formation of mold on walls, whose spores presumably get destroyed when they come into contact with the nanosilver in paint. Humans may thus get exposed to nanosilver when they come into contact with walls painted with nanosilver-containing paint. It is anticipated that humans will be exposed to the nanosilver throughout the use of the products that contain the nanomaterial.

5.3.2 Exposure via consumer products

For nanotechnology consumer products, there are several existing inventories that contain a wide variety of globally available products. The most extended publicly available inventory is the database of the Nanotechnology project of the Woodrow Wilson International Centre for Scholars (www.nanotechproject.org). Since this project on Emerging Nanotechnologies launched the first online inventory of manufacturer identified nanotech goods in March 2006, the number of items has increased 175%, from 212 to 580 products in December 2007. By August 2009, the total number of products was further increased to 803, a rise of 279% when compared to the first inventory in 2006. This clearly indicates how fast the market for nano-containing products is growing. However, the inventory database has also seen some decreases because of proposed intentions of regulatory agencies in the US, Europe and elsewhere to regulate the industries using nanomaterials in their products. Wijnhoven *et al.* (2009) mentioned that companies based in the US produce most of the nano-containing consumer products (317), followed by companies in Asia (127), Europe (92), and elsewhere around the world (32). For nano-products produced outside Europe, including those produced in the US, consumers in Europe are only allowed to obtain them through European distributors or ordering the products directly from manufacturers or sellers online.

Several nanomaterials such as metal oxides (e.g., titanium dioxide, zinc oxide, silica), metals (e.g., silver, gold, nickel) and organic nanomaterials (e.g., nanovitamins, nanoclays, carbon nanotubes) (Dekkers *et al.*, 2007a, b) are used in consumer products. Nanosilver appears to

be incorporated into the highest number of different products, with manufacturers claiming nanosilver incorporation in 233 consumer products and in 33 food products as of 2008 (Wijnhoven *et al.*, 2009). This was 30% of the Woodrow Wilson inventory, far more than other materials such as carbon, gold or silica. As of August 2009 (the last time the inventory was updated), 259 consumer products contained nanosilver out of 1015 products listed in the database (25%). The application of nanosilver in these consumer products is mainly based on the antibacterial property of silver. Apart from the Food and Beverages category, the product categories in which nanosilver is represented include: electronics, filtration, purification, neutralization, sanitization, personal care and cosmetics, household products/home improvement, textiles and shoes. Table 5.4 lists a summary of products that contain nanosilver. As can be concluded from this table, most nanosilver containing consumer products are in the product categories textiles and shoes (34), personal care and cosmetics (30) and electronics (29) with clothing, skin care and personal care as subcategories with the largest number of products. Also the categories household products/home improvement (19) and filtration, purification, neutralization, and sanitization (13) contain a substantial amount of products with nanosilver. Some products are difficult to classify and can be categorized in more than one group, therefore it is possible that discrepancies exist between this and former inventories.

One of the earliest consumer products to include nanosilver was Samsung's Silver Wash washing machine (Figure 5.8). Samsung claimed that the washing machine achieves 99.9% sterilization, and kills 650 different types of bacteria. Samsung also states that the washing machine coats silver nanoparticles onto the fabrics, which maintain antibacterial activity for up to a month.



Figure 5.8: Samsung's Silver Wash washing machine (<http://ww2.samsung.co.za/silvernano/silvernano/washingmachine.html>). Samsung also claims that the machine releases up to 400 billion silver ions per wash cycle.

Geranio *et al.* (2009) investigated the amount and the form of silver released during washing from nine fabrics with different ways of nanosilver incorporated into or onto the fibers. The effect of pH, surfactants, and oxidizing agents was also evaluated. Results from their study indicated that little dissolution of silver nanoparticles occurred under conditions relevant to washing (pH 10) with dissolved concentrations 10 times lower than at pH 7. Bleaching agents such as hydrogen peroxide or peracetic acid could greatly accelerate the dissolution of silver. The amount and form of silver released from the fabrics as ionic and particulate silver depended on the type of nanosilver incorporated into the textile. The percentage of the total silver emitted during one washing of the textiles varied considerably among products (from less than 1 to 45%). In the washing machine, the majority of the silver (at least 50% but mostly >75%) was released in the size fraction >450 nm, indicating the dominant role of mechanical stress. A conventional silver textile did not show any significant difference in the size distribution of the released silver compared to many of the textiles containing nanosilver. A recent study (Benn & Westerhoff, 2008) revealed that the silver can easily leak into

wastewater during washing, thus, potentially disrupting helpful bacteria used in wastewater treatment facilities, or endangering aquatic organisms in lakes and streams. Benn and colleagues found that some brands of socks lose nearly 100% of their silver content within four washings, while two other brands lost less than 1% over the same number of washings (Benn & Westerhoff, 2008).

Nanosilver is used in washing machines because of its antimicrobial activity (Chen & Schluesener, 2008). Several Swedish Agencies, including the Swedish Environmental Protection Agency, have protested against this application because wastewater may be contaminated with nanosilver. The USEPA has recently decided to regulate this specific form of nanotechnology (Federal Register Notice 73 Fed. Reg. 69,644, November 19, 2008). In the US, silver-ion generating devices such as washing machines, with the declared aim to kill bacteria, will no longer be considered a simple washing device, but a pesticide. This notice is not an action to regulate nanotechnology; it is the silver's bactericidal effect rather than the size that led to the decision. In view of potential effects in aquatic ecosystems, new purification methods need to be developed to eliminate possible negative effect of nanosilver.

Table 5.4: Product categories with examples of products containing nanosilver. Values in brackets indicate the number of subcategories. Reprinted from *Nanotoxicology*, Vol. 3 (2), Wijnhoven, S.W.P., Peijnenburg, W.J.G.M., Herberts, C.A., Hagens, W.I., Oomen, A.G., Heugens, E.H.W., Roszek, B., Bisschops, J., Gosens, I., van de Meent, D., Dekkers, S., de Jong, W.H., van Zijverden, M., Sips, A.J.A.M., Geertsma, R.E., *Nanosilver – a review of available data and knowledge gaps in human and environmental risk assessment*, pp109-138, Copyright 2009 with permission from Informa Healthcare.

Categories	Subcategories	Examples
Personal care and cosmetics (30)	Skin care (14) Oral hygiene (6) Hair care (3) Cleaning (2) Coating (2) Baby care (2) Over the counter health products (1)	(Body) cream, hand sanitizer, hair care products, beauty soap, face masks Tooth brush, teeth cleaner, toothpaste Hair brush, hair masks Elimination wipes and spray Make-up instrument, watch chain Pacifier, teeth developer Foam condom
Textile and shoes (34)	Clothing (28) Other textiles (2) Toys (4)	Fabrics and fibers, socks, shirts, caps, jackets, gloves, underwear Sheets, towels, shoe care, sleeves and braces Plush toys
Electronics (29)	Personal care (13) Household appliances (8) Computer hardware (6) Mobile devices (2)	Hair dryers, wavers, irons, shavers Refrigerators, washing machines Notebooks, (laser) mouse, keyboards Mobile phones

Table 5.4: continued

Categories	Subcategories	Examples
Household products/home improvement (19)	Cleaning (9)	Cleaning products for bathrooms, kitchens, toilets, detergents, fabric softener
	Coating (4)	Sprays, paint supplements
	Furnishing (3)	Pillows
	Furnishing/coating/flooring (3)	Showerheads, locks, water taps, floors, tiles
Filtration, purification, neutralization, sanitation (14)	Filtration (8)	Air filters, ionic sticks
	Cleaning (6)	Disinfectant and aerosol sprays

Park *et al.* (2009) monitored and analyzed the exposure characteristics of silver nanoparticles during a liquid-phase process (Figure 5.9) in a commercial production facility in Korea. The facility produces approximately 3000 kg of silver nanoparticles month. Based on the measured exposure data, the source of silver nanoparticles emitted during the production processes was indentified and a mechanism for the growth of silver nanoparticle released was proposed. The authors concluded that silver nanoparticles were released from the reactor during the liquid-phase production process and agglomerates of silver nanoparticles were formed in the atmosphere of the workplace. The increase in particle number concentration during the liquid-phase process was higher than that during processes that involved the handling of a dry powder. The data reported in this study could potentially be used in conjunction with other similar studies to establish occupational safety guidelines in the nanotechnology workplace, especially in a liquid-phase production facility.

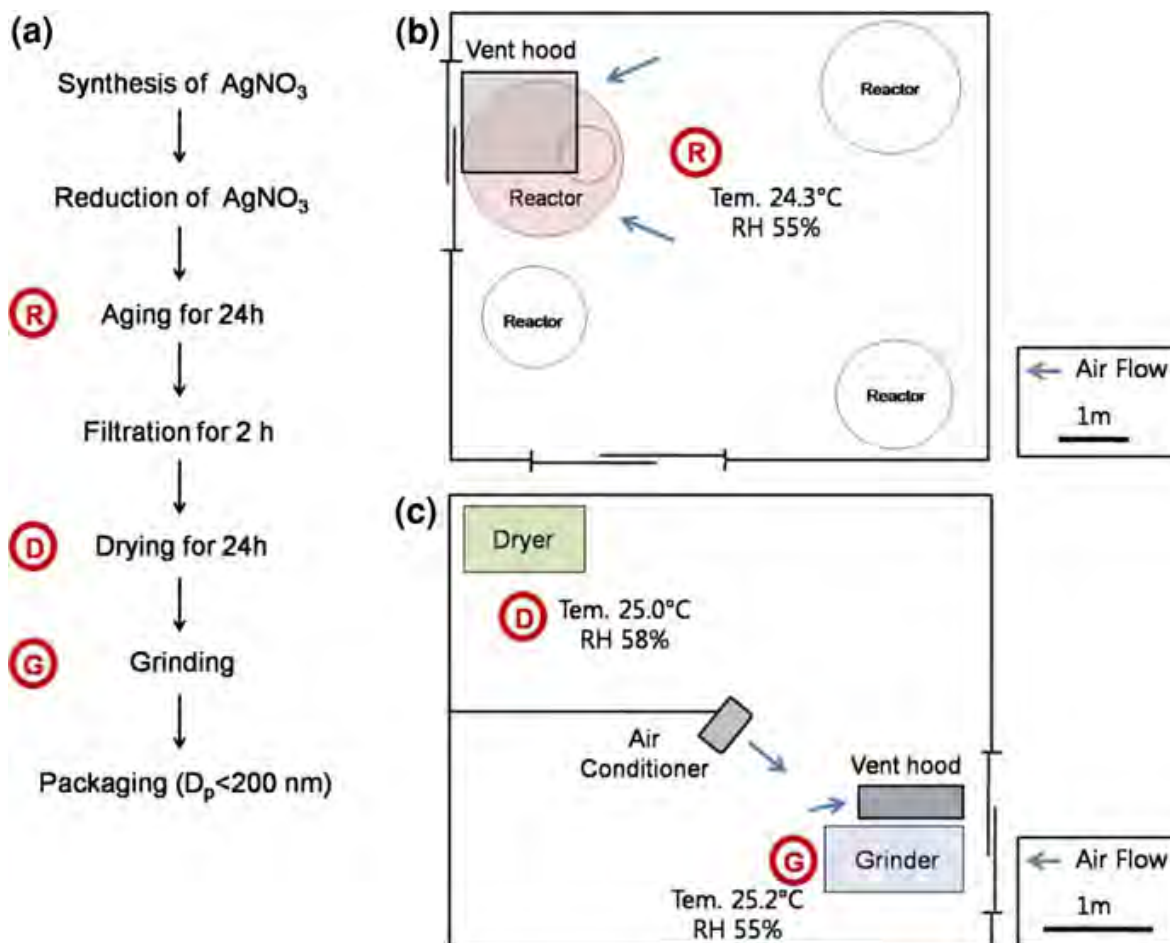


Figure 5.9: Illustration showing production flow process and measurement locations in the Korean silver nanoparticle manufacturing facility. (a) Production flow process of the silver nanoparticle. The points marked R, D and G were monitored, (b) Reaction room, (c) Drying room. Reprinted from *J. Nanoparticle Res.*, Vol. 11 (7), Park, J., Kwak, B.K., Bae, E., Lee, J., Kim, Y., Choi, K., Yi, J. Characterization of exposure to silver nanoparticles in a manufacturing facility, pp1705-1712, Copyright 2009 with permission from Springer.

5.3.3 Exposure via Medical Applications

Silver has been known for decades for its antimicrobial properties in curative and preventive medicine. The most widespread uses of silver are silver salts, silver complexes, and metallic silver in pharmaceutical and homeopathic products (e.g., ointments, suspensions). Silver antibiotic salts such as silver sulfadiazide are used as prophylaxis of infections in patients with burns (Church *et al.*, 2006). Medical devices such as catheters, orthopedic implants, heart valves and wound care products are prone to bacterial adhesion, colonization, biofilm formation and adhesion of glycoproteins from tissue and blood plasma. Silver coatings, using silver salts or ion beam implantation of metallic silver, have been devised to address these

problems. Some coatings have shown disappointing clinical results as effective strategies to prevent medical device-related infections. For instance, in 2000, a voluntary recall of a silver-coated sewing cuff fabric for heart valve replacement was initiated due to elevated rates of paravalvular leakage (Schaff *et al.*, 2002). Advanced silver nanotechnologies have substituted the use of bulk silver in curative and preventive medicine in an attempt to engineer out the risks caused by medical device-related infections.

Several other medical devices and products for wound care management that incorporate nanosilver are on the market. Table 5.5 provides a summary of consumer products with medical applications. In general, nanosilver is deposited, impregnated or coated onto medical devices or fabrics rendering them suitable for controlling infections. Advanced nanotechnologies, such as physical vapor deposition, chemical vapor deposition, or ink-jet technology, are used to create thin layers of nanosilver on a broad range of substrates, e.g., metals, ceramics, polymers, glass, and textiles. Silver nanotechnologies that have been launched for antimicrobial coatings are Bactiguard® (Bactiguard AB, Sweden), HyProtect™ (Bio-Gate AG, Germany), Nucryst's nanocrystalline platform technology (Nucryst Pharmaceuticals Corp., USA), Spi-Argent™ (Spire Corp. USA), Surfaccine® (Surfaccine Development Company LLC, USA), and SylvaGard® (AcryMed Inc., USA). Nanosilver is extensively used for wound management, particularly in medical devices for the treatment of burns (Tredget *et al.*, 1998; Figure 5.10), chronic wounds (Yin *et al.*, 1999; Sibbald *et al.*, 2001), burns in children (Dunn & Edwards-Jones, 2004), burn injuries in neonates (Rustogi *et al.*, 2005), rheumatoid arthritis-associated leg ulcers (Coelho *et al.* 2004), diabetic ulcers (Thomas, 2007), venous ulcers (Sibbald *et al.*, 2007), toxic epidermal necrolysis (Asz *et al.*, 2006), for healing of donor sites (Innes *et al.*, 2001), and for meshed skin grafts (Demling & Leslie DeSanti, 2002).

Table 5.5: Medical devices containing nanosilver. Values between brackets indicate the number of devices. Adapted from Nanotoxicology, Vol. 3 (2), Wijnhoven, S.W.P., Peijnenburg, W.J.G.M., Herberts, C.A., Hagens, W.I., Oomen, A.G., Heugens, E.H.W., Roszek, B., Bisschops, J., Gosens, I., van de Meent, D., Dekkers, S., de Jong, W.H., van Zijverden, M., Sips, A.J.A.M., Geertsma, R.E., Nanosilver – a review of available data and knowledge gaps in human and environmental risk assessment, pp109-138, Copyright 2009 with permission from Informa Healthcare.

Medical Domains	Examples
Anesthesiology	Catheter for administration of local anesthetic (1)
Cardiology	Battery used in implantable cardioverter-defibrillator (1)
Nephrology	Hemodialysis catheter (2)
Urology	Urinary catheter (2) Battery used in implantable electrical pulse generator (1)
Wound care	Burn and wound dressing, professional use (15) Burn and wound dressing, over the counter (2) Bum glove (1) Bum sock (1) Tubular stretch knit (1) (Adhesive) strip, professional use (2) (Adhesive) strip, over the counter (2) Gel(1) Compress (2) IV/catheter dressings (2)

The application of nanosilver in medical products is emerging in the field of medical devices and pharmaceutical research and development (Table 5.6). Other potential applications of nanosilver coated/deposited/impregnated medical devices are infusion ports, orthopedic protruding fixation devices, endovascular stents, urological stents, endoscopes, electrodes, peritoneal dialysis devices, subcutaneous cuffs, surgical and dental instruments. Silver nanoparticles can be deposited on various natural and synthetic textile and fabrics which can be useful in hospitals to control infection (Lee *et al.*, 2003). The incorporation of nanosilver into medical products has been of great interest in recent years. Properties of nano-structured silver can be controlled and tailored in a predictable manner and imparted with biological properties and functionalities that bring new and unique capabilities to a variety of medical applications ranging from implant technology and drug delivery, to diagnostics and imaging.



Figure 5.10: Anti-microbial burn dressing manufactured by Anson Nano-Biotechnology (Zhuhai) Co., Ltd., China. The manufacturers claim that the core material is nanosilver antibacterial granule. The manufacturer does not provide information on the size or amount of silver nanoparticles in their products (<http://www.ansonano.com/productinfo.asp?newsid=156349068&lan=zh-en&skin=5&open=0>).

Table 5.6: Emerging applications of nanosilver in medical products. Reprinted from Nanotoxicology, Vol. 3 (2), Wijnhoven, S.W.P., Peijnenburg, W.J.G.M., Herberts, C.A., Hagens, W.I., Oomen, A.G., Heugens, E.H.W., Roszek, B., Bisschops, J., Gosens, I., van de Meent, D., Dekkers, S., de Jong, W.H., van Zijverden, M., Sips, A.J.A.M., Geertsma, R.E., Nanosilver – a review of available data and knowledge gaps in human and environmental risk assessment, pp109-138, Copyright 2009 with permission from Informa Healthcare.

Medical Domains	Examples	References
Anesthesiology	Coating of breathing mask Coating of endotracheal tube for mechanical ventilatory support	Patent –
Cardiology	Coating of driveline for ventricular assist devices Coating of central venous catheter for monitoring	– –
Dentistry	Additive in polymerizable dental materials Silver-loaded SiO ₂ nanocomposite resin filter	Patent Jia <i>et al.</i> , 2008
Diagnostics	Nanosilver pyramids for enhanced biodetection Ultrasensitive and ultrafast platform for clinical assays for diagnosis of myocardial infarction Fluorescence-based RNA sensing Magnetic core/shell Fe ₃ O ₄ /Au/Ag nanoparticles with tunable plasmonic properties	Walt, 2005 Aslan & Geddes, 2006 Aslan <i>et al.</i> , 2006 Xu <i>et al.</i> , 2007
Drug delivery	Remote laser light-induced opening of microcapsules	Skirtach <i>et al.</i> , 2006
Eye care	Coating of contact lens	Weisbarth <i>et al.</i> , 2007
Imaging	Silver/dendrimer nanocomposite for cell labeling Fluorescent core-shell Ag@SiO ₂ nanoballs for cellular imaging Molecular imaging of cancer cells	Lesniak <i>et al.</i> , 2005 Aslan <i>et al.</i> , 2007 Tai <i>et al.</i> , 2007
Neurosurgery	Coating of catheter for cerebrospinal fluid drainage	Bayston <i>et al.</i> , 2007 Galiano <i>et al.</i> , 2007

Table 5.6: continued

Medical Domains	Examples	References
Orthopedics	Additive in bone cement Implantable material using clay-layers with starch-stabilized silver nanoparticles Coating of intramedullary nail for long bone fractures Coating of implant for joint replacement Orthopedic stockings	Alt <i>et al.</i> , 2004 Podsiadlo <i>et al.</i> , (2005) Alt <i>et al.</i> , 2006 Chen <i>et al.</i> , 2006 Pohle <i>et al.</i> , 2007
Patient care	Superabsorbent hydrogel for incontinence material	Lee <i>et al.</i> , 2007
Pharmaceutics	Treatment of dermatitis Inhibition of HIV-1 replication Treatment of ulcerative colitis Treatment of acne	Bhol <i>et al.</i> , 2004 Bhol & Schechter, 2007 Elechiguerra <i>et al.</i> , 2005 Sun <i>et al.</i> , 2005 Bhol & Schechter, 2007 Patent
Surgery	Coating of hospital textile (surgical gowns, face mask)	Li <i>et al.</i> , 2006
Urology	Coating of surgical mesh for pelvic reconstruction	Cohen <i>et al.</i> , 2007
Wound care	Hydrogel for wound dressing	Yu <i>et al.</i> , 2007

5.3.4 Exposure via occupation

Small quantities of silver and/or nanosilver are absorbed by humans through diet or inhalation at occupational sites. Occupations that have a potential for exposure to nanosilver include metallurgists, solderers, electroplaters, jewelers, cosmeticians, and individuals working in a silver nanoparticle manufacturing facility or electronics manufacturing facility. The most common clinical presentations due to occupational exposure are argyria and argyrosis (Pala *et al.*, 2008). Other clinical presentations involve exposure to soluble silver compounds, which may cause liver and kidney damage, irritation of the eyes, skin, respiratory and intestinal tract, and hematological changes (Drake & Hazelwood, 2005; Lansdown, 2007).

Pala *et al.* (2008) presented a case study of a 71-year old craftsman, working from the age of 17, producing silver-containing items such as vases, plates, trays and frames by using cutting tools, welding and hammering silver sheets. The craftsman's work bench was approximately 30-40 cm from his face, and he was exposed to silver at least 8 hours per day. Pala *et al.* (2008) conducted an ocular examination of the craftsman, and diagnosed bilateral conjunctival-corneal argyrosis (Figure 5.11) without systemic intoxication.



Figure 5.11: Conjunctival-corneal argyrosis in the craftsman occupationally exposed to silver. Reprinted from J. Occup. Health, Vol. 50, Pala, G., Fronterre, A., Scafa, F., Scelsi, M., Ceccuzzi, R., Gentile, E., Candura, S.M., Case Study: Ocular Argyrosis in a Silver Craftsman, pp521-524, Copyright 2008 with permission from Japan Society for Occupational Health.

Moss *et al.* (1979) studied ocular manifestations and functional effects of occupational argyrosis. Thirty employees of an industrial plant involved in the manufacture of silver nitrate and silver oxide underwent ophthalmologic evaluation in an effort to evaluate the frequency and extent of ocular argyrosis. The most frequently noted ocular abnormality was pigmentation of the conjunctiva, present in 20 workers; corneal pigmentation occurred in 15 workers. A direct relationship existed between the levels of pigmentation and duration of employment. Ocular pigmentation was seen more frequently than cutaneous pigmentation. Ten workers noted decreased night vision, but electrophysiologic and psychophysiologic studies of seven of these ten workers demonstrated no functional deficits.

Tsai *et al.* (2009) studied airborne exposures associated with manual handling of nanoparticles in three fume hoods operating under a range of operational conditions. The handling tasks the authors studied included transferring particles from beaker to beaker by spatula and pouring. Measurements studied included the room background, researcher's breathing zone, and upstream and downstream from the handling location. The test results by the authors found that the handling of dry powders consisting of nano-sized particles inside laboratory fume hoods can result in a significant release of airborne nanoparticles from the

fume hood into the laboratory environment and the researcher's breathing zone. Many variables were found to affect the extent of particle release including hood design, hood operation (sash height, face velocity), work practices, type and quantity of the material being handled, room conditions, and the adequacy of the room exhaust.

5.4 Projected Quantities, Geographic and Demographic Distribution in the US

Specific data on silver emissions to the environment and the distribution of the emitted nanosilver across the US are not available. As previously mentioned in Section 5.1, the survey was designed to collect information covering various aspects of the projected quantities, geographic and demographic distribution. With the lack of response, the only source from which information can be extracted is the Project of Emerging Nanotechnologies (PEN). On their website (www.nanotechproject.org/121), an interactive map for the US highlights the distribution of more than 1200 nanotechnology companies, universities, research laboratories and other organizations working with nanotechnology could be found (Figure 5.12). The map was launched in 2007 and updated in 2009 and it includes 955 companies, 182 university and government laboratories and 81 other types of organizations. This map is not limited to nanosilver distribution but it is generic for all produced nanomaterials. The main findings were: a) all the states in the US contain at least one organization deal with nano materials, b) the top 4 states contain organizations working with nano materials are California, Massachusetts, New York and Texas, c) the top 6 cities (including more than 30 entries) are: Boston, MA; San Francisco, CA; San Jose, CA; Raleigh, NC; Middlesex-Essex, MA, Oakland, CA, d) the top 3 sectors working in nanotechnology are, materials, tools and instruments, and medicine and health and e) California is the lead state, with more than double the entries in any other state. This information is valuable for predicting source points for nanomaterials activities. But the final destinations of nanomaterials as well as produced quantities still need to be addressed. This information is greatly lacking and is as seen as a knowledge gap.

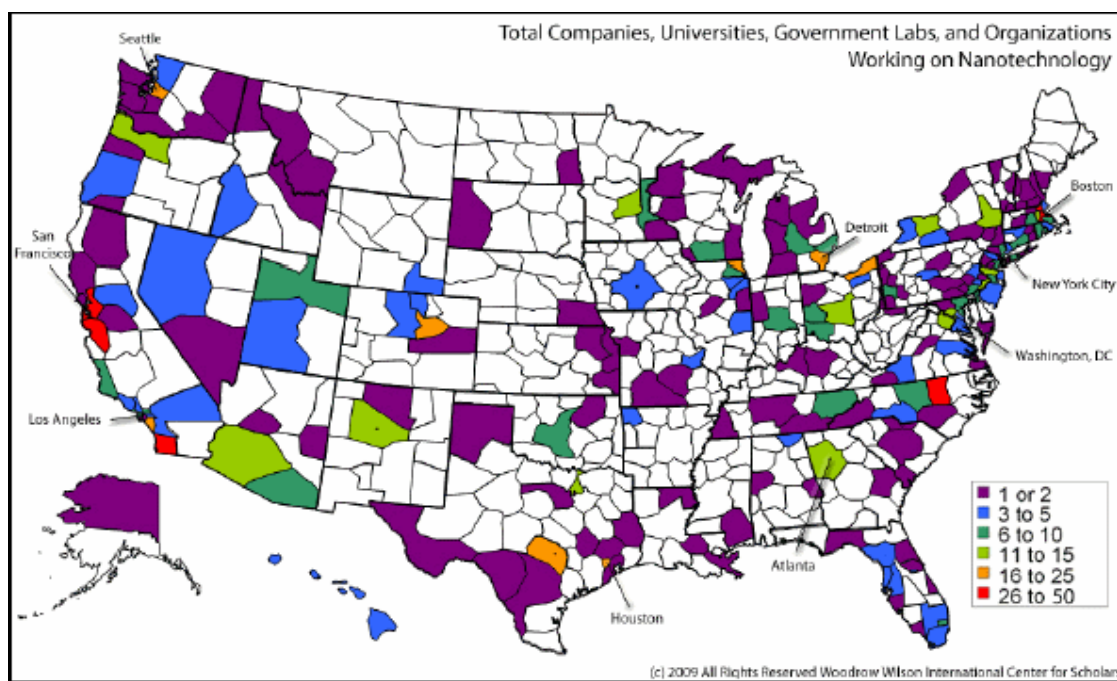


Figure 5.12: Number of companies, universities, laboratories and/or organization working in nanotechnology across the US. (<http://www.nanotechproject.org/inventories/map/>)

All available data related to quantities of released nanosilver are modeled data. Mueller & Nowack (2008) modeled the release of three types of nanoparticles including (nanosilver) into air, water and soil in Switzerland. The estimated worldwide production volume, allocation of the production volume to product categories, particle release from products, and flow coefficients within the environmental compartments were used as the model's input parameters. The authors presented a schematic (Figure 5.13) for the flows of the nanosilver to the environmental compartments (air, water and soil), Sewage treatment plant (STP), Waste incineration plants (WIP) and the landfills for the high emission scenario. In the case of nanosilver, the most prominent flows are between the products and the STP (3.27 t/year), the STP and the WIP (2.65 t/year) and the WIP to landfills (3.26 t/year). The predicted concentrations of nanosilver in all different environmental compartments were extremely low ($1.7 \times 10^{-3} \text{ g m}^{-3}$ in air, 0.03 g L^{-1} in water and 0.02 g kg^{-1} in soil). In another study, Blaser *et al.* 2008, estimated the cumulative nanosilver release based on the estimated silver use in plastics and textiles in the year 2010. It was estimated the nanosilver-containing plastics and textiles account for up to 15% of the total nanosilver released into water in the European Union. The Rhine River was used as a case study and the predicted concentrations are presented in Table 5.7. In this study, Blaser *et al.* (2008) estimated that 9–20 tons of

silver from biocidal uses would be discharged to the European environment in wastewaters, with the remainder going to sewage sludge.

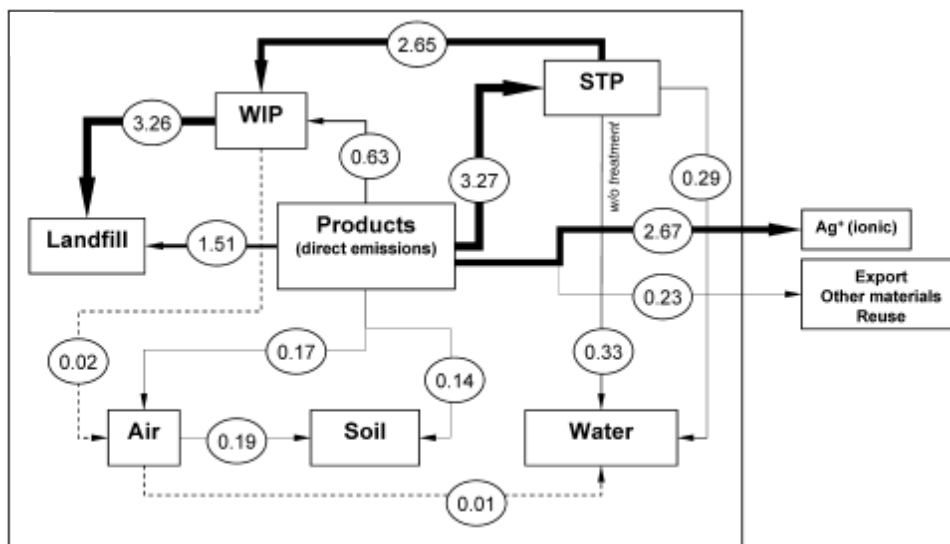


Figure 5.13: Nanosilver flows during high emission scenarios. Reprinted from Environ. Sci. Technol., Vol. 42 (12), Mueller, N.C., Nowack, B., Exposure modeling of engineered nanoparticles in the environment, pp4447-4453, Copyright 2008 with permission from American Chemical Society.

Table 5.7: Predicted environmental concentrations in Rhine River. Reprinted from Sci. Tot. Environ., Vol. 390, Blaser, S.A., Scheringer, M., MacLeod, M., Hungerbuhler, K., Estimation of cumulative aquatic exposure and risk due to silver: contribution of nano-functionalized plastics and textiles, pp396-409, Copyright 2008 with permission from Elsevier.

Compartment	Unit	Minimum scenario	Intermediate scenario	Maximum scenario
Sewage treatment plants	g/L	2	9	18
River water	ng/L	40	140	320
River sediment	mg/kg	2	6	14
Interstitial water in sediment	ng/L	9	30	70

Luoma (2008) provided various important tables on estimates of environmental exposure information, masses of silver discharged to the aquatic environment from different sources in 1978, discharges of silver into South San Francisco Bay from one waste treatment facility and from the combined POTW discharges from the surrounding urban area in the 1980s and in 2007, typical silver concentrations in water bodies of the world and a comparison of discharges from silver nanotechnologies for several different near-term scenarios for the USA and for San Francisco Bay. Some of the information in these tables revealed that no individual product will release silver at rates equal to those released by photographic development in the 1980s. The authors however state that the amount of silver released from

all products containing nanosilver in the monitored areas in 2007 might significantly exceed the release of silver from just the photographic industry in the 1980s. The author gave an example by calculating the maximum predicted release scenario of nanosilver from 3 consumer products to be high as 457 metric tons/year for the USA and 128 metric tons/year after wastewater treatment compared to 124 ton/year in 1978 from the photographic industry. Luoma (2008) predicted similar quantities of nanosilver to be released for 100 consumer products such as silver socks if used by 10% of the population in the US.

6. Toxicity and Health Effects

6.1 Silver Toxicity

Among all forms of silver that can potentially be found in the environment, the majority of toxicological data in the literature is only available for its two most common forms – elemental silver (Ag(0)) and monovalent silver ion (Ag⁺). Even though silver has been used widely for its medicinal, antibacterial and antiviral properties for hundreds of years, there is relatively limited information available on its toxicity in the literature. Existing environmental and human studies suggest that some forms of silver, especially those that dissociate and release free silver ions (Ag⁺), are more toxic than others. Many researchers have theorized that the toxic effects (especially acute) of silver-containing materials are directly proportional to the rate of release of monovalent silver ions. Drake & Hazelwood (2005) showed that metallic silver appeared to pose minimal risk to health, whereas soluble silver compounds were more readily absorbed and, hence, had the potential to produce adverse effects. Various studies have shown that 1-5 g Ag⁺/L is enough to kill sensitive aquatic and marine species (Bryan & Langston 1992; Wood *et al.* 1994). Some studies have shown that accumulation of silver in species exposed to a slightly lower concentration of silver may lead to adverse effects on growth (Eisler, 1997). Other investigations have shown that the concentrations of Ag⁺ ions, especially in the environment, are too low to lead to toxicity (WHO, 2002).

The wide variety of uses of silver in everyday life allows for exposure through various routes of entry into the body, including inhalation, ingestion and dermal exposure. Ingestion is the primary route for entry for silver compounds and colloidal silver proteins (Silver, 2003). Wijnhoven *et al.* (2009) estimate a dietary intake of 70-90 g of silver/day. Inhalation, ingestion and dermal contact of dusts or fumes containing silver occurs primarily in the manufacturing sector such as chemical plants that produce silver-containing compounds and its surrounding communities (ATSDR, 1990; Drake & Hazelwood, 2005). Dermal contact also occurs in medical settings such as from the application of burns creams, use of dental amalgams and acupuncture needles, catheters, accidental punctures, and from contact with jewelry and silverware (Catsakis & Sulica, 1978; Drake & Hazelwood 2005; Wan *et al.*, 1991). The most common health effects associated with chronic exposure to silver are a

permanent grey or blue grey discoloration of the skin (argyria; Figure 6.1) and other organs (ATSDR, 1990; Drake & Hazelwood 2005; White *et al.*, 2003). Lower-level exposure to silver also results in the metal being deposited in the skin and other parts of the body such as liver, brain, muscles and kidneys, and may cause changes in blood cells (Fung & Bowen, 1996; Venugopal & Luckey, 1978). Exposure to high levels of silver in the air can result in breathing problems, lung and throat irritation, and stomach pains. Skin contact with silver can cause mild allergic reactions including rashes, swelling, and inflammation in some people. Since bulk silver in any form is not thought to be toxic to the immune, cardiovascular, nervous or reproductive systems, and it is not considered to be carcinogenic, many researchers and regulatory agencies consider silver to be relatively non-toxic except for argyria and other symptoms mentioned previously (ATSDR, 1990; Chen & Schluesener 2008; Furst & Schlauder, 1978).



Figure 6.1: Systemic argyria of the skin from ingestion of colloidal silver (bottom hand) when compared to normal pigmentation (top hand) (Wadhera & Fung, 2010).

6.2 Nanosilver Toxicity

Even though silver is usually not available in concentrations high enough to pose a risk to human health and the environment, nanosilver has physical and surface properties which could pose a threat to human and environmental health (Lee *et al.*, 2007). Nanosilver has characteristics such as its size, surface area, solubility, ability to aggregate, chemical composition and surface chemistry that are different from the characteristics of bulk silver. Because of the different physicochemical properties and biological activities of nanosilver when compared with the regular metal, it cannot be excluded that the increased reactivity of nanosilver (because of the large surface area) leads to increased toxicity due to the activity of free silver ions released by the nanoparticles. Nanomaterials including nanosilver, primarily because of their extremely small size, which is comparable to the size of viruses, may have the ability to enter, translocate within, and damage living organisms. Some nanoparticles could penetrate the lung or skin and enter the circulatory and lymphatic systems of humans and animals, reaching body tissues and organs, and potentially disrupting cellular processes and causing disease.

The health effects of the nanoparticles used in consumer products are not yet known, though various studies have revealed adverse health effects of materials previously considered safe. Silver, however, has been shown to be toxic to humans or animal cells when in nanoparticle form, with reported observations of a cytotoxic response nearly identical to that for chrysotile asbestos (Soto *et al.*, 2005). Inhalation of silver nanoparticles leads to their migration to the olfactory bulb, where they locate in mitochondria, translocation to the circulatory system, liver, kidneys, and heart (Oberdöster *et al.*, 2005a, 2005b; Takenaka *et al.*, 2001). Silver nanoparticles have been found in the blood of patients with blood diseases and in the colon of patients with colon cancer (Gatti, 2004; Gatti *et al.*, 2004). There are contradictory studies on silver nanoparticles and ion cytotoxicity from laboratories around the world. Silver is known to have a lethal effect on bacteria, but the same property that makes it antibacterial may render it toxic to human cells. Concentrations of silver that are lethal for bacteria are also lethal for both keratinocytes and fibroblasts (Poon & Burd, 2004). *In vitro* studies have demonstrated that nanosilver has effects on reproduction, development, and has an effect on DNA among others.

Recent research with zebra fish showed that highly purified, single silver 12 nm nanoparticles affected early development of fish embryos (Lee *et al.* 2007). Silver nanoparticles have the potential to cause chromosomal aberrations and DNA damage and are capable of inducing proliferation arrest in zebrafish cell lines (Asharani *et al.* 2007). More nanosilver *in vitro* and *in vivo* toxicity studies have been performed in mammalian species have shown that silver nanoparticles have the capability to enter cells and cause cellular damage (Hussain *et al.*, 2005; Ji *et al.*, 2007).

Recent epidemiological studies have shown a strong correlation between particulate air pollution levels, respiratory and cardiovascular diseases, various cancers, and mortality (Brook *et al.*, 2004). Adverse effects of nanoparticles on human health depend on individual factors such as genetics and existing disease, as well as exposure, and nanoparticle chemistry, size, shape, agglomeration state, and electromagnetic properties. Animal and human studies show that inhaled nanoparticles are less efficiently removed than larger particles by the macrophage clearance mechanisms in the lungs, leading to lung damage (Asgharian & Price, 2007; Card *et al.* 2008; Oberdörster *et al.*, 2007). Nanoparticles can also translocate through the circulatory, lymphatic, and nervous systems to many tissues and organs, including the brain (Takenaka *et al.*, 2001). The key to understanding the toxicity of nanoparticles is that their minute size, smaller than cells and cellular organelles, allows them to penetrate these basic biological structures, disrupting their normal function. Examples of toxic effects include tissue inflammation, and altered cellular redox balance toward oxidation, causing abnormal function or cell death (Samberg, *et al.*, 2010; Udea *et al.*, 2002).

The same properties that make nanomaterials appealing also cause problems with studying the toxicity due to exposure to such particles. Researchers have shown that while exposure to nanosilver is toxic under certain experimental conditions, other researchers have shown that nanosilver is non-toxic under similar experimental conditions. From a review of the toxicological studies being conducted in the literature, it is becoming increasingly apparent that the main difference in the outcome of the toxicity studies is due to variations in physicochemical features of the nanosilver being used in various studies. The physicochemical features of the nanoparticles must be characterized under the experimental setting so that definitive associations between these parameters and any biological responses observed may be identified. There are difficulties in monitoring nanomaterial behavior when dispersed in physiological solutions as the latter often contain particulate and charged

materials that will mask the true size distribution and charge measurements of the nanomaterials themselves. Agglomeration can also be temperature dependent and so measurements should be made at a constant temperature, which requires temperature-controlled equipment. Many of the techniques currently available to assess surface area, morphology and composition are reliant on dry samples and are difficult to apply to nanomaterials in solution.

Due to the lack of reliable nanosilver toxicity data in the literature, it is impossible to assess the environmental risks associated with the production and use of nanosilver. An important research question is the validation of the hypothesis that toxic effects of nanoparticles are proportional to the activity of the free silver ions released by the nanoparticles. Apart from nanosilver toxicity assessment in the aqueous environments, more research is needed to investigate the effects of nanosilver in terrestrial environments as no toxicity data for nanosilver in soils were found in the literature. Additional research is also needed on ecologically relevant species to investigate whether silver nanoparticles present a threat to environmental health in general. It should be determined whether nanosilver in products is actually capable of reaching the aqueous and terrestrial environment. Specifically, the strength of the bonds between nanosilver and the product it is incorporated into should be investigated. Additional questions to consider include release patterns and release kinetics of nanosilver from specific applications and whether the physicochemical properties change under certain circumstances leading to more/less release of nanosilver into the aqueous environment.

6.2.1 Toxicity of Nanosilver to Organisms

There is a significant body of literature discussing the toxicity of silver nanoparticles to various bacterial species; however, the diverse methods of synthesis, capping agents and dispersants used may make direct and meaningful comparisons difficult. Results using different bacterial strains, even if they are of the same species, may not be comparable.

Wang *et al.* (2010) studied the impact of silver on the metabolism of anaerobic cultures of *Shewanella oneidensis*. The authors found that *S. oneidensis* MR-1 reduced toxic silver ions in solution to elemental nanosilver particles, which was later confirmed using X-ray diffraction analyses. Low silver ion concentrations (1 to 50 μM) had a limited impact on growth, while higher ion concentrations (100 μM) reduced both the doubling time and cell

yields. At the higher concentration, the authors determined that the silver nanoparticles were accumulated within the cell, while at lower concentrations, the nanoparticles were exclusively reduced and precipitated outside the cell wall. Whole organism metabolite fingerprinting, using the method of Fourier transform infrared spectroscopy analysis of cells grown in a range of silver concentrations, confirmed that there were significant physiological changes at higher silver concentrations. Molecular analyses confirmed a dramatic drop in cellular yields of both the phospholipid fatty acids and their precursor molecules at high concentrations of silver, suggesting that the structural integrity of the cellular membrane was compromised at high silver concentrations. The authors concluded that this was a result of intracellular accumulation of the silver nanoparticles.

Elechiguerra *et al.* (2005) studied the interaction of silver nanoparticles with HIV-1 virus. The authors demonstrated that the silver nanoparticles undergo a size-dependent interaction with HIV-1 (Figure 6.2), with nanoparticles exclusively in the range of 1-10 nm attached to the virus. Figure 6.2 shows high angle annular dark field (HAADF) scanning transmission electron microscope image of a HIV-1 virus in the presence and absence of silver nanoparticles. The regular spatial arrangement of the attached nanoparticles, the center-to-center distance between nanoparticles, and the fact that the exposed sulfur-bearing residues of the glycoprotein knobs would be attractive sites for nanoparticle interaction suggested that silver nanoparticles interact with the HIV-1 virus via preferential binding to the gp120 glycoprotein knobs. Due to this interaction, silver nanoparticles inhibited the virus from binding to host cells.

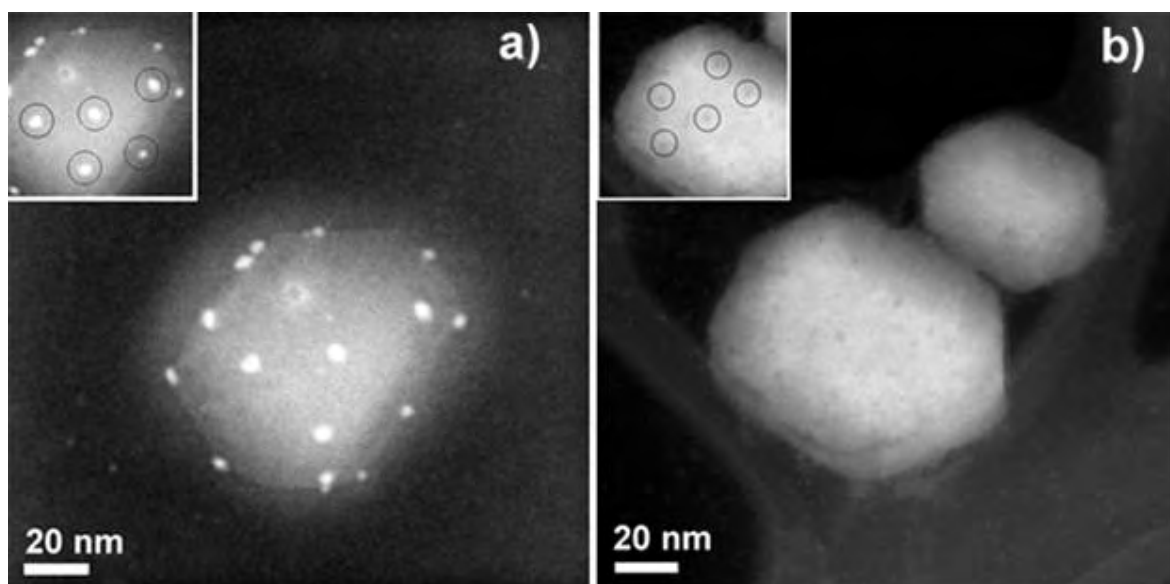


Figure 6.2: HAADF image of an HIV-1 virus that is (a) exposed to silver nanoparticles, (b) without silver nanoparticle treatment. Reprinted from *J. Nanobiotechnology*, Vol. 3, Elechiguerra, J.L., Burt, J.L., Morones, J.R., Camacho-Bragado, A., Gao, X., Lara, H.H., Yacaman, M.J., Interaction of silver nanoparticles with HIV-1, pp6, Copyright 2005 with permission from BioMed Central.

Nanosilver has been recently recognized as a more potent antimicrobial form of silver (Alt *et al.*, 2004; Aymonier *et al.*, 2002; Baker *et al.*, 2005; Melaiye *et al.*, 2005; Sondi *et al.*, 2004; Wright *et al.*, 2002). As an example, Wright *et al.* (2002) demonstrated that wound dressing coated with sputtered nanosilver reduced infections in burns. The antibacterial action of Ag^{+1} is thought to have several mechanisms. Recent observations have suggested the primary mechanism of action is cell death due to the uncoupling of oxidative phosphorylation (Holt & Bard, 2005), which confirms work from other investigators; however, others have reported interaction with membrane-bound enzyme and protein thiol groups that may result in compromised cell wall integrity that would lead to deterioration of proton gradient-driven oxidative phosphorylation (Bragg & Rainnie, 1974; Liao *et al.*, 1997; Silver, 2003; Zeiri *et al.*, 2004).

Lok *et al.* (2006) have recently examined the effect of nanosilver 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 nanosilver was considerably lower than that for Ag^{+} ions.

6.2.2 Ecological or Multispecies Studies of Nanosilver Toxicity

The same unique physical and chemical properties of nanomaterials that make them of interest in industrial applications have also increased concern that nanomaterials may have unique biological properties resulting in potential toxicity in the event of unintended use or release into the environment (Lovern & Klaper, 2006; Moore, 2006). Following environmental release, engineered nanomaterials are likely to deposit in aquatic systems and represent a possible danger to aquatic life (Moore, 2006). Soluble forms of many of these metals may be toxic to aquatic organisms, implying that the potential exists for nanoparticulate formulations of these metals to induce toxicological effects in aquatic species. For example, the toxicity of silver to aquatic organisms has been shown to be primarily because of exposure to silver ions (Navarro *et al.*, 2008). Although regulations such as the Clean Water Act exist for protecting aquatic life from dissolved forms of these metals, it is unclear if they are appropriate for use with nanomaterials as the mechanisms of toxicity, if any, may be different from those due to exposure to bulk metals.

Griffitt *et al.* (2008) reported the effects of particle composition on the toxicity of metallic nanomaterials to aquatic organisms. They used zebrafish, daphnids, and an algal species of various trophic levels and feeding strategies as a model. To understand whether observed effects were caused by dissolution, the particles used in their toxicity experiments were characterized before testing, and particle concentration and dissolution were determined during exposures. Organisms were exposed to silver, copper, aluminum, nickel, and cobalt as both nanoparticles and soluble salts as well as to titanium dioxide nanoparticles. Results from the toxicity experiments indicated that nanosilver and nanocopper caused toxicity in all organisms tested, with 48-h median lethal concentrations as low as 40 and 60 $\mu\text{g/L}$, respectively, in *Daphnia pulex* adults, whereas titanium dioxide did not cause toxicity in any of the tests. The authors reported that susceptibility to nanomaterial toxicity differed among species, with filter-feeding invertebrates being markedly more susceptible to nanometal exposure compared with larger organisms (i.e., zebrafish). The observed toxicity was found to vary with the role of dissolution, with dissolution playing a minor role for studies involving silver and copper, however, it played a major role in studies involving nickel. The authors also observed that nanomaterial forms of metals were less toxic than soluble forms based on mass added.

Griffitt *et al.* (2009) also examined the interplay of nanoparticle composition and dissolution on response of the zebrafish gill following exposure to toxic (nanocopper or nanosilver) or nontoxic (nano-TiO₂) nanometals. Female zebrafish were exposed to the 48-h no observable effects concentration (NOEC) concentrations of nanocopper and nanosilver or to soluble Cu and Ag that matched the concentration of dissolved metals released during nanoparticle exposure. The authors observed that both nanocopper and nanosilver exposures increased metal content associated with gill tissue. Silver concentrations were much higher following nanosilver exposures suggesting that intact silver nanoparticles were associated with the gill. Morphological and transcriptional responses of the gills differed among various nanomaterials and between nanoparticulate and soluble species. Nanocopper increased mean gill filament width three to fourfold between 24 and 48 hours, whereas nanosilver did not alter gill filament width at either time point. Soluble silver and copper exposure both increased gill filament widths by approximately twofold over control values. Gill filament widths were higher in soluble silver exposures than in nanosilver exposures, despite both tanks containing highly similar concentrations of soluble silver. Global gene expression analysis performed by the authors demonstrated that the exposure to each nanometal or soluble metal produced a distinct gene expression profile at both 24 and 48 h, suggesting that each exposure was producing biological response by a different mechanism. The differences in responses among the exposures indicated that each particle was having a distinct biological effect that did not appear to be driven solely by release of soluble metal ions into the water column.

The authors note that exposure to silver nanoparticles produced significantly higher levels of silver associated with the gills than did exposure to only the soluble fraction. This suggested that the nanoparticles themselves were contributing to the gill burden of silver through a mechanism that did not involve the formation of silver ions. The authors theorize several mechanisms by which nanoparticulates may increase the gill silver levels. Nanoparticles may be trapped in the mucus layer of the gill as demonstrated for larger particles (Sanderson *et al.*, 1996; Tao *et al.*, 1999). The authors reason that nanoparticles trapped in this manner may not actually enter the cells, but mucus entrained particles can also increase intracellular metal content by enhanced dissolution due to changes in water chemistry in the gill microenvironment including mucus complexation (Tao *et al.*, 2002). It is possible that nanoparticles are actually taken up by gill epithelial cells. Martens & Servizi (1993) demonstrated that sediment particles less than 500 nm were present intracellularly in

salmonid gill epithelial cells. Though the results from their study demonstrated that not all nanoparticles interacted with the gill in the same manner, the authors cautioned that further work would be required to ascertain the mechanism by which silver and copper nanoparticles interacted with the gill. Griffitt *et al.* (2009) noticed that exposure to silver nanoparticles also significantly increased whole body silver content; however, it was not clear whether this is due to translocation of silver from the gills to the rest of the body, as had been shown previously in rainbow trout (Morgan *et al.*, 2004), or due to ingestion of particulates and gastrointestinal (GI) absorption.

Harris & Bali (2008) investigated the limits of uptake of metallic silver by two common metallophytes, *Brassica juncea* and *Medicago sativa* and assessed the form and distribution of the metal once sequestered by the plants. *B. juncea* accumulated up to 12.4 wt.% silver when exposed to an aqueous substrate containing 1,000 ppm AgNO₃ for 72 hours; silver uptake was largely independent of exposure time and substrate silver concentration. *M. sativa* accumulated up to 13.6 wt. % silver when exposed to an aqueous substrate containing 10,000 ppm AgNO₃ for 24 hours. In contrast to *B. juncea*, there was a general trend for *M. sativa* to show an increase in metal uptake with a corresponding increase in the substrate metal concentration and exposure time. In both cases the silver was stored as discrete nanoparticles, with a mean size of approximately 50 nm. Haverkamp *et al.* (2007) demonstrated a way for plants to synthesize mixed metal nanoparticles by adding solutions containing the appropriate metal ions. In their study, the authors demonstrated a way for *B. juncea* to synthesize nanoparticles containing gold, silver and copper as an alloy. This work could potentially lead to a biosynthetic process to force plants to produce nanoparticles of metal alloys for a range of nanotechnology applications. Assuming that other commonly consumed plants have a similar capacity for nanosilver uptake, animals and humans consuming such plants will be susceptible to the toxicity caused by the intake of nanosilver.

Gao *et al.* (2009) conducted experiments that likely mimicked the introduction of manufactured nanomaterials into aquatic systems to assess the effect of nanoparticle dispersion/solubility and water chemical composition on nanomaterial toxicity. Aqueous suspensions of fullerenes, nanosilver, and nanocopper were prepared in both deionized water and filtered (0.45 µm) natural river water samples collected from the Suwannee River basin, to emphasize differences in dissolved organic carbon (DOC) concentrations and solution ionic strengths. Two toxicity tests, the *Ceriodaphnia dubia* and MetPLATE bioassays were

used in the experiments. Results obtained from exposure studies showed that water chemistry affected the suspension/solubility of nanomaterials as well as the particle size distribution, resulting in a wide range of biological responses depending on the type of toxicity test used. MetPLATE results for nanosilver showed decreasing trends in toxicity with increasing DOC concentrations and ionic strength. The biological responses in *C. dubia* was contrasting in that increasing DOC concentrations reduced toxicity, while the latter increased with increasing ionic strength. The results showed that laboratory experiments that use DI-water and drastic nanomaterial suspension methods may not be realistic as nanomaterial dispersion and suspension in natural waters vary significantly with water chemistry and the reactivity of the nanomaterials.

In a 96-hour acute exposure study with *Daphnia magna*, Gaiser *et al.* (2009) determined that nanosilver caused more mortality than bulk silver. In *Cyprinus carpio*, the authors mentioned that silver was detected in the liver, intestine, gills and gall bladder after treatment with both sizes of nanoparticles. However, the authors noted a trend towards higher uptake of the nanosilver than the micro-sized particles.

Lee *et al.* (2007) synthesized silver nanoparticles using sodium citrate and sodium borohydride as reducing agents, and evaluated their effects on zebrafish embryos. The authors concluded that Brownian motion and transport into and out of embryos through chorion pore canals occurred; however, restricted diffusion out of the embryos due to the viscosity inside the embryo resulted in the accumulation of nanoparticles. The authors determined the locations of individual nanoparticles (5-46 nm) within embryos using dark-field single nanoparticle optical microscopy and spectroscopy (SNOMS), determining that the embryonic response was dose-dependent. The authors showed that deformities increased with nanoparticle concentration up to 0.19 nM, then decreased with increasing concentration (up to the maximum concentration tested, 0.71 nM) based on an increase in dead zebrafish. Specific deformities were correlated with the concentration of nanoparticles. All tested concentrations of nanoparticles resulted in finfold abnormality and tail/spinal cord defects, while head edema occurred with 0.44-0.71 nM and eye deformity only occurred with 0.66-0.71 nM nanoparticles. They concluded that the great sensitivity of zebrafish early embryos to silver nanoparticles indicates that this species may be useful for *in vivo* toxicity assays to screen other nanomaterials.

Asharani *et al.* (2008) also investigated the toxicity of silver nanoparticle using zebrafish as a model. Silver nanoparticles were synthesized using starch and bovine serum albumin (BSA) as capping agents to study their deleterious effects and distribution pattern in zebrafish embryos. TEM/EDX of the embryos showed that nanoparticles accumulated in the brain, heart, yolk, and blood of embryos. They also conclude that silver nanoparticles effect normal embryo development and have a dose-dependent toxicity in embryos.

Very little is known on the specific effects of nanosilver in the environment, especially its fate and transport in the environment and its effect on biota and the ecosystem. It is currently impossible to reliably assess the environmental risks associated with the production and use of nanosilver, and its release into the environment.

6.2.3 Studies Concerning Human Health Including Mammalian Models

No specific mammalian models for nanosilver were available in the literature, although several were identified for nanoparticles in general. The toxicity of nanoparticles to humans and mammals depends on various factors such as the size, their composition, ease of aggregation, physical surface characteristics, chemical surface characteristics such as crystallinity, presence of functional groups, etc. The toxicity of the nanoparticle is also heavily dependent on the mammal's genetic complement, its susceptibility and its ability to adapt to changes in the environment, and to fight toxic substances. Diseases associated with inhaled nanoparticles might include asthma, bronchitis, emphysema, lung cancer, and neurodegenerative diseases. Nanoparticles in the gastrointestinal tract have been linked to Crohn's disease and colon cancer. Nanoparticles that enter the circulatory system are related to occurrence of arteriosclerosis, blood clots, arrhythmia, heart diseases, and ultimately cardiac death. Translocation to other organs such as liver, spleen, etc., may lead to diseases of these organs as well. Exposure to some nanoparticles is associated with the occurrence of autoimmune diseases such as systemic lupus erythematosus, scleroderma, and rheumatoid arthritis (Buzea *et al.*, 2007). Figure 6.3 provides a schematic of a human body with pathways of exposure to nanoparticles, affected organs, and associated diseases from epidemiological, *in vivo* and *in vitro* studies.

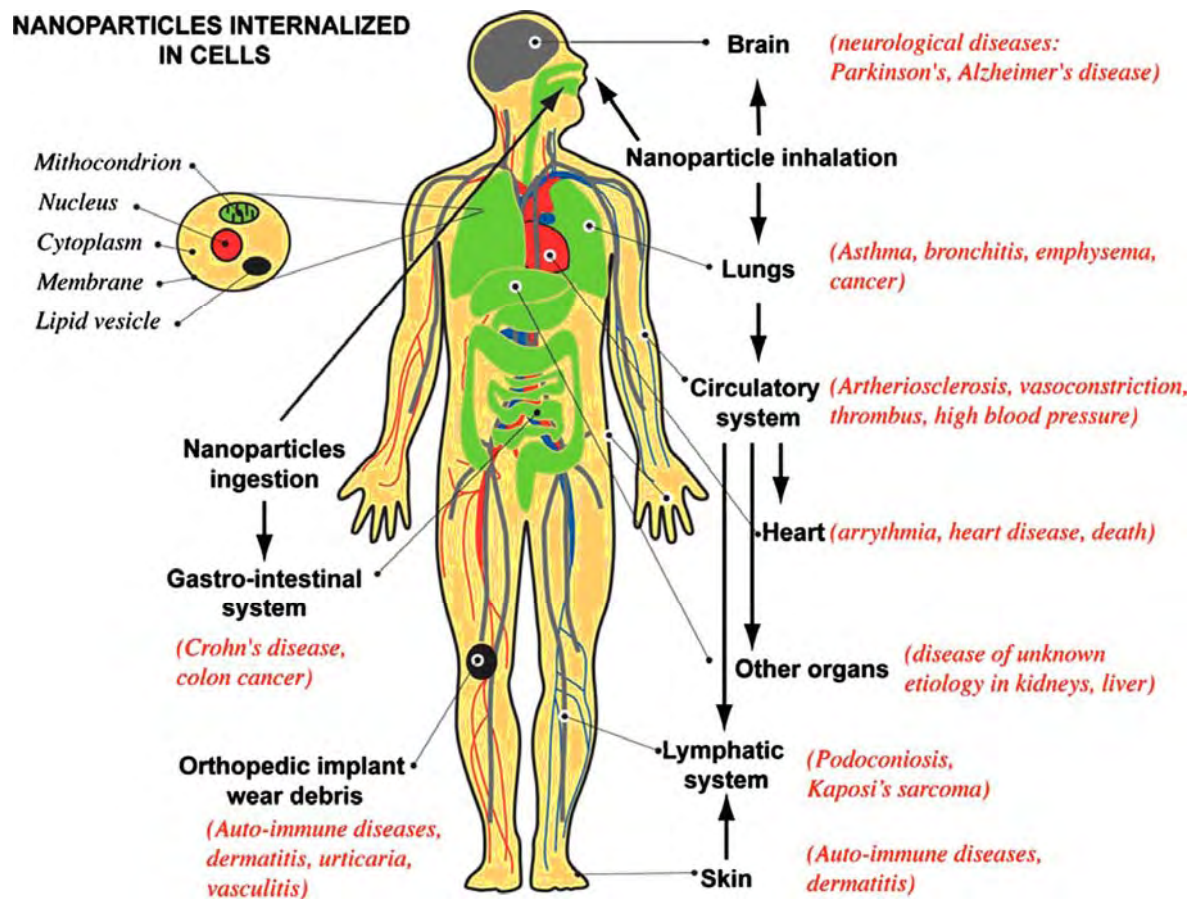


Figure 6.3: A schematic of the human body with pathways of exposure to nanoparticles, affected organs, and associated diseases from epidemiological, *in vivo* and *in vitro* studies. Reprinted from *Biointerphases*, Vol. 2 (4), Buzea, C., Pacheco, I.I., Robbie, K. *Nanomaterials and nanoparticles: Sources and toxicity*, ppMR17-MR71, Copyright 2007 with permission from American Institute of Physics.

The increased biological activity of nanoparticles can be either positive or desirable (e.g., antioxidant activity, carrier capacity for therapeutic penetration of blood-brain barrier, and the stomach wall or tumor pores), and dispersed throughout the whole body including entering the central nervous system, or negative and undesirable (e.g., toxicity, induction of oxidative stress, or cellular dysfunction) or a mix of both (El-Ansary & Al-Daihan, 2009; Oberdörster *et al.*, 2005b). Nanoparticles have been found to be distributed to the colon, lungs, bone marrow, liver, spleen, and the lymphatics after intravenous injection (Hagens *et al.*, 2007). Distribution in the human body is generally followed by rapid clearance from the systemic circulation, predominantly by action of the liver and splenic macrophages (Moghimi *et al.*, 2005). Clearance and opsonization, the process that prepares foreign materials to be more efficiently engulfed by macrophages, occur under certain conditions for

nanoparticles depending on size and surface characteristics (Moghimi *et al.*, 2005). When inhaled, nanoparticles are found to be distributed to the lungs, liver, heart, spleen, and brain (Hagens *et al.*, 2007). Nanoparticles are cleared in the alveolar region via phagocytosis by macrophages facilitated by chemotactic attraction of alveolar macrophages to the deposition site (El-Ansary & Al-Daihan, 2009; Curtis *et al.*, 2006; Garnett & Kallinteri, 2006). The average half-life ($t_{1/2}$) for nanoparticles in the respiratory tract is ≈ 700 days in humans (El-Ansary & Al-Daihan, 2009; Oberdörster *et al.*, 2005b). After intraperitoneal injection, nanoparticles were found to cross the transplacental membrane or cross the peritoneal cavity into uterus. This affected the embryos cranial development and even caused embryo death (Vega-Villa *et al.*, 2008). After oral exposure, nanoparticles were found in the kidneys, liver, spleen, lungs, brain, and the gastrointestinal (GI) tract (Hagens, *et al.*, 2007). Some nanoparticles passed through the GI tract and were rapidly excreted in feces and urine, indicating that they can be absorbed across the GI barrier and into the systemic circulation (Hagens, *et al.*, 2007). There is some evidence, but no proof, that silver nanoparticles are adsorbed within the first few feet of the small intestine, and do not proceed far enough into the gastrointestinal tract to cause problems. However, some nanoparticle systems can accumulate in the liver during the first-pass metabolism (El-Ansary & Al-Daihan, 2009; Oberdörster *et al.*, 2005a).

In sharp contrast to the emphasis on the application of silver nanoparticles, information on the toxicological implication of the use of silver nanoparticles is limited (Chen & Schluesener, 2008; Wijnhoven *et al.*, 2009). Between the different toxicological studies that are reported in the literature so far, the compositions of the silver nanoparticles vary widely. Also the descriptions of used silver formulations diverge from detailed to very limited, with variable attention paid to the size, solubility and aggregation of the nanoparticles. This information may be highly relevant, since a good dispersion of the silver nanoparticles is required for effective toxicological and/or antibacterial activities, and might influence its subsequent toxicity (Lok *et al.*, 2007; Wijnhoven *et al.*, 2009). Toxicity determination of nanosilver particles may be dependent on the size distribution of the particles (Ji *et al.*, 2007; Wijnhoven *et al.*, 2009). Although the oxidation state of the silver nanoparticles may influence their biological and/or toxicological activity, little attention has been paid to the oxidation state of the silver nanoparticles in the literature. Other factors such as ionic strength, pH and the presence/absence of other salts may also play a role in the oxidation state of the silver nanoparticles. Only oxidized silver nanoparticles exert an antibacterial

effect, most likely due to the combination of nanocarrier material (i.e., silver nanoparticle) and the Ag^+ ions which are tightly adsorbed/chemisorbed on the particle surface (Lok *et al.*, 2007; Wijnhoven *et al.*, 2009). It should be noted that reduced silver nanoparticles appeared very unstable and can easily be oxidized (Lok *et al.*, 2007; Wijnhoven *et al.*, 2009). It is because of the antimicrobial properties of oxidized nanosilver that nanosilver is mostly used and studied. According to various studies in the literature, these properties are supposed to be dependent upon the biological activity of silver ions (Ag^+ ; Lansdown, 2007; Wijnhoven *et al.*, 2009). It may be assumed that most of the silver nanoparticles used in the studies discussed in this report are in the oxidized form. Colloidal silver represents another formulation with silver particles. The size of the silver particles in colloidal suspension is assumed to be mainly in the range of 250-400 nm. These particles are aggregates/agglomerates of smaller sized nanoparticles (~100 nm), that under certain conditions can disaggregate/disagglomerate. Reports on colloidal silver have also been included in this report as well.

A case report was published regarding elevated liver enzymes following topical use of a nanosilver preparation on a young burn victim (Trop *et al.*, 2006). Six days after treatment the patient developed grayish discoloration with bluish-lips (argyria) 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 $\mu\text{g}/\text{kg}$) and serum (107 $\mu\text{g}/\text{kg}$) 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 $\mu\text{g}/\text{kg}$, respectively) were still elevated at 7 weeks. In preclinical studies with pigs, no elevated plasma levels or adverse reactions were reported with the same nanosilver preparation (Burrell, 1997). While clinical studies contrasted the efficacy of the nanosilver 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 (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 & Bingham, 1982). It is not clear at this time if the report of hepatotoxicity by Trop *et al.* (2006) with nanosilver is an isolated incidence or the beginning of a trend with nanoscale versus other forms of silver. In the only report on carcinogenicity of silver (Furst & 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.

6.2.3.1 Respiratory Tract Toxicity

Human exposure to inhaled ambient particles, including nanosilver, may have adverse health effects (Buzea *et al.*, 2007; Dockery, 2005; Donaldson *et al.*, 2004; Lippmann *et al.*, 2003; Shah, 2007; Vermeylen *et al.*, 2005). Pulmonary and cardiovascular diseases may result when inhaled particles interfere with the normal function of bodily systems (Peters *et al.*, 1997, 2001 and 2005). The health consequences of particle inhalation vary greatly with particle composition and concentration, among other factors. After inhalation, nanoparticles deposit throughout the entire respiratory tract, starting from nose and pharynx, down to the lungs (Buzea *et al.*, 2007; Oberdöster, 2001; Elder *et al.*, 2006). Lungs consist of airways, which transport air in and out, and alveoli, which are gas exchange surfaces. Human lungs have an internal surface area between 75 and 140 m², and about 300 × 10⁶ alveoli (Hoet *et al.*, 2004). Due to their large surface area, the lung is the primary entry portal for inhaled particles. Spherically shaped solid material with particle diameters smaller than 10 μm can reach the gas exchange surfaces (Buzea *et al.*, 2007; Hoet *et al.*, 2004; Oberdöster, 2001). Larger diameter particles tend to be deposited further up in the respiratory tract as a result of gravitational settling, impaction, and interception (Lippmann, 1990). Many larger diameter fibers are deposited at saddle points in the branching respiratory tree. Smaller-diameter particles are more affected by diffusion, and these can collect in the smaller airways and alveoli. Fibers having a small diameter may penetrate deep into the lung, though very long-aspect ratio fibers will remain in the upper airways (Buzea *et al.*, 2007; Hoet *et al.*, 2004).

The nasopharyngeal region captures mainly microparticles and nanoparticles smaller than 10 nm, while the lungs will receive mainly nanoparticles with diameters between 10 and 20 nm (Buzea *et al.*, 2007; Oberdöster, 2001). Figure 6.4 shows the deposition of nanoparticles in the respiratory tract as a function of their size. A small fraction of the applied dose of

nanosized particles can pass from the epithelial surface of the air space into blood, but the fraction increases if the barrier is disrupted, for example, by an inflammatory stimulus. The amount that gets into blood has also been shown to be size dependent (Chen *et al.*, 2006), with smaller (~55 nm) particles having greater fractional penetration than larger particles (~200 nm).

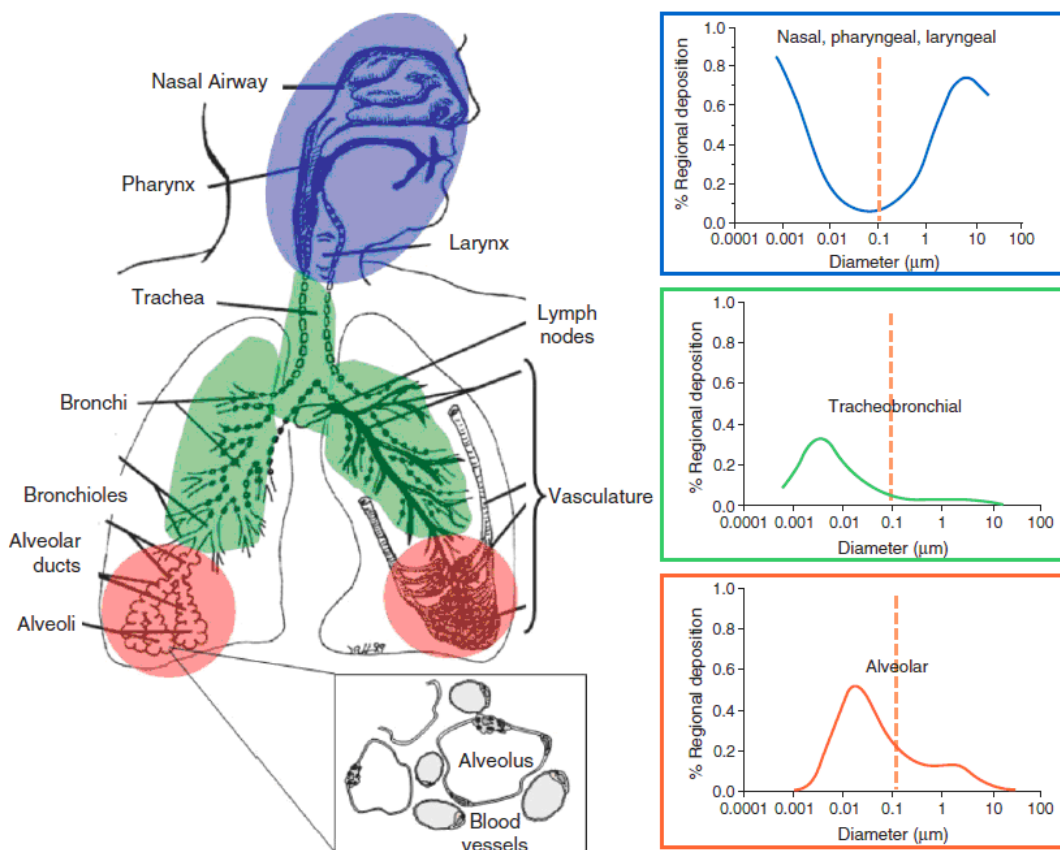


Figure 6.4: Deposition of particles in the respiratory tract as a function of their size, with inset illustrating the proximity of the air spaces (alveoli) to the vasculature (in pink). Reprinted from WIREs Nanomedicine and Nanobiotechnology, Vol. 1 (4), Elder, A., Vidyasagar, S., DeLouise, L., Physicochemical factors that affect metal and metal oxide nanoparticle passage across epithelial barriers, Copyright 2009 with permission from Wiley.

As summarized elsewhere (Asgharian & Price, 2007; Card *et al.*, 2008; Oberdörster *et al.*, 2007), inhaled particles of different sizes exhibit different fractional depositions within the human respiratory tract. Although inhaled ultrafine particles (<100 nm) deposit in all regions, tracheobronchial deposition is highest for particles <10 nm in size, whereas alveolar deposition is highest for particles approximately 10–20 nm in size (Asgharian & Price, 2007; Card *et al.*, 2008; Oberdörster *et al.*, 2007). Particles <20 nm in size also efficiently deposit in the nasopharyngeal-laryngeal region. Human studies of potential adverse pulmonary

effects resulting from exposure to engineered nanoparticles appear to be limited, although a number of investigations into pulmonary deposition patterns of inhaled nanoparticles in the healthy and diseased lung have been conducted (Anderson *et al.*, 1990; Card *et al.*, 2008; Chalupa *et al.*, 2004; Daigle *et al.*, 2003; Moller *et al.*, 2008). Computational models predict increased deposition of inhaled nanoparticles in diseased or constricted airways (Card *et al.*, 2008; Farkas *et al.*, 2006), and consistent with this prediction, obstructive lung disease and asthma have both been demonstrated to increase their pulmonary retention (Anderson *et al.*, 1990; Card *et al.*, 2008; Chalupa *et al.*). Nonetheless, Pietropaoli *et al.* (2004) did not observe differences between healthy and asthmatic subjects in respiratory parameters assessed up to 45 h after a 2-h inhalation of ultrafine carbon particles (up to 25 $\mu\text{g}/\text{m}^3$), nor was airway inflammation observed in either group (measured as exhaled nitric oxide). The same study reported that exposure of healthy subjects to a higher concentration of ultrafine carbon particles (50 $\mu\text{g}/\text{m}^3$ for 2 h) resulted in decreased mid-expiratory flow rate and carbon monoxide diffusing capacity 21 h after exposure, albeit still in the absence of airway inflammation (Card *et al.*, 2008; Pietropaoli *et al.*, 2004). Figure 6.5 shows a simplified depiction of potential factors that may influence the effects of nanoparticles in the respiratory system.

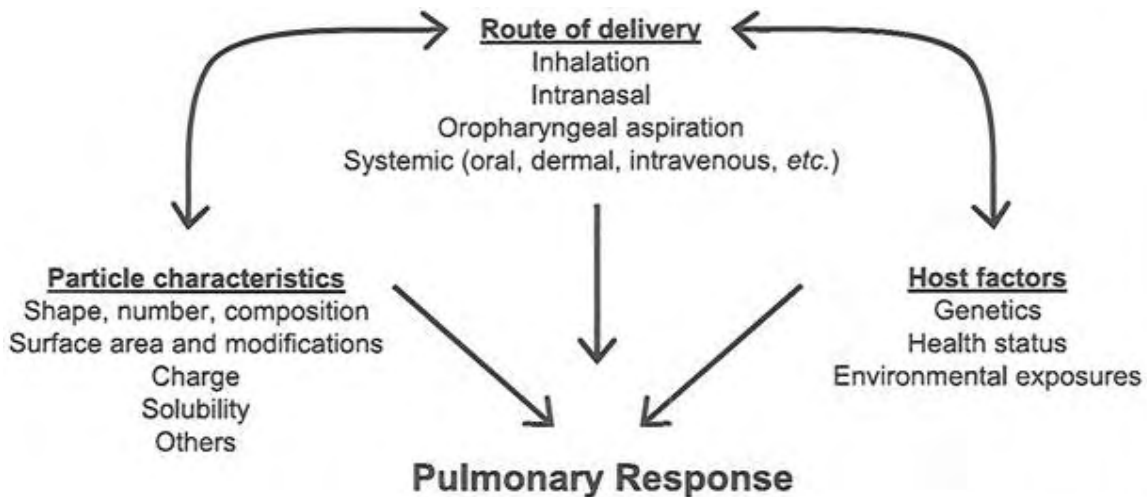


Figure 6.5: A simplified depiction of potential factors that may influence the effects of engineered nanoparticles on the respiratory system. Reprinted from Am. J. Physiol. Lung Cell Mol. Physiol., Vol. 295, Card, J.W.; Zeldin, D.C., Bonner, J.C., Nestmann, E.R., Pulmonary applications and toxicity of engineered nanoparticles, ppL400-L411, Copyright 2008 with permission from American Physiological Society.

There is a need to conduct inhalational studies of exposure to silver nanoparticles to ensure the health of workers and consumers. The dispersion of inhalable ambient nano-sized

particles evenly has been an obstacle in evaluating the effect of the inhalation of nano-sized particles on the respiratory system. Ji *et al.* (2007) exposed Sprague-Dawley rats to silver nanoparticles following OECD test guideline 412 (OECD, 1993), based on 28 days of repeated inhalation with Good Laboratory Practice (GLP) application. Their study used a device that generated silver nanoparticles by evaporation/condensation using a small ceramic heater to distribute the desired concentrations of silver nanoparticles to chambers containing experimental animals. The concentrations and distribution of the silver nanoparticles with respect to the size were also measured directly using a differential mobility analyzer and ultrafine condensation particle counter. Eight week old rats were divided into 4 groups (10 rats in each group): a fresh air control, a low dose group ($1.73 \times 10^4/\text{cm}^3$), middle dose group ($1.27 \times 10^5/\text{cm}^3$), and a high dose group ($1.32 \times 10^6/\text{cm}^3$, which equates to approximately $61 \text{ g}/\text{m}^3$). The animals were exposed to the silver nanoparticles for 6 hours/day, 5 days/week, for a total of 4 weeks. At the conclusion of the exposure period, the clinical and histopathological parameters were examined; later, the tissue distribution of silver nanoparticles in the blood, lungs, brain, olfactory bulb and liver was also investigated. The male and female rats did not show any significant changes in body weight relative to the concentration of silver nanoparticles. No distinct clinical and histopathological effects on the respiratory system of silver nanoparticles were seen during the 28 days inhalation study in rats (Ji *et al.* 2007). The authors report that the content of silver in the liver of male rats increased in a concentration-dependent manner following inhalation of silver nanoparticles for 5 days/week for 4 weeks, and concluded that exposure to silver nanoparticles at a concentration near the current American Conference of Governmental Industrial Hygienists (ACGIH) silver dust limit ($100 \text{ g}/\text{m}^3$) did not appear to have any significant health effects. The study might not have been complete as it lacked specific examinations of the respiratory system such as respiratory rate, airway resistance, tidal volume, hemoglobin oxygen saturation as well as inflammation status.

In a similar study conducted by Takenaka *et al.* (2001), nanosilver accumulation was seen in the lungs of the rats (1.7 mg) of which 4% was still left after seven days, but again additional toxicity parameters were not included. The tissue distribution of inhaled silver nanoparticles seemed to indicate that silver or silver nanoparticles can be translocated to other organs such as the liver, olfactory bulb and brain, as seen in the previous acute silver nanoparticle (15 nm modal diameter) inhalation study (Takenaka *et al.*, 2001). The lung silver concentration

exhibited a dose-dependent increase following silver nanoparticle inhalation exposure. Three possible translocation routes to the blood were suggested by the authors: (1) translocation of silver nanoparticles from the tracheobronchial region by a mucociliary escalator, with subsequent ingestion into the gastrointestinal tract, (2) translocation of the particles into the lymph nodes, and (3) entry into the blood via alveolar epithelial cells. Significant increase of silver in the liver indicated the blood circulation of silver deposited in the lungs. Sung *et al.* (2008) performed a 90 days rat inhalational study (18 nm sized silver nanoparticles 6 hours/day at concentrations of 0.7, 1.4 and 2.9×10^6 particles/cm³). The authors showed lung function decrease (including tidal volume, minute volume and peak inspiration flow), inflammatory lesions in the lung morphology and effects of inflammatory markers.

One acute colloidal silver aerosol inhalation study of rabbits (whole-body exposure; concentration was not provided) reports ultrastructural damage and disruption of the tracheal epithelium (Konradova, 1968). The authors did not provide toxicity data on subacute or subchronic exposure to silver dust. Respiratory effects have been observed in humans following the inhalation of silver compounds (Rosenman *et al.*, 1979, 1987), yet the causal relationship is difficult to establish due to a lack of information on the concentration, diameter, and chemical composition of silver in workplace air along with occupational history of the workers.

Potential factors in the increased inflammatory profile observed for nanoscale materials in some studies include their size, increased number, and higher surface area per unit mass compared with that of larger particles of the same material (Borm *et al.*, 2006; Card *et al.*, 2008; Nel *et al.*, 2006; Oberdörster *et al.*, 2005a). The increased ratio of surface area to mass for nanoparticles meant that a greater percentage of the atoms or molecules of a given particle were present on the surface of the particle; thereby, providing an increased number of potential reactive groups at the particle surface that may influence toxicity. Although this appeared to be a useful metric for assessing the toxic potential of some nanoparticles, there is consensus among experts in the field that no single dose metric (i.e., particle number, size, surface area, or other) has emerged to be useful for assessment of the reactivity and potential toxicity of nanoparticles in general (Maynard & Aitken, 2007; Warheit *et al.*, 2007).

6.2.3.2 Neuronal Uptake

Inhaled nanoparticles are known to reach the nervous system via the olfactory nerves (Borm *et al.*, 2006; Oberdörster *et al.*, 2004, 2005a,b) and/or blood-brain barrier (Borm *et al.*, 2006; Buzea *et al.*, 2007; Peters *et al.*, 2006). Nanoparticles that reach the lungs are predominantly cleared via the mucociliary escalator into the gastrointestinal tract and then eliminated in the feces (Semmler *et al.*, 2004) lymphatic system (Lui *et al.*, 2006), and circulatory systems (Buzea *et al.*, 2007; Oberdörster *et al.*, 2005b). From the lymphatic and circulatory systems, nanoparticles may be distributed to organs, including kidneys from where partial or total clearance may occur.

As mentioned in Section 6.2.3.1, silver was found in the brain of rats systemically exposed to silver nanoparticles via inhalation (Takenaka *et al.* 2001; Ji *et al.* 2007), but no toxicity endpoints were monitored in the brain. Passage of the blood brain barrier (BBB) was also not investigated. According to a recent review on neurotoxicity of silver (Lansdown, 2007), most animal studies indicate that after silver exposure, silver was contained within the blood brain barrier but did not pass it.

In the only known human exposure study available, epileptic seizures and coma following daily ingestion of colloidal silver for 4 months was reported (Mirsattari *et al.* 2004), along with high levels of silver in plasma, erythrocytes and cerebrospinal fluid. The authors suggest that silver caused signs of irreversible neurological toxicity which eventually led to death after the patient remained in a persistent vegetative state for close to 6 months.

6.2.3.3 Dermal Toxicity

Though nanosilver-based dressing and surgical sutures have received approval for clinical application and good control of wound infection has been achieved, their dermal toxicity is still a topic of concern. Despite laboratory and clinical studies confirming the dermal biocompatibility of nanosilver-based dressings, several other researchers have demonstrated the cytotoxicity of these materials (Chen *et al.*, 2006; El-Ansary & El-Daihan, 2009; Limbach *et al.*, 2007; Muangman *et al.*, 2006; Oberdörster *et al.*, 2005b; Supp *et al.*, 2005; Wright *et al.*, 2002). Paddle-Ledinek *et al.* (2006) exposed cultured keratinocytes to extracts of several types of silver containing dressings. The results showed that extracts of nanocrystalline coated dressings are among those that are the most cytotoxic. Keratinocyte

proliferation was significantly inhibited, and cell morphology was affected (El-Ansary & El-Daihan, 2009; Paddle-Ledienk *et al.*, 2006).

Acticoat® is a topical wound dressing consisting of a polyethylene mesh coated with nanosilver (average size 15 nm). There is one reported case of silver poisoning after the use of Acticoat® for treatment of severe burns to the legs (Trop *et al.* 2006; Wijnhoven *et al.*, 2009). On day 6 post-injury, the patient developed a grayish discoloration in the treated area, complained of being tired and had no appetite. On day 7, silver levels in urine and blood were found to be elevated (28 and 107 mg/kg, respectively). Acticoat® was removed and the discoloration of the face gradually faded and liver function test returned to normal values. Elevated blood silver levels were seen 7 weeks post-injury, but were negligible after 10 months. These observed adverse effects may be associated with the release of Ag⁺ ions from the nanosilver dressing. Absorption of silver from Acticoat® was confirmed in 30 patients treated in another study (Vlachou *et al.* 2007; Wijnhoven *et al.*, 2009). Despite measurable amounts of serum silver levels (median 59 g/l), very limited changes in hematological or biochemical indicators of toxicity associated with the silver absorption were observed.

In a moist environment, silver is released from the Acticoat® dressing (possibly as nanocrystals) and improves microbial control of the wound. Acticoat® has been tested in small clinical trials (Innes *et al.*, 2001; Tredget *et al.*, 1998; Wijnhoven *et al.*, 2009) with contradictory results. No adverse effects were found in the Tredget *et al.* study, but silver absorption was not assessed (Tredget *et al.*, 1998). Innes *et al.* (2001) reported delayed re-epithelialization and temporary scars while in another study, an increase in re-epithelization was found in meshed skin grafts (Demling & Leslie DeSanti, 2002). A case of delayed wound healing was also reported by Trop *et al.* (2006). All the studies mentioned in this paragraph were small scale and used different controls; thus, inter-study comparison was not possible.

In a porcine model of wound healing, nanosilver wound dressing promoted rapid wound healing of full-thickness wounds on the back of pigs (Wright *et al.*, 2002; Wijnhoven *et al.*, 2009). The proteolytic environment of the wounds treated with nanosilver was characterized by reduced levels of metalloproteinases and enhanced cellular apoptosis. Application of Acticoat® on cultured skin substitutes grafted on nude mice did not inhibit nor promote

wound healing (Supp *et al.*, 2005). In the mice, application of a 1% nanosilver cream (96.1% is <50 nm) induced apoptosis of inflammatory cells but not of keratinocytes (Supp *et al.*, 2005).

The potential cytotoxicity of silver nanoparticles in human epidermal keratinocytes and their inflammatory and penetrating potential into porcine skin *in vivo* was assessed by Samberg *et al.* (2010). The authors used eight different types of silver nanoparticles [unwashed/uncoated (20, 50, and 80 nm particle diameter), washed/uncoated (20, 50, and 80 nm), and carbon-coated (25 and 35 nm)]. Skin was dosed topically for 14 consecutive days. Human epidermal keratinocytes (HEK) viability was assessed by MTT, alamarBlue (aB), and CellTiter 96 AQueous One (96AQ). Release of the proinflammatory mediators interleukin (IL)-1 β , IL-6, IL-8, IL-10, and tumor necrosis factor- α (TNF- α) were measured. The effect of the unwashed silver nanoparticles on HEK viability after a 24-hr exposure indicated a significant dose-dependent decrease ($p < 0.05$) at 0.34 $\mu\text{g/ml}$ with aB and 96AQ, and at 1.7 $\mu\text{g/ml}$ with MTT. Both the washed silver nanoparticles and carbon-coated silver nanoparticles showed no significant decrease in viability at any concentration assessed by any of the three assays. For each of the unwashed nanoparticles, a significant increase ($p < 0.05$) in IL-1 β , IL-6, IL-8, and TNF- α concentrations were noted. Macroscopic observations showed no gross irritation in porcine skin, whereas microscopic and ultrastructural observations showed areas of focal inflammation and localization of nanosilver on the surface and in the upper stratum corneum layers of the skin. Samberg *et al.* (2010) showed that nanosilver was nontoxic when dosed in washed nanosilver particle solutions or carbon-coated nanosilver particles. The authors also concluded that toxicity of nanosilver in HEKs was influenced by the residual contaminants in the nanosilver solutions, and that the particles themselves may not have been responsible for an increase in cell mortality.

6.2.3.4 Gastrointestinal Tract Toxicity

Jeong *et al.* (2010) investigated the effects of silver nanoparticles on the histological structure and properties of the mucosubstances in the intestinal mucosa of Sprague-Dawley rats. For the experiment, the rats were divided into four groups (10 rats in each group): control group, low-dose group (30 mg/kg), middle-dose group (300 mg/kg), and high-dose group (1,000 mg/kg). Silver nanoparticles (60 nm) were administered for 28 days, following OECD test guideline 407 and using GLP. The control contained no silver nanoparticles; however, the treated samples showed luminal and surface particles, and the tissue contained silver

nanoparticles. A dose-dependent increased accumulation of silver nanoparticles was observed in the lamina propria in both the small and large intestine, in the tip of the upper villi in the ileum and in the protruding surface of the fold in the colon. The silver nanoparticle-treated rats exhibited higher numbers of mucus granules than the controls, resulting in more mucus materials in the crypt lumen and ileal lumen. The authors concluded that silver nanoparticles induced the discharge of mucus granules along with an abnormal mucus composition in the goblet cells of the intestines.

In a previous inhalation study, Ji *et al.* (2007) found no significant toxicity in Sprague–Dawley rats that had been repeatedly exposed to silver nanoparticles via inhalation, based on 6 h each day, 5 days a week, for a total of 4 weeks. Plus, no significant changes were found in the male and female body weights or hematology and blood biochemical values relative to various concentrations of silver nanoparticles during the 28-day experiment (Ji *et al.*, 2007). In another oral toxicity study using male and female Sprague–Dawley rats, Kim *et al.* (2008) found that oral exposure to silver nanoparticles (60 nm) at concentrations of 30, 300, and 1,000 mg/kg for 28 days had no effect on micronucleated polychromatic erythrocytes or bone marrow cells. However, the repeated oral doses of silver nanoparticles did induce liver toxicity and had a coagulation effect on peripheral blood. The histopathologically evaluated liver toxicity included dilatation of the central vein, bile duct, and hyperplasia (Kim *et al.* 2008).

6.2.3.5 Other Organ Toxicity

Tang *et al.* (2009) investigated the distribution and accumulation of silver nanoparticles in rats with subcutaneous injection. Rats were injected with either silver nanoparticles or silver microparticles at 62.8 mg/kg, and then sacrificed at predetermined time points. The main organs of the experimental animals, including the kidney, liver, pancreas, brain, lung and heart, were harvested for ultrastructural analysis by transmission electron microscopy (TEM) and for silver content analysis by inductively coupled plasma mass spectrometry (ICP-MS). Results indicated that the silver nanoparticles were carried to the organs by blood circulation, and was distributed throughout the main organs, especially in the kidney, liver, spleen, brain and lung in the form of particles. Silver microparticles, however, could not invade the blood stream or organ tissues. Ultrastructural observations indicate that those nanoparticles that had accumulated in organs could enter different kinds of cells, such as renal tubular epithelial cells and hepatic cells. The nanoparticles also induced blood-brain barrier (BBB) destruction

and astrocyte swelling, and caused neuronal degeneration. The results suggest that the cellular uptake of silver nanoparticles is dependent on its size.

Death has been observed in rats following exposure to very high doses of colloidal silver after intravenous administration (LD₅₀, 67 mg/kg) (Schmaehl & Steinhoff, 1960) and after oral ingestion (1680 mg/kg/day for four days; ATSDR, 1990). Following the intravenous injection of colloidal silver, rats died from lung edema; while liver, spleen and kidney showed signs of brown discoloration (Schmaehl & Steinhoff 1960). The cause of death following oral intake was not reported. Chronic subcutaneous administration of colloidal silver (1.75-2.5 mg weekly) appeared relatively well tolerated, apart from the development of argyria. Very limited health effects were observed in a 28-day inhalation toxicity study (Ji *et al.*, 2007) and a 28-day oral toxicity study (Kim *et al.*, 2008) with silver nanoparticles in Sprague-Dawley rats despite absorption into major organs. In the Ji *et al.* study rats inhaled 12-16 nm sized silver nanoparticles 6 h/day, 5 d/week, for four weeks. Three exposure levels were used, a low exposure of 1.73×10^4 /cm³, a medium exposure of 1.27×10^5 /cm³, and a high exposure of 1.3×10^6 nanoparticles/cm³ (approximately 61 g/m³, which is near the American Conference of Governmental Industrial Hygienists (ACGIH) silver dust threshold limit of 0.1 mg/m³). A study on the 28-day oral toxicity of silver nanoparticles (60 nm) at doses of 30 mg/kg, 300 mg/kg, and 1,000 mg/kg, following OECD test guideline 407 and good laboratory practices (GLP), indicated that exposure to over 300 mg of silver nanoparticles could result in slight liver damage, plus a gender-related difference was noted in the accumulation of silver in the kidneys, with a twofold higher accumulation in the female kidneys when compared with the male kidneys (Kim *et al.* 2008).

6.2.3.5.1 Kidney Toxicity

Previously, Kim *et al.* (2008) reported gender differences in the accumulation of silver nanoparticles in kidneys of Sprague-Dawley rats after subacute exposure. It is of interest that subacute inhalation of silver nanoparticles did not produce gender differences with respect to accumulation in various tissues. In a study by Kim *et al.* (2009), the tissue distribution of silver nanoparticles showed a dose-dependent accumulation of silver in all the tissues examined, including testes, kidneys, liver, brain, lungs, and blood. A gender-related difference in the accumulation of silver was noted in the kidneys, with a twofold higher concentration in female kidneys compared males after subacute exposure to silver nanoparticles via inhalation or oral ingestion. To investigate the gender specific accumulation

of silver nanoparticles in kidneys of Fischer 344 rats, detailed histopathological studies were conducted by silver enhancement staining. Female rats showed a higher accumulation of silver nanoparticles in all kidney regions, including cortex, outer medulla, and inner medulla. In particular, the glomerulus in the cortex contained a higher accumulation in females than males. The silver nanoparticles were also preferentially accumulated in the basement membranes of the renal tubules in the cortex, middle and terminal parts of the inner medulla, and outer medulla. Silver nanoparticles were detected in the cytoplasm and nuclei of interstitial cells in the inner medulla of the kidney.

6.2.3.5.2 Liver Toxicity

Significant amounts of silver in the liver were observed after inhalation (Takenaka *et al.*, 2001; Ji *et al.*, 2007; Wijnhoven *et al.*, 2009). At each time point analyzed, 9-21% of the nanosilver lung content was observed in the liver (Takenaka *et al.*, 2001). Histopathology of the liver revealed cytoplasmic vacuolization in both sexes with a clear dose dependent increase in females. Several cases of hepatic focal necrosis were seen in the high dose groups (Ji *et al.* 2007). No effect on the liver enzyme alkaline phosphatase (ALP) was observed. In contrast, repeated oral doses of 60 nm silver nanoparticles during 28 days did induce liver toxicity, as shown by increases in ALP and histopathological observations of dilatation of the central vein, bile-duct hyperplasia and increased foci (Kim *et al.*, 2008). In the case report of Trop *et al.* (2006), elevated liver enzymes (aspartate amino transferase, alanine aminotransferase and gamma-galactosyl transferase) after the use of Acticoat® were reported. Levels returned to normal following cessation of exposure. The patient did not receive any other potentially hepatotoxic medication.

6.2.3.5.3 Immune System Toxicity

No treatment related effects on hematology and blood cell subset distribution (% lymphocytes, monocytes, etc.) was seen after inhalation of nanosilver particles (Wijnhoven *et al.*, 2009). Of note, nanosilver particles were detectable in the spleen in the Takenaka study (Takenaka *et al.*, 2001), but not in the Ji study (Ji *et al.*, 2007). In the 90-day inhalation study of Sung *et al.* (2008), the presence of nanosilver particles in the lung may have induced a local inflammatory response in the high dose group. Parameters on potential systemic immune effects were not monitored in this study (Sung *et al.*, 2008). In mice, application of a 1% nanosilver cream (96.1% is <50 nm) inhibited DNB-induced allergic contact dermatitis (Bhol & Schechter, 2005). It was found that the expression of two cytokines (TNF and IL-12) was suppressed (histopathological staining) and apoptosis of inflammatory cells but not

keratinocytes was induced. Similar concentration-dependent anti-inflammatory effects have also been seen in guinea pigs by the same group (Bhol *et al.*, 2004). These latter data may suggest that silver nanoparticles are especially effective at inhibiting inflammations and may thus be used to treat immunologic and inflammatory diseases (Shin *et al.*, 2007). Further studies may be necessary to determine effective doses since local inflammatory responses may be induced when applying high doses of nanosilver particles (Sung *et al.* 2008).

At high concentrations, nanoparticles tend to cluster, forming aggregates often larger than 100 nm. Larger nanoparticles (>100 nm) can be readily phagocytized by alveolar macrophages (Buzea *et al.*, 2007; Oberdörster, 1988; Takenaka *et al.*, 2001). Results of studies involving inhalation or intratracheal instillation of high concentrations of nanoparticle (silver, iron, India ink, or titanium dioxide) smaller than 100 nm, which aggregate in larger particles, suggest that most nanoparticles are indeed stopped by alveolar macrophages (Takenaka *et al.*, 2001). Rat studies based on inhalation of low concentrations of 15 nm diameter silver nanoparticles showed that soon after inhalation (30 min), nanoparticles are distributed in the blood and brain, and subsequently, to organs, such as heart and kidney, while the lungs are rapidly cleared off of the nanoparticles (Buzea *et al.*, 2007; Takenaka *et al.*, 2001). Hence, minute concentrations of nanoparticles with size smaller than 100 nm can have a higher probability of translocating to the circulatory system and organs than high concentrations of the same particles, which are likely to form aggregates and will be stopped from translocation by macrophage phagocytosis (Buzea *et al.*, 2007).

Very limited changes in hematological or biochemical indicators of toxicity were associated with the silver absorption from Acticoat® in humans (Vlachou *et al.*, 2007), despite measurable amounts of silver in serum. Another case report possibly involving uptake of silver particles is the finding of small electron-dense particles, probably silver nanoparticles, in mast cells following 20 years of local acupuncture (Kakurai *et al.*, 2003). The mast cells showed focal or partial loss of granule content suggesting degranulation (activation) associated with pruritus (itching) and an inflammatory reaction.

6.2.3.5.4 Other blood effects

Apart from a small increase in blood calcium, no additional effects of systemic exposure of nanosilver on hematology and blood chemistry parameters have been reported after inhalation exposure (Ji *et al.*, 2007). Oral administration of 60 nm silver nanoparticles

induced some changes in the red blood cell compartment (increased red blood cell count, hemoglobin, and hematocrit) and on coagulation parameters (decreased active partial prothrombin time) (Kim *et al.*, 2008).

6.2.3.5.5 Reproductive System Toxicity

In the Ji *et al.* study (2007), no effect on the histopathology of the epididymis was noted. No additional *in vivo* data on the potential toxic effect of nanosilver on female or male reproductive function (e.g., female egg development or male sperm formation) is readily available.

6.2.3.5.6 Genotoxicity, Carcinogenicity

In a chronic subcutaneous administration of colloidal silver (1.75-2.5 mg weekly), eight of the 26 (31%) rats that survived longer than 16 months developed malignant tumors (Schmaehl & Steinhoff, 1960; Wijnhoven *et al.*, 2009). In six of the rats, the tumor arose at the site of subcutaneous injection. This was significantly higher than the historical control tumor levels that were between 1-3% (Schmaehl & Steinhoff, 1960; Wijnhoven *et al.*, 2009). In contrast, no tumor induction at the site of injection was found in rats after intramuscular injection of a suspension of fine silver powder (<300 mesh) in trioctanoin (Furst & Schlauder, 1978). Kim *et al.* (2008) investigated the *in vivo* genotoxicity using a bone marrow micronucleus test after oral administration of 60 nm silver nanoparticles for 28 days at various doses. They found no statistically significant effects.

6.2.4 Cell Culture Nanosilver Toxicity

In vitro cell line studies by Samberg *et al.* (2010) have shown decreased mitochondrial function after exposure to silver nanoparticles in murine neuroblastoma cells (Schrand *et al.*, 2008), hepatic cells (Hussain *et al.*, 2005), germline stem cells (Braydich-Stolle *et al.*, 2005), human skin carcinoma cells (Arora *et al.*, 2008), and human epidermal keratinocytes (HEKs) and fibroblasts (Burd *et al.*, 2007). Although *in vivo* studies have not been performed with silver nanoparticles, polyvinylpyrrolidone (PVP)-stabilized silver nanoparticles with a mean size of 25 nm were shown to penetrate into the upper layers of the epidermis in excised human skin in static diffusion cells (Larese *et al.*, 2009). The *in vitro* cytotoxicity of silver nanoparticles (15 nm diameter) in mammalian mouse C18-4 germline stem cells indicated that a silver nanoparticle concentration of more than 5 µg/ml reduced the mitochondrial function and cell viability while increasing the LDH leakage (Braydich-Stolle *et al.*, 2005). It also suggested that the use of silver nanoparticles in bone cement or implantable devices as

antimicrobial agents (Alt *et al.*, 2004) could be toxic for the bone-lining cells and other tissues.

Carlson *et al.* (2008) designed a study to evaluate size-dependent cellular interactions of known biologically active silver nanoparticles (Ag-15nm, Ag-30nm, and Ag-55nm). Alveolar macrophages provide the first defense and were studied for their potential role in initiating oxidative stress. Cell exposure produced morphologically abnormal sizes and adherence characteristics with significant nanoparticle uptake at high doses after 24 h. Toxicity evaluations using mitochondrial and cell membrane viability along with reactive oxygen species (ROS) were performed. After 24 h of exposure, viability metrics significantly decreased with increasing dose (10-75 µg/ml) of Ag-15nm and Ag-30nm nanoparticles. A more than 10-fold increase of ROS levels in cells exposed to 50 µg/ml Ag-15nm suggests that the cytotoxicity of Ag-15nm is likely to be mediated through oxidative stress. Activation of the release of traditional inflammatory mediators were examined by measuring levels of cytokines/chemokines, including tumor necrosis factor (TNF- α), macrophage inhibitory protein (MIP-2), and interleukin-6 (IL-6), released into the culture media. After 24 h of exposure to Ag-15nm nanoparticles, a significant inflammatory response was observed by the release of TNF- α , MIP-2, and IL-1 β . There was no detectable level of IL-6 upon exposure to silver nanoparticles. A size-dependent toxicity was produced by silver nanoparticles, and one predominant mechanism of toxicity was found to be largely mediated through oxidative stress. The toxicity of silver exhibited in liver cells was also shown to be mediated by oxidative stress (Hussain *et al.*, 2005), and silver nanoparticles were found to induce toxicity in germline stem cells (Braydich-Stolle *et al.*, 2005)

There are various *in vitro* studies found in the literature on the effects of silver nanoparticles with size varying between 1 and 100 nm (Hussain *et al.*, 2005; Park *et al.*, 2007; Skebo *et al.*, 2007; Suzuki *et al.*, 2007). There is no consensus on the cytotoxicity of nanosilver. Most publications do show reduced cell viability following exposure. Additional toxic effects seen in the *in vitro* studies are glutathione depletion, mitochondrial deviations or destruction and damage to cell membranes. *In vitro* exposure of human peripheral blood mononuclear cells to silver nanoparticles (1-2.5 nm, 72 h) resulted in inhibition of phytohemagglutinin (PHA) induced proliferation (at a concentration of 15 ppm) (Shin *et al.*, 2007).

Hussain *et al.* (2005) evaluated the *in vitro* toxicity of several nanoparticles, including nanosilver (15 and 100 nm) on a rat liver derived cell line (BRL 3A). Following 24 h after exposure, the mitochondrial function and membrane integrity were significantly decreased (at ≥ 5 mg/ml and ≥ 10 mg/ml, respectively). LDH leakage was dose dependent and more severe for 100 nm than for 15 nm silver nanoparticles. Visual microscopic evaluation indicated that not all nanoparticles accumulated in the cell, but some remained associated with membranes. The observed cytotoxicity was attributed to be mediated by oxidative stress, as indicated by the detection of GSH depletion, reduced mitochondrial potential, and increased reactive oxygen species (ROS) levels. A similar concentration-dependent cytotoxicity was observed when the effects of the same nanosilver particles on a mouse cell line with spermatogonial stem cell characteristics was studied (Braydich-Stolle *et al.*, 2005). Here, a concentration dependent effect on mitochondrial function, cell viability and membrane integrity was seen, albeit at somewhat lower concentrations. In another study, nanosilver particles (~30 nm) were classified again to be amongst the most cytotoxic nanoparticles when tested on a murine alveolar macrophage cell line, a human alveolar macrophage cell line and epithelial lung cell line (Soto *et al.*, 2005, 2007).

Using a human alveolar epithelial cell line (A549), Park *et al.* (2007) confirmed that various metallic nanoparticles (Ag, TiO₂, Ni, Zn, Al) induce variable extents of cellular toxicity in a dose dependent manner. Nanosilver (mean diameter 150 nm, 24 h exposure, and concentrations up to 200 mg/ml) were found to be among the least cytotoxic among the metallic nanoparticles. Neuroendocrine cells were found to be sensitive to the cytotoxic activity of silver nanoparticles (15 nm; Hussain *et al.*, 2006). Inhibition of dopamine production was only seen at the highest cytotoxic levels. Addition of 1.0% silver nanoparticles (5-50 nm) to bone cement, a dose at which antibactericidal activity was seen, did not result in additional cytotoxicity towards mouse fibroblasts (L929), or on growth of human osteoblast cell line (hFOB 1.19) (Alt *et al.*, 2004).

Acticoat® dressing was found to be cytotoxic to primary keratinocytes cultured on a pliable hyaluronate derived membrane (Laserskin) (Lam *et al.*, 2004). Reduced mitochondrial metabolism, as well as reduced viability of human keratinocytes and fibroblast cultured on a collagen substrate were detected (Supp *et al.*, 2005). Similar effects (cytotoxicity and disordered morphology) on keratinocytes were reported for extracts of various silver-containing dressings (including Acticoat®) (Paddle-Ledinek *et al.*, 2006). Fibroblasts appear

to be more sensitive for these effects than keratinocytes (Poon & Burd, 2004). When the complexity of the environment increased, e.g., after 3-d culture in collagen lattices, the toxic effect of silver appears to decrease (Poon & Burd, 2004).

Carlson *et al.* (2008) reported size-dependent alveolar macrophage cells interactions with silver nanoparticles. Their result shows that after 24 hr of exposure, viability significantly decreased with increasing dose (10–75 $\mu\text{g}/\text{mL}$) of 15 and 30 nm silver nanoparticles. There was more than a 10 \times increase of ROS levels in cells exposed to 50 $\mu\text{g}/\text{mL}$ of 15 nm silver nanoparticles, which suggests that oxidative stress may be responsible for the observed cytotoxicity.

Soto *et al.* (2005) characterized a range of manufactured nanoparticulate materials, including Ag, TiO₂, Fe₂O₃, Al₂O₃, ZrO₂, Si₃N₄, and a range of carbonaceous nanoparticulate materials: single-wall and multi-wall carbon nanotube aggregates and aggregated nanoparticles of black carbon, as well as commercial (mineral grade) chrysotile asbestos nanotube aggregates, using transmission electron microscopy. These nanoparticulate materials ranged in primary particle sizes from roughly 3 to 150 nm (except for the nanotube materials with lengths in excess of 15 μm). Aggregate sizes ranged from 25 nm to 20 μm . Comparative cytotoxicological assessment of these nanomaterials was performed utilizing a murine (lung) macrophage cell line. Considering the chrysotile asbestos to be a positive control, and assigning it a relative cytotoxicity index of unity (1.0), relative cytotoxicity indexes were observed as follows at concentrations of 5 $\mu\text{g}/\text{ml}$: 1.6 and 0.4 for Ag and TiO₂, respectively; 0.7–0.9 for the Fe₂O₃, Al₂O₃ and ZrO₂, 0.4 for the Si₃N₄, 0.8 for the black carbon, and 0.9 to 1.1 for the carbon nanotube aggregate samples. Observations of a cytotoxic response, nearly identical to that for chrysotile asbestos, for multi-wall carbon nanotube aggregates which very closely resemble anthropogenic multi-wall carbon nanotubes in the environment, raise some concern for potential health effects, especially for long-term exposure.

Kim *et al.* (2009) demonstrated the cytotoxicity induced by silver nanoparticles and the role that oxidative stress plays in the process in human hepatoma cells. Toxicity induced by silver (Ag⁺) ions was studied in parallel using AgNO₃ as the Ag⁺ ion source. Using cation exchange treatment, the authors confirmed that the silver nanoparticle solution contained a negligible amount of free Ag⁺ ions. Metal-responsive metallothionein 1b (MT1b) mRNA expression

was not induced in nanosilver-treated cells, while it was induced in AgNO₃-treated cells. These results indicate that nanosilver-treated cells have limited exposure to Ag⁺ ions, despite the potential release of Ag⁺ ions from silver nanoparticles in cell culture. The nanoparticles agglomerated in the cytoplasm and nuclei of treated cells, and induced intracellular oxidative stress. Nanosilver exhibited cytotoxicity with a potency comparable to that of Ag⁺ ions in *in vitro* cytotoxicity assays. The toxicity of silver nanoparticles was prevented by use of the antioxidant N-acetylcysteine, and nanosilver-induced DNA damage was also prevented by N-acetylcysteine. AgNO₃ treatment induced oxidative stress-related glutathione peroxidase 1 (GPx1) and catalase expression to a greater extent than nanosilver exposure, but treatment with AgNO₃ and silver nanoparticles induced comparable superoxide dismutase 1 (SOD1) expression levels. These findings suggest that nanosilver cytotoxicity is primarily the result of oxidative stress and is independent of the toxicity of Ag⁺ ions.

Foldbjerg *et al.* (2009) investigated the toxicity of silver nanoparticles *in vitro*. Silver ions (Ag⁺) have been used in medical treatments for decades whereas silver nanoparticles have been used in a variety of consumer products within recent years. This study was undertaken to compare the effect of well characterized, PVP-coated nanosilver (69nm±3 nm) and Ag⁺ in a human monocytic cell line (THP-1). Characterization of the nanosilver was conducted in both stock suspension and cell media with and without serum and antibiotics. By using the flowcytometric annexin V/propidium iodide (PI) assay, both nanosilver and Ag⁺ were shown to induce apoptosis and necrosis in THP-1 cells depending on dose and exposure time.

Kawata *et al.* (2009) evaluated *in vitro* toxicity of nanosilver at non-cytotoxic doses in human hepatoma cell line, HepG2, based on cell viability assay, micronucleus test, and DNA microarray analysis. They also used polystyrene nanoparticles (PS-NPs) and silver carbonate (Ag₂CO₃) as test materials to compare the toxic effects with respect to different raw chemical composition and form of silver. The cell viability assay demonstrated that silver nanoparticles accelerated cell proliferation at low doses (<0.5 mg/L), which was supported by the DNA microarray analysis showing significant induction of genes associated with cell cycle progression. Only nanosilver exposure exhibited a significant cytotoxicity at higher doses (>1.0 mg/L) and induced abnormal cellular morphology, displaying cellular shrinkage and acquisition of an irregular shape. Only nanosilver exposure increased the frequency of micronucleus formation up to 47.9 ± 3.2% of binucleated cells, suggesting that silver

nanoparticles appear to cause much stronger damages to chromosome than PS-NPs and ionic Ag^+ .

In a study by Asharani *et al.* (2009), normal human lung fibroblast cells (IMR-90) and human glioblastoma cells (U251) were exposed to different doses of nanosilver *in vitro*. Uptake of nanosilver occurred mainly through endocytosis, accompanied by a time dependent increase in exocytosis rate. The electron micrographs revealed a uniform intracellular distribution of nanosilver both in cytoplasm and nucleus. Nanosilver treated cells exhibited chromosome instability and mitotic arrest in human cells. There was efficient recovery from arrest in normal human fibroblasts whereas the cancer cells ceased to proliferate. Toxicity of nanosilver is mediated through intracellular calcium (Ca^{2+}) transients along with significant alterations in cell morphology and spreading and surface ruffling. Down regulation of major actin binding protein, filamin, was observed after silver nanoparticle exposure. Silver nanoparticle induced stress resulted in an increase in the number of metallothionein and heme oxygenase -1 genes (upregulation). The results suggest that cancer cells are susceptible to damage with lack of recovery from nanosilver-induced stress. Silver nanoparticles are found to be acting through intracellular calcium transients and chromosomal aberrations, either directly or through activation of catabolic enzymes. Figure 6.6 shows the proposed mechanism of nanosilver toxicity based on the results from this study. The signaling cascades are believed to play key roles in cytoskeleton deformations and ultimately to inhibit cell proliferation.

According to the criteria of the USEPA (U.S. EPA, 1994), silver is not classifiable as a human carcinogen (group D). Silver powder and colloidal silver do not induce cancer in animals, and silver chloride is considered nonmutagenic in the Ames assay. Silver compounds have been generally considered not to have carcinogenicity in humans and animals. No evidence of the carcinogenicity of silver nanoparticles has been reported despite the growing commercialization of nanosilver. The upregulation (increase in quantity of cells upon external stimulation) of a number of the genes associated with DNA repair and the increase in micronuclei in the nanosilver exposed cells at relatively low doses (<1.0 mg/L) clearly suggested the DNA damaging effects (chromosome aberration) of silver nanoparticles. Both nanosilver and Ag^+ contribute to the toxic effects of silver nanoparticles. The nanosilver concentration assessed in the study by Kawata *et al.* (2009) would be higher than those occurring in air and water environment. Since the internal kinetics of nanoparticles

has not been elucidated, the local concentration in tissues might reach higher level as the result of accumulation. Given that the physicochemical properties of nanosilver such as particle size, particle agglomeration, and dispersibility significantly influence the degree and actions of toxicity of silver nanoparticles, further research is necessary to assess the effects of these variables.

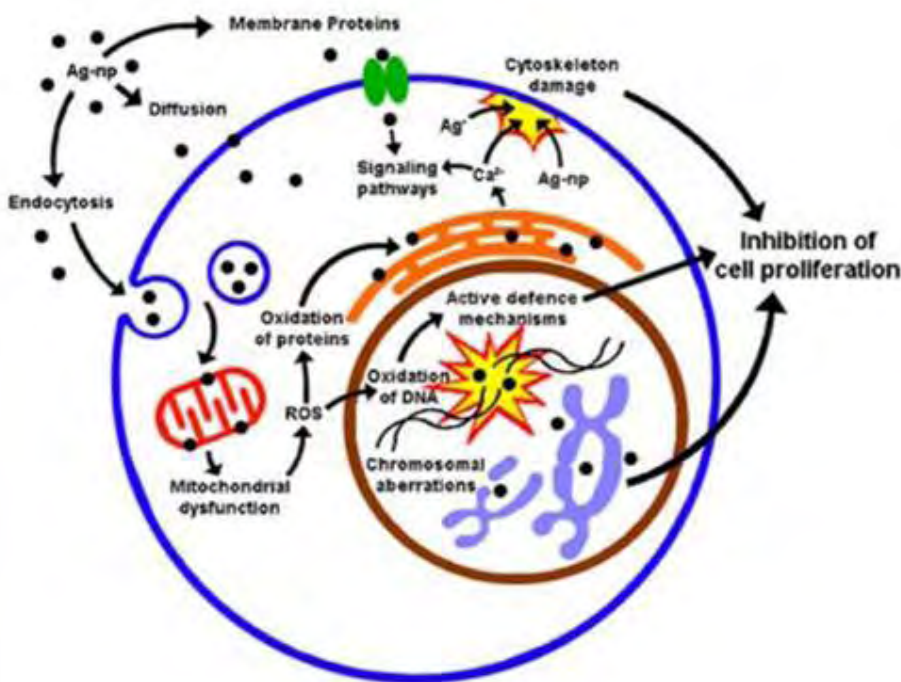


Figure 6.6: The proposed mechanism of nanosilver toxicity based on the experimental data obtained by Asharani *et al.* (2009). Reprinted from BMC Cell Biology, Vol. 10, Asharani, P.V., Hande, M.P., Valiyaveetil, S. Anti-proliferative activity of silver nanoparticles, pp65-79, Copyright 2009 with permission from BioMed Central.

6.3 Conclusions on Nanosilver Toxicity

Several factors influence the ability of a metal to produce toxic effects on the body. These include the solubility of the metal, its ability to bind to biological sites, and the degree to which the complexes formed are sequestered or metabolized and excreted. For nano-sized particles, additional parameters such as size and surface area are recognized as important determinants for toxicity (Ji *et al.* 2007; Wijnhoven *et al.*, 2007). Nanoparticles can pass through biological membranes. After administration, nanoparticles are small enough to penetrate even very small capillaries throughout the body. Silver nanoparticles are used because of the antibacterial activity of silver. The antibacterial action of silver (ions) may

have several mechanisms. It has been suggested that the primary mechanism of action is cell death due to the uncoupling of oxidative phosphorylation (Holt & Bard, 2005) or the induction of free radical formation (Kim *et al.*, 2007). Interference with respiratory chain at the cytochrome C level, and/or with components of microbial electron transport system, has also been reported (Muangman *et al.*, 2006). Interactions with membrane bound enzymes and protein thiol groups that may result in compromised cell wall integrity have been postulated (Bragg & Rainnie, 1974; Lok *et al.*, 2006; Silver, 2003; Wijnhoven *et al.*, 2007; Zeiri *et al.*, 2004). It has also been suggested that silver ions bind to DNA and may cause DNA strand breaks and DNA replication (ATSDR, 1990; Russell & Hugo, 1994). The reason why eukaryotic (mammalian) cells appear less sensitive to this action of silver can be explained by the higher structural and functional redundancy and size of eukaryotic compared to prokaryotic (bacterial) cells. This may increase the silver concentration needed to achieve comparable toxic effects on eukaryotic cells than for bacterial cells (Alt *et al.*, 2004). There may be a therapeutic window in which bacterial cells are successfully attacked, at which harmful effects on eukaryotic cells cannot yet be observed. The effective concentration for silver nanoparticles is much lower in comparison to Ag⁺ ions (nmol vs. mmol levels) (Lok *et al.*, 2006). Given the potential higher toxicity and the specific concerns associated with the use of nano-sized materials particular attention to the toxicity of silver nanoparticles may be warranted.

Information on the toxicological implication of the use of silver nanoparticles is limited (Chen & Schluesener, 2008; Wijnhoven *et al.*, 2007). Toxicity of silver nanoparticles is mostly determined *in vitro* with particles ranging in size from 1-100 nm. The available *in vivo* animal studies are generally relative short term (max 28 days), except for one 90 day inhalation study of the use of one size of silver nanoparticles (Sung *et al.*, 2008). Only limited health effects of the use of nanosilver in humans have been documented. Argyria or argyrosis was rarely reported, and appeared to occur only after intake of large amounts of silver particles (usually colloidal, a suspension with nanosilver of different sizes). Potential target organs for nanosilver toxicity may involve the liver, kidneys and the immune system. Accumulation and histopathological effects were seen in the liver of rats systemically exposed to silver 10-15 nm nanoparticles (Ji *et al.*, 2007), while an effect on liver enzymes was noted in one human case study with dermal exposure to particles with an average of the same size (Trop *et al.*, 2006). Accumulation, histopathological effects and increased liver enzymes were reported after oral exposure to 60 nm nanosilver (Kim *et al.*, 2008). It is not

known if the silver reaches the liver as silver nanoparticles or as ions, nor has the location of the silver (nanoparticles) within the liver been studied. Effects on the immune system, especially cytokine excretion, have been noted *in vitro* and *in vivo*, where application of a 1% nanosilver cream with <50 nm particles, inhibited DNB-induced allergic contact dermatitis (Bhol & Schechter, 2005), and accumulation in the spleen has also been noted (Takenaka *et al.*, 2001). It has been suggested that silver nanoparticles are especially effective at inhibiting inflammations and may be used to treat immunologic and inflammatory diseases (Shin *et al.*, 2007). There were only very limited changes in hematological or biochemical indicators of toxicity associated with the systemic silver absorption from 15 nm nanosilver wound dressings in humans (Vlachou *et al.*, 2007), and after inhalation in rats (Ji *et al.*, 2007). Oral administration of 60 nm silver particles in rats induced some local inflammatory effects (Kim *et al.*, 2008). Whether silver nanoparticles indeed have a (systemic) effect on immune function *in vivo* needs to be further explored.

No reports on effects of silver nanoparticles on the cardiovascular, renal/urinary or gastrointestinal systems for humans have been found, however, specific studies addressing these organs have not been identified. There are very limited well controlled human studies on the potential toxicities of nanosilver. Additional long term, higher dosed studies, preferably using multiple particle sizes, are needed to better characterize the risk of the use of silver nanoparticles on humans.

7. Life Cycle Analysis for Comprehensive Environmental Assessment

Materials that incorporate particles manufactured at the nano scale (nanomaterials) may have many potential benefits to society with their development and deployment in science, engineering and technology. The use of nanosilver in socks potentially benefits society by preventing foot odor, and may reduce the number of washings, which results in less water usage. Their benefits, however, need to be weighed with any potential cost to the environment and public health. In the case of the above-mentioned example, there is plenty of evidence that the nanosilver impregnated in the socks leaches out after a few wash cycles (Benn & Westerhoff, 2008). The leached nanosilver makes its way into wastewater treatment plants, and depending on its fate and transport characteristics, may ultimately wind up in streams and sediments where it may cause some risk to the ecosystem. The unknown health effects and risks associated with these materials have drawn considerable attention from researchers, consumers and regulators. As a result, scientists at the USEPA and elsewhere have recognized the need to develop risk assessment processes to study the potential health and environmental impacts of manufacturing nanomaterials as well as using these materials in other products. In addition to the toxicological concerns, there are other aspects that have to be considered during the risk assessment process as well such as the cost of transportation, including the amount of emissions that are emitted from trucks and trains. To address these issues, researchers have begun implementing more comprehensive assessment tools such as Life Cycle Assessment (LCA) to assess the cradle-to-grave cost/risk associated with any nanomaterial (Khanna *et al.*, 2007; Krishnan *et al.*, 2008; Lloyd & Lave, 2003; Lloyd *et al.*, 2005; Osterwalder *et al.*, 2006; Roes *et al.*, 2007). An LCA can establish the comparative impact of products or processes in terms of specified impact categories using a well-defined and documented methodology (Baumann *et al.*, 2004; ISO, 2006; Meyer *et al.*, 2009; Rebitzer *et al.*, 2004; U.S. EPA, 1993). Typical impact categories include global warming/climate change, stratospheric ozone depletion, human toxicity, ecotoxicity, photo-oxidant formation, acidification, eutrophication, land use, and resource depletion (Meyer *et al.*, 2009; Rebitzer *et al.*, 2004). A potential advantage of LCA-based evaluations for nanomaterials is that they can address both the health and environmental consequences associated with the inclusion of nanocomponents. The ultimate goal is to ensure that the

potential benefits of nanocomponents are realized in a manner that is safe for both consumers and the environment without resulting in unintended consequences (Meyer *et al.*, 2009).

A second method that is commonly used to assess the total risk associated with manufacturing nanomaterials as well as using these materials in other products is the use comprehensive environmental assessment (CEA), which combines LCA with the risk assessment paradigm (NRC, 1983) to examine the environmental impacts of technology in a broad, systematic manner (Davis, 2007; Davis & Thomas, 2006). The general features of CEA are highlighted in Figure 7.1. In the figure, as listed in Column 1, the life cycle of a product typically comprises several stages, including feedstock production or extraction, manufacturing processes, distribution, storage, use, and disposal of the product and waste byproducts. At any stage of the life cycle, pollutants may enter one or more environmental pathways: air, water, soil, and food web (Column 2). It is important to identify these primary contaminants and the transport and transformation processes they undergo. The idea is to characterize the primary as well as secondary or byproduct pollutants associated with the entire life cycle for all relevant environmental media (Column 3). The potential for humans and other organisms (biota) to become exposed or come into contact with primary and secondary pollutants via all pertinent routes, for example, inhalation, ingestion, and dermal absorption is also considered (Column 4). In this column, microenvironmental and high-end exposure scenarios should be considered, not just “typical” or “average” exposure levels. This scenario-driven approach to exposure assessment is a feature that distinguishes the CEA approach from LCA. Once exposure potential has been characterized, the health and ecological hazards associated with respective contaminants need to be described qualitatively and quantitatively (Column 5). To characterize risk quantitatively, the dose–response characteristics of a toxicant must be considered in relation to exposure potential. Some pollutants may pose low risk because the exposure potential is low or the hazard potential is low, or both. In other cases, risk may be relatively high when exposure potential is low but hazard potential is high, or *vice versa* (Davis, 2007; Davis & Thomas, 2006).

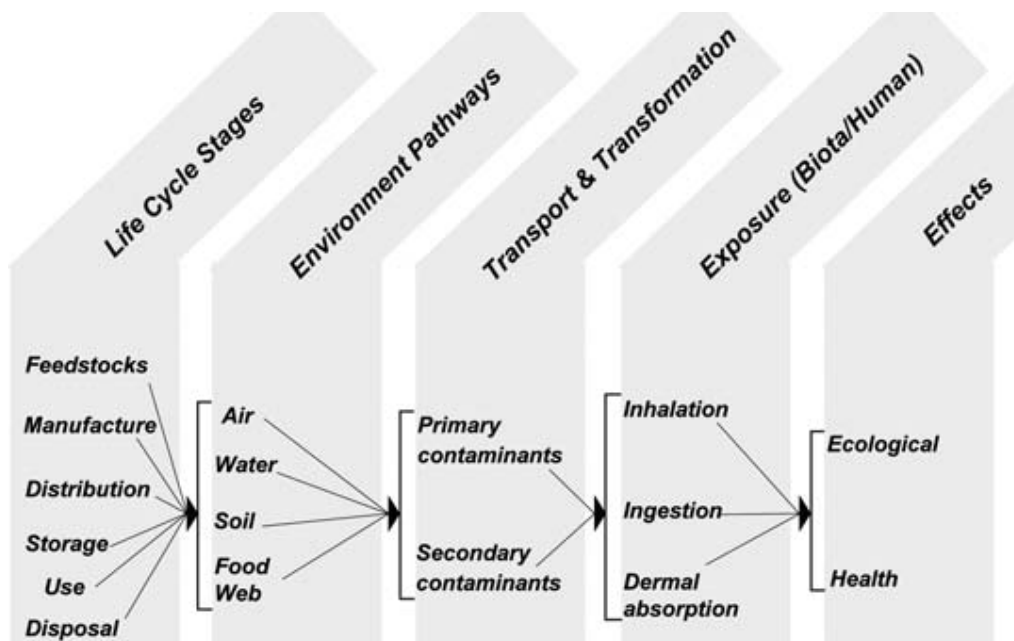


Figure 7.1: Comprehensive environmental assessment (CEA). CEA combines LCA and the risk assessment paradigm. Reprinted from Ann. N.Y. Acad. Sci., Vol. 1076, Davis, J.M., Thomas, V.N., Systematic approach to evaluating trade-offs among fuel options. The lessons of MTBE, pp498-515, Copyright 2006 with permission from Wiley Interscience.

7.1 Nanosilver life cycle assessment

Meyer *et al.* (2009) propose using life cycles with four main aspects (Figure 7.2): material selection, manufacturing, application, and disposal/recycle. The material selection aspect of nanosilver LCA involves both the composition (organic such as polymers, dendrimers, etc.; inorganic such as metals, metal oxides, etc.; carbon such as carbon tubes or a combination of any of these) and geometry of the nanocomponents, which can be a variety of shapes (sphere, rod, etc.) and is dependent on the synthesis methods (Meyer *et al.*, 2009; Nowack & Bucheli, 2007; U.S. EPA, 2007). The manufacturing aspect of nanosilver LCA involves the synthesis techniques discussed in Section 4.1. The application aspect of nanosilver LCA involves using the nanomaterials in either naturally dispersive or composite form for a range of applications, most of which are described in Chapter 3. The disposal/recycle aspect of nanosilver LCA involves incineration, disposal in a landfill or removal during wastewater treatment among others (Mueller & Nowack, 2008; U.S. EPA, 2007).

Gill (2007) presented an alternate life cycle assessment/materials flow analysis (Figure 7.3) at the Geology Symposium 2007 held in Sacramento, CA. Similar to the four aspects of LCA

proposed by Meyer *et al.* (2009), the LCA/materials flow analysis presented by Gill (2007) had the materials and disposal aspects of the LCA. Other aspects in the LCA proposed by Gill (2007) include material processing, manufacturing, distribution, use and/or reuse. These four aspects fall into the two main aspects (manufacturing and application) proposed by Meyer *et al.* (2009). Much more inventory information will be required for the more complex LCA proposed by Gill (2007).

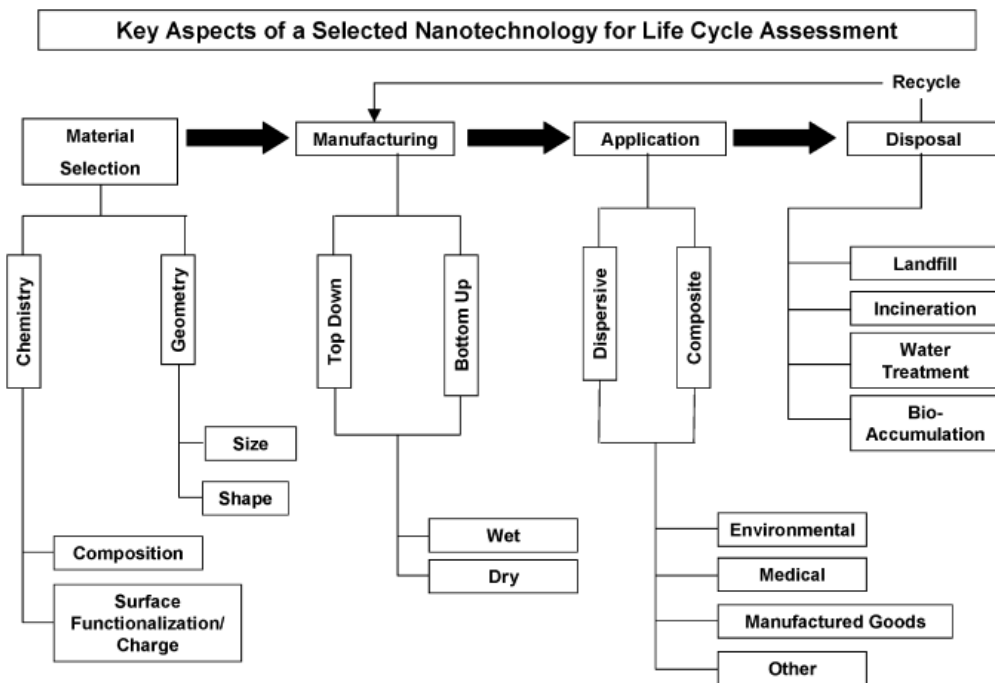


Figure 7.2: Choices associated with a nanotechnology throughout its life cycle as proposed by Meyer *et al.* (2009). Reprinted from *Environ. Sci. Technol.*, Vol. 43 (5), Meyer, D.E., Curran, M.A., Gonzalez, M.A., An examination of existing data for the industrial manufacture and use of nanocomponents and their role in the life cycle impact of nanoproducts, pp1256-1263, Copyright 2009 with permission from American Chemical Society.

USEPA's Nanotechnology Research Program within the ORD is conducting a series of LCAs on various products made from nanomaterials to gain knowledge about potential release into the environment. The assessments are holistic and comprehensive and track a product from its inception (cradle) through its final disposal (grave). LCAs are essential to analyze, evaluate, understand, and manage the overall health and environmental impacts of products. The LCAs are focused on answering questions which include: (i) what are the trade-offs associated with nanomaterials? and (ii) is the large-scale production of an environmentally

taxing material justified if it has beneficial applications for society or if it can reduce costs or enhance performance?

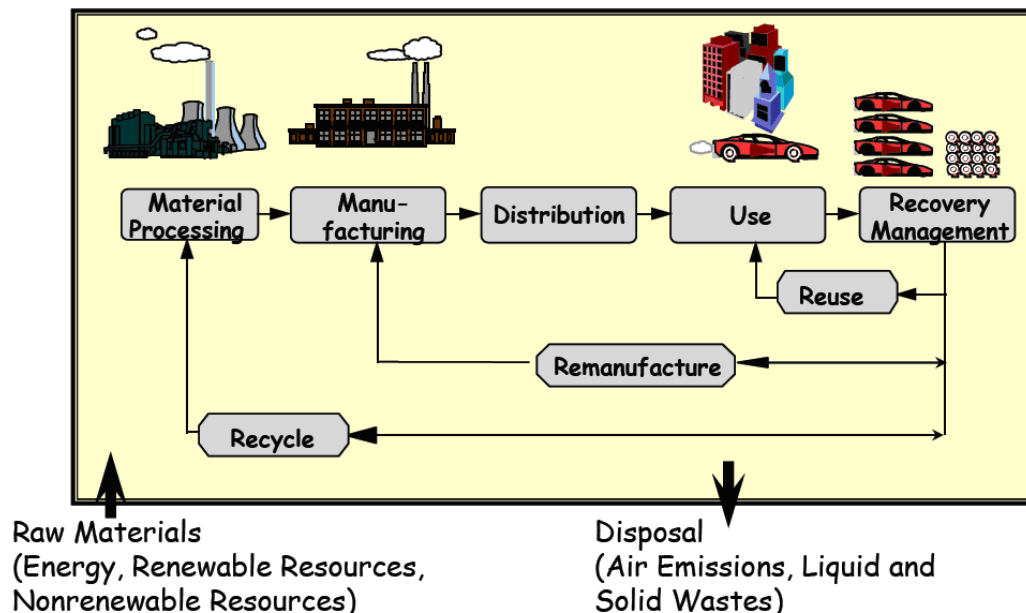


Figure 7.3: Choices associated with a nanotechnology throughout its life cycle as proposed by Gill (2007). (Adapted from Gill, 2007).

Characterization of nanomaterials on a life cycle basis is challenging because this is a new field of study. A large number of data gaps exist when considering the application of LCA to nanomaterials (Bauer *et al.*, 2008; Khanna *et al.*, 2007; Kloepffer *et al.*, 2007; von Gleich *et al.*, 2008). Finding adequate data to model the potential fate and effects of unintended releases of nanomaterials into the environment may be difficult to obtain. USEPA researchers are working to locate and provide the necessary data. Specifically, only minimal data exist detailing the material inputs and environmental releases related to the manufacture, release, transport, and ultimate fate of nanomaterials (Meyer *et al.*, 2009). Studies have mainly focused on “cradle-to-gate” assessments investigating the production of either nanocomponents or nanomaterials up to the point these materials leave the “gate,” or the manufacturing source. However, many nanomaterials are not yet in full production to create a consumer product; therefore, much of the data must be estimated.

As mentioned in Chapter V, the project on emerging nanotechnologies that was established in April 2005 as a partnership between the Woodrow Wilson International Center for Scholars and the Pew Charitable Trusts has published a fair amount of information on the inventory of consumer products containing nanomaterials including nanosilver. There is still a lack of information in regards to the characteristics of the particles (shape, size and surface chemistry), synthesis methods, production quantities, production losses, production consumption and the geographic distribution of these nanosilver-containing products. All these data are required in order to perform risk assessment and life cycle analysis.

Additional data is available in a database maintained by Nanowerk (<http://www.nanowerk.com>). The site lists 2437 items containing nanoparticles as of March 31, 2010 in nine categories. Figure 7.4 illustrates the number of items in each category, while Table 7.1 lists the company with specifics on nanosilver in items containing elemental silver nanoparticles. Even with this information, the database does not list the necessary information to conduct an LCA of nanosilver either.

Table 7.1: Companies selling nanosilver as listed in the Nanowerk database (as of March 31, 2010).

Company	APS	Phase	Specification
Shanghai Huzheng Nano Technology	1nm	suspension	Ag content <10,000ppm
Vive Nano	1-10nm	suspension	Carboxy-functionalized silver nanoparticles, sodium as carboxy counterion. 1.5 mg/mL in water or powder
nanoComposix, Inc.	10-127nm	suspension	Monodisperse
Nanocs	10nm	suspension	Dextran coated, 0.01% Ag
Nanocs	10nm	suspension	In aqueous, 0.01% Ag
Nanostructured & Amorphous Materials, Inc.	10nm	powder	P(99.9), w/~0.3% (PUP) surfactant (alcohol dissolvable)
QuantumSphere, Inc.	10-60nm	powder	
Shanghai Huzheng Nano Technology	10nm	suspension	Ionic solution; mono silver, pH 7
Shanghai Huzheng Nano Technology	10nm	suspension	Ionic solution; silver ion or nitrate ion, pH <4.5 or >9
Argonide Corporation	100nm	powder	Exploding wire synthesis
Inframat Advanced Materials LLC	100-127nm	powder	P(99.95)
Inframat Advanced Materials LLC	100-127nm	powder	P(99.95)
Nanostructured & Amorphous Materials, Inc.	100nm	powder	Ag coated, SiO ₂ cored 40:60
Nanostructured & Amorphous Materials, Inc.	100nm	powder	Ag coated, SiO ₂ cored 30:70
Nanostructured & Amorphous Materials, Inc.	100nm	powder	Ag coated, SiO ₂ cored 20:80
nGimat	100nm	powder	Nanospray technology

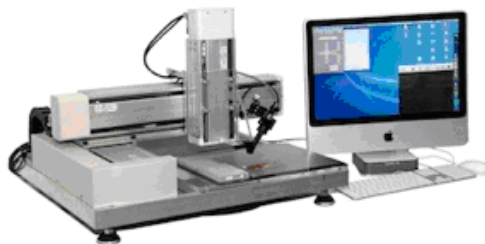
Table 7.1: Continued

Company	APS	Phase	Specification
SkySpring Nanomaterials	100nm	powder	P(99.95%) spherical
Inframat Advanced Materials LLC	127nm	powder	P(99.95)
Nano Technology Inc.	127nm	powder	Spherical
NanoMetal	127nm	powder	Spherical
ABC Nanotech	15-35nm	suspension	dispersed in alcohol; solid content <10wt%
AgPure Nanosilver	15nm	suspension	<5% Ag concentration
AgPure Nanosilver	15nm	suspension	<10% Ag concentration
AgPure Nanosilver	15nm	suspension	<20% Ag concentration
Nano-Vision Tech	15-25nm	suspension	P(99.99); pH 8-9; Suspended in water, alcohol; 500ppm
Nano-Vision Tech	15-25nm	suspension	P(99.99); pH 7-8; Suspended in water, alcohol; 2000ppm
Shanghai Huzheng Nano Technology	15nm	suspension	Colloid; Ag content 350ppm
IoLiTec	150nm	powder	P(99.95%)
NTbase	150nm	powder	P(99.99) spherical
Microspheres-Nanospheres	2-250nm	suspension	Spheres sizes available from 2-250nm
Nanocs	20nm	suspension	Dextran coated, 0.01% Ag
Nanocs	20nm	suspension	In aqueous, 0.01% Ag
PlasmaChem GmbH	20nm	suspension	colloidal suspension 0.5 mg/ml
SkySpring Nanomaterials	20-30nm	powder	P(99.95%) spherical, partially passivated
SkySpring Nanomaterials	20-30nm	powder	P(99.95%) spherical, PVP coated
NaBond	25nm	powder	P(>99.9%)
Nanogap	25-45nm	powder	P(85%)
NovaCentrix	25-30nm	powder	Strong crystallinity, spherical
Nanogap	28-57nm	powder	P(85%)
NanoSonic, Inc.	28nm	suspension	Suspended in water
Nanogap	3.5-7.5nm	powder	P(85%)
Auto Fibre Craft	30-54nm	powder	Dry, uncoated, pure powder silver in elemental form
Chengdu Alpha Nano Technology	30-50nm	powder	P(>99.9%) w/0.3% PVP
Nanocs	30nm	suspension	Dextran coated, 0.01% Ag
Nanocs	30nm	suspension	In aqueous, 0.01% Ag
Nanostructured & Amorphous Materials, Inc.	30-50nm	powder	P(99.9), w/~0.3% (PUP) surfactant (alcohol dissolvable)
Nanostructured & Amorphous Materials, Inc.	30-50nm	powder	P(99.9), w/~0.3% (PUP) surfactant (alcohol dissolvable), surface coated with 2% wt oleic acid
IoLiTec	35nm	powder	P(99.5%)
Shenzen Junye Nano Material	35nm	powder	P(>99.9%)
Sunano	35nm	powder	P(>99.8%)
Sunano	35nm	suspension	P(>99.9%) Aqueous Solution with Non-Ionic Surfactant; 1.6 wt%
Nanocs	40nm	suspension	In aqueous, 0.01% Ag
Nanocs	40nm	suspension	Dextran coated, 0.01% Ag
NanoDynamics, Inc.	40nm	suspension	P(99.99), crystalline
Applied Nanotech Holdings	45nm	powder	P(>99%)
Bio-Gate	5-50nm	suspension	P(99.9)
nanoCompound	5nm	powder	P(>99.9);Spherical
Nanogap	5-10nm	powder	P(85%)
Polytech & Net GmbH	5-20nm	suspension	Yellow color; PH 6-8; antibacterial rate 99.9%
Nano-Size Ltd.	50nm	powder	Cubic
Nano-Size Ltd.	50nm	suspension	Cubic, suspended in benzyl alcohol
Nanocs	50nm	suspension	Dextran coated, 0.01% Ag

Table 7.1: Continued

Company	APS	Phase	Specification
Nanocs	50nm	suspension	In aqueous, 0.01% Ag
Seashell Technology, LLC	50-127nm		Spherical
SkySpring Nanomaterials	50-60nm	powder	P(99.95%) spherical
MTI Corporation	55nm	powder	
Nano Ocean Tech	6nm	powder	Surface ligand dodecanethiol
Nano Ocean Tech	6nm	suspension	In water/surface ligand COOH
Neo-Ecosystems	70nm	powder	P(99%)
Nano-Size Ltd.	80nm	suspension	Cubic, suspended in benzyl alcohol
NanoDynamics, Inc.	80nm	powder	P(99.99), crystalline
Nanostructured & Amorphous Materials, Inc.	80-127nm	powder	P(99.95), spherical
Blue Nano	90-110nm	powder	
MKnano	90nm	powder	P(99.9%)
Nanostructured & Amorphous Materials, Inc.	90-127nm	powder	P(>99), spherical

Nanomaterial Database Search



(choose from one category only)

sonoplot

Fullerenes (129 items):
Material: <input type="text" value="Select One"/> <input type="button" value="Search"/>
Carbon Nanotubes (580 items):
Material: <input type="text" value="Select One"/> Configuration: <input type="text" value="Select One"/> <input type="button" value="Search"/>
Nanoparticles of Elements (454 items):
Material: <input type="text" value="Silver"/> <input type="button" value="Search"/>
Nanoparticles of Binary Compounds (673 items):
Material: <input type="text" value="Select One"/> <input type="button" value="Search"/>
Nanoparticles of Complex Compounds (169 items):
Material: <input type="text" value="Select One"/> <input type="button" value="Search"/>
Quantum Dots (171 items):
Material: <input type="text" value="Select One"/> <input type="button" value="Search"/>
Biomedical Quantum Dots (209 items):
Material: <input type="text" value="Select One"/> <input type="button" value="Search"/>
Nanowires (24 items):
Material: <input type="text" value="Select One"/> <input type="button" value="Search"/>
Nanofibers (28 items):
Material: <input type="text" value="Select One"/> <input type="button" value="Search"/>

Figure 7.4: The Nanomaterial Database maintained by Nanowerk (www.nanowerk.com). The database currently has 2437 items listed under nine categories.

To obtain information to perform an LCA, a survey was created and sent to a total of 122 companies. Response was low; only one company replied. This lack of information identifies a data gap which must be filled to successfully perform an LCA. An alternative may be to estimate the information necessary to perform the LCA.

As an example, it may be possible to use the estimates generated by Blaser *et al.* (2008), who conducted a risk analysis of nanosilver incorporated in textiles and plastics to freshwater ecosystems after estimating the amount of silver use in 2010. Blaser *et al.* (2008) assessed the risk in four stages. First, the system boundaries were defined, mass flows of silver were quantified, and three emission scenarios were defined. Second, the behavior of silver in natural freshwater was reviewed, and a mass balance model was applied to calculate predicted environmental concentrations (PECs) for freshwater and freshwater sediments. PECs were also estimated for sewage treatment plants (STPs) and sewage sludge. The uncertainty of the results was assessed and predicted concentrations were compared to empirical data. Third, toxicity data for environmentally relevant silver compounds were compiled and predicted no-effect concentrations (PNECs) were determined where possible. Finally, the potential for risk caused by the release of silver into freshwater was evaluated using all available data. Figure 7.5 illustrates the quantified mass flows of silver triggered mainly by the use of biocidal products with other silver uses. This information can directly be used to estimate the disposal/recycle aspect of the LCA.

Mueller & Nowack (2008) used a life-cycle perspective to model the quantities of engineered nanoparticles into the environment. The quantification was based on a substance flow analysis from nanomaterials to air, soil and water in Switzerland. Production and product distribution was estimated by multiplying the total nanoparticle production (based on the Woodrow Wilson Center database; www.nanotechproject.org) by a weighting factor. The weighting factor was determined by combining three sources: (i) the inventory of the Wilson Center, (ii) a search of the www.products.ec21.com database, and (iii) a web search. Figure 7.6 illustrates the flow of nanosilver from the nanomaterial containing products to different environmental compartments – waste incineration plants (WIP), sewage treatment plants (STP) and landfill. As in the case of the data estimated by Blaser *et al.* (2008), the data from the model generated by Mueller & Nowack (2008) can directly be used to estimate the disposal/reuse aspect of the nanosilver LCA.

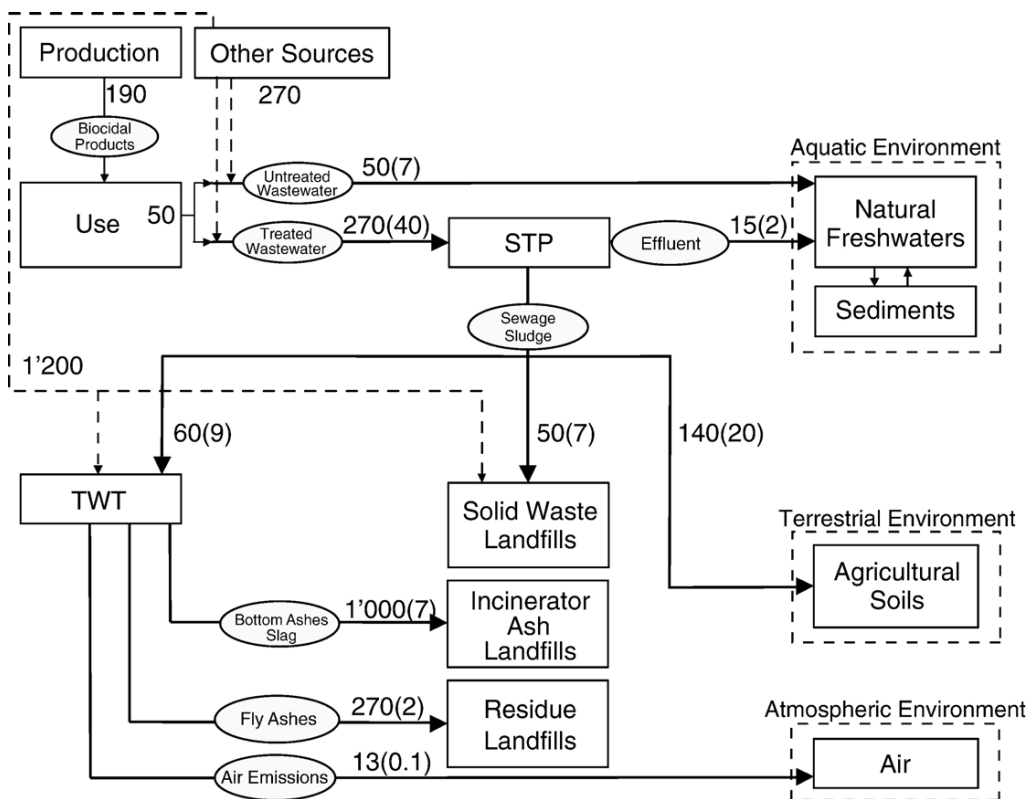


Figure 7.5: Quantified mass flows of silver triggered by the use of biocidal products and by other silver uses. Reprinted from *Sci. Tot. Environ.*, Vol. 390, Blaser, S.A., Scheringer, M., MacLeod, M., Hungerbuhler, K., Estimation of cumulative aquatic exposure and risk due to silver: contribution of nano-functionalized plastics and textiles, pp396-409, Copyright 2008 with permission from Elsevier.

Sayes & Ivanov (2010) developed a method to obtain Quantitative Structure-Activity Relationship (QSAR) models to predict cellular membrane damage caused by exposure to two different types of nanomaterials – TiO₂ and ZnO. It is hypothesized that nanosilver can also be predicted in this manner. QSAR models are mathematical models that relate a response to certain physicochemical properties of chemicals that can either be measured or estimated. The authors use a mathematical modeling approach that uses physical properties of the nanomaterials such as its engineered size and size in water (or buffer) and one chemical property – zeta potential as descriptors (or independent variables) to describe cellular membrane damage as measured by lactate dehydrogenase release. More elaborate QSAR models can be developed to predict various toxicity endpoints of interest. Once these models have been developed, such models can be used to estimate the toxicity of a nanoparticle at any given site. This information can be used to estimate the toxicity component of an LCA.

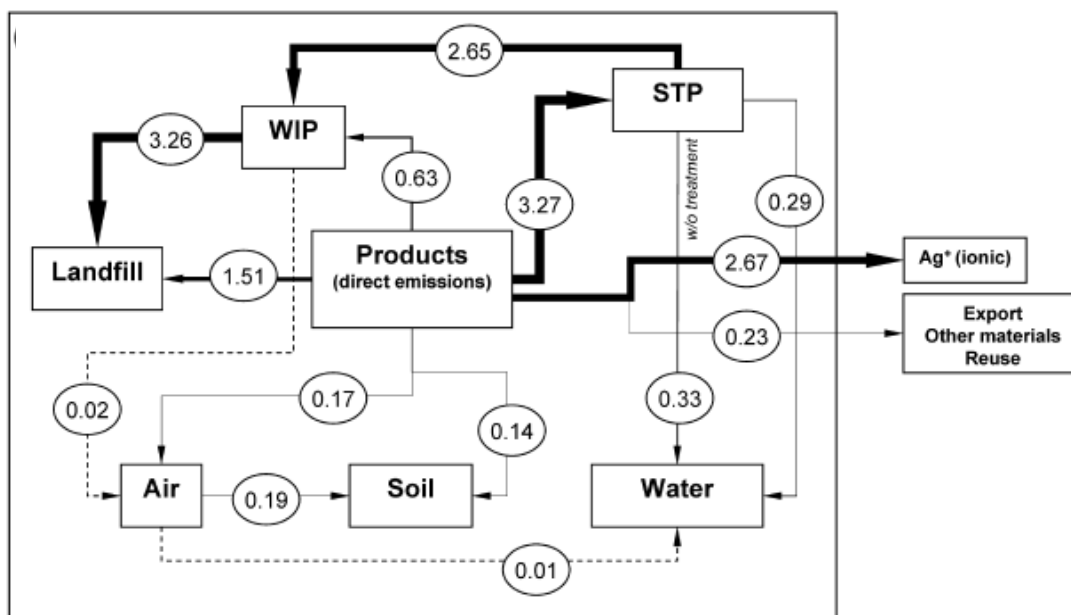


Figure 7.6: Nanosilver flows from the nanomaterial containing products to various environmental compartments (WIP, STP and landfill). All flows are in tons/year. The thickness of the arrows is proportional to the amount of silver flowing between the compartments. Dashed arrows represent the lowest volume. Reprinted from Environ. Sci. Technol., Vol. 42 (12), Mueller, N.C., Nowack, B., Exposure modeling of engineered nanoparticles in the environment, pp4447-4453, Copyright 2008 with permission from American Chemical Society.

7.2 Nanosilver comprehensive environmental assessment

The USEPA’s mission and mandates call for an understanding of the health and ecological implications of engineered nanomaterials, which necessitates conducting a comprehensive environmental assessment on such nanomaterials. To serve as a foundation for creating a long-term research strategy to provide the information needed for comprehensive environmental assessments of nanomaterials, the USEPA completed two case studies that focused on nanoscale titanium oxide (nanoTiO₂) and released a preliminary draft report that is currently undergoing external peer review (U.S. EPA, 2009). The first case study focused on the use of nanoTiO₂ as an agent for removing arsenic from drinking water while the second case study focused on nanoTiO₂ as an active ingredient in topical sunscreen. The USEPA followed up on the two nanoTiO₂ case studies with a case study that focused on nanoscale silver as possibly used in aerosol disinfectant sprays, and released a preliminary draft report that is currently undergoing internal (interagency) peer review (U.S. EPA, 2010).

The process for selecting nanosilver in disinfectant spray as a case study involved a workgroup representing several USEPA program offices, regional offices, and ORD laboratories and centers. The USEPA workgroup considered several candidate nanomaterials and voted for their preferences based on, among other things, apparent relevance of the nanomaterial to USEPA programmatic interests. The organization of the draft report reflected the CEA approach: Chapter 1 provided an overall introduction while Chapter 2 provided introductory material on silver and nanosilver. Chapter 3 in the draft report highlighted stages of the product life cycle (feedstocks, manufacturing, distribution, storage, use, disposal), followed by fate and transport processes in Chapter 4, exposure-dose characterization in Chapter 5, and ecological and health effects in Chapter 6. The draft report contained lists of questions that reflect information gaps or possible research issues. This report is not expected to be released to the public until 2011.

8. Data Gaps

Nanosilver is being used in an extensive number of applications and this number is still growing. Eventually, all known applications for metallic silver may involve the use of nanosilver instead of silver to take advantage of nanosilver's unique properties. In spite of all beneficial uses for nanosilver, its impact on the environment is of concern. For that reason, research is being conducted in order to answer many questions such as the fate, transport and toxicity of nanosilver. This report is a state of the art review that covers all available information on various aspects of nanosilver research, including synthesis methods, applications, fate and transport, exposure, toxicity, life cycle analysis (or comprehensive environmental assessment) and risk assessment. Even though there is information available on each individual aspect listed previously, there are many research gaps that have to be filled to gain a comprehensive understanding of benefits and risks of using nanosilver. This section highlights some of these data gaps that exist:

There is a plethora of synthesis methods for nanosilver, but the ones that are most commonly utilized in the industry are not yet known. This is extremely important to know since each synthesis method will result in nanoparticles with specific surface properties that will govern their fate, transport, toxicity and environmental interactions and impacts. These synthesis methods may also involve the use of different raw materials and may yield reaction byproducts or waste that may be toxic. Such information is also necessary while performing a cost-benefit analysis, life cycle analysis or comprehensive environmental assessment. Thus, this is the first important question that the researchers should answer in order to gear the efforts towards the investigation of the right types of silver nanoparticles.

There are plenty of techniques to characterize pure silver nanomaterials. The majority of these techniques will, of course, work for pure nanosilver suspensions, but there are still there is lack of methodologies and analytical tools for the detection and characterization of silver nanoparticles in environmental samples. Thus, there is a need for developing methods to measure the nanosilver concentration, speciation (e.g. ionic versus metallic), size, shape, surface charge, crystal structure, surface chemistry and surface transformations in complicated matrices such as rivers, streams, wastewater and/or landfill leachate, soils and sediments.

It is important to characterize the nanoparticles, perform dose-metrics as well as quantify the physicochemical properties of the nanomaterials. Nanoparticles have novel properties compared to conventional chemicals. The characterization of these properties is important in order to enable realistic estimations of exposure to humans and the ecosystem. This information is also important to establish dose-response relationships for estimating the toxicity of these nanoparticles.

There is still a lack of information in regards to the characteristics of the silver nanoparticles used in consumer products (shape, size, surface chemistry and synthesis methods), production quantities, production losses, production consumption and the geographic and demographic distribution of these nanosilver-containing products in the US and elsewhere. All these data are necessary in order to perform risk assessment and life cycle analyses. The manufactures of nanosilver and nanosilver-containing products are yet not cooperative in providing this necessary information. State or Federal laws that mandate providing this information to regulatory agencies may be necessary to obtain information from manufacturers.

There is limited number of studies on the leachability of silver nanoparticles (or other forms of silver) from consumer products containing nanosilver under various environmental conditions that a given product may potentially encounter. The speciation of the leached silver is also unknown for many consumer products. Additional questions to consider include the investigation of the release patterns and release kinetics of nanosilver from specific applications and whether the physicochemical properties change under certain circumstances leading to more/less release of nanosilver into the aqueous environment.

There is no information available yet on the fate and transport of silver nanoparticles in waste streams, solid waste landfills, soils and sediments.

There is a significant body of literature discussing the toxicity of silver nanoparticles to various bacterial species; however, the diverse types of reducing agents, capping agents and dispersants used to synthesize and stabilize the nanosilver may make direct and meaningful comparisons difficult. Results using different bacterial strains, even if they are of the same species, may not be comparable. Between the different toxicological studies that are reported in the literature so far, the compositions of the silver nanoparticles vary widely. The descriptions of used silver formulations diverge

from detailed to very limited, with variable attention paid to the size, solubility and aggregation of the nanoparticles.

Due to a lack of reliable nanosilver toxicity data in the literature, it is impossible to assess the environmental risks associated with the production and use of nanosilver. An important research question is the validation of the hypothesis that toxic effects of nanoparticles are proportional to the activity of the free silver ions released by the nanoparticles.

Various toxicity mechanisms of nanosilver have been proposed but the exact mechanisms are yet to be determined.

Apart from nanosilver toxicity assessment in the aqueous environments, more research is needed to investigate the effects of nanosilver in terrestrial environments as no toxicity data for nanosilver in soils were found in the literature. Additional research is also needed on ecologically relevant species to investigate whether silver nanoparticles present a threat to environmental health in general. It should be determined whether nanosilver in products is actually capable of reaching the aqueous and terrestrial environment. Specifically, the strength of the bonds between nanosilver and the product it is incorporated into should be investigated.

No specific mammalian models for nanosilver were available in the literature although several were identified for nanoparticles in general.

Although the oxidation state of the silver nanoparticles may influence their biological and/or toxicological activity, little attention has been paid to the oxidation state of the silver nanoparticles in the literature.

Toxicity of silver nanoparticles is mostly determined *in vitro* with particles ranging in size from 1-100 nm. The available *in vivo* animal studies are relatively short term studies (maximum of 28 days) except for one 90-day inhalation study on the use of a single size of silver nanoparticles.

Only limited health effects on the use of nanosilver in humans have been documented.

Limited risk assessment studies for nanosilver are available in the literature as a result there is a lack of adequate data to model the potential fate and effects of unintended releases of nanosilver into the environment. Specifically, only minimal data exist detailing the material inputs and environmental releases related to the manufacture, release, transport, and ultimate fate of silver nanoparticles.

9. Bibliography

1. Abu Bakar, N.H.H., Ismail, J., Abu Bakar, M. Synthesis and characterization of silver nanoparticles in natural rubber. *Mater. Chem. Phys.*, 2007, 104(2-3), 276-283.
2. AGC Glass. 2007. <http://www.agc-flatglass.eu/AGC+Flat+Glass+Europe/English/Homepage/News/Press+room/Press-Detail-Page/page.aspx/979?pressitemid=1031>. Date accessed Feb-2010
3. Ahmad, A., Mukherjee, P., Senapati, S., Mandal, I., Khan, I.M., Kumar, R., Sastry, M. 2003. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*, *Colloids Surf B: Biointerfaces*, 28:313-318.
4. Alt, V., Bechert, T., Steinrucke, P., Wagener, M., Seidel, P., Dingeldein, E., Domann, E., Schnettler, R. 2004. An in vitro assessment of the antibacterial properties and cytotoxicity of nanoparticulate silver bone cement. *Biomaterials*, 25, 4383-4391.
5. Alt, V., Wagener, M., Salz, D., Bechert, T., Steinrücke, P., Schnettler, R. 2006. Plasma polymer - high-porosity silver composite coating for infection prophylaxis in intramedullary nailing. In: Leung, K.S., Taglang, G., Schnettler, R., Alt, V., Haarman, H.J.T.M., Seidel, H., Kemf, I. (Eds). *Practice of intramedullary locked nails. New developments in techniques and applications*. Berlin: Springer, p 297-303.
6. Amendola, V., Polizzi, S., Meneghetti, M. 2007. Free Silver Nanoparticles Synthesized by Laser Ablation in Organic Solvents and Their Easy Functionalization, *Langmuir*, 23: 6766-6770.
7. Ameen, K.B., Rajasekar, K., Rajasekharan, T. Silver nanoparticles in mesoporous aerogel exhibiting selective catalytic oxidation of benzene in CO₂ free air. *Catal Lett.* 2007,119, 289-295.
8. Anand, M., Bell, P.W., Fan, X., Enick, R.M., Roberts, C.B. Synthesis and steric stabilization of silver nanoparticles in neat carbon dioxide solvent using fluorine-free compounds. *J. Phys. Chem. B.* 2006, 110, 14693-14701.

9. Anderson, P.J., Wilson, J.D., Hiller, F.C. Respiratory tract deposition of ultrafine particles in subjects with obstructive or restrictive lung disease. *Chest* 97: 1115–1120, 1990.
10. Arangoa, M.A., Campanero, M.A., Popineau, Y., Irache, M. Evaluation and characterisation of gliadin nanoparticles and isolates by reversed-phase HPLC, *J. Cereal Sci.*, 2000, 31(2), 223.
11. Arnaud, C.H. Probing nanotoxicity, *Chemical & Engineering News*, 2010, 88(13): 32-34.
12. Arora, S., Jain, J., Rajwade, J.M., Paknikar, K.M., 2008. Cellular responses induced by silver nanoparticles: in vitro studies. *Toxicol. Lett.* 179 (2), 93–100.
13. Asgharian, B., Price, O.T. Deposition of ultrafine (nano) particles in the human lung. *Inhal Toxicol* 19: 1045–1054, 2007.
14. Asharani, P.V., Nair, G., Zhiyuan, H., Manoor. P., Valiyaveetil, S. 2007. Potential health impacts of silver nanoparticles. Abstracts of Papers, 234th ACS National Meeting, Boston, MA, USA, August 19-23, 2007. pp:TOXI-099.
15. Asharani, P.V., Wu, Y.L., Gong, Z., Valiyaveetil, S. Toxicity of silver nanoparticles in zebrafish models, *Nanotechnology*, 2008, 19(25):255102.
16. Asharani, P.V., Hande, M.P., Valiyaveetil, S. Anti-proliferative activity of silver nanoparticles. *BMC Cell Biology*, 2009, 10:65 (14 pp).
17. Aslan, K., Geddes, C.D. 2006. Microwave-accelerated and metal-enhanced fluorescence myoglobin detection on silvered surfaces: Potential application to myocardial infarction diagnosis, *Plasmonics*, 1:53-59.
18. Aslan, K., Gryczynski, I., Malicka, J., Matveeva, E., Lakowicz, J.R., Geddes, C.D. 2005. Metal-enhanced fluorescence: An emerging tool in biotechnology, *Curr. Opin. Biotechnol.*, 16:55-62.
19. Aslan, K., Huang, J., Wilson, G.M., Geddes CD. 2006. Metal-enhanced fluorescence-based RNA sensing, *J. Am. Chem. Soc.*, 128:4206-4207.
20. Aslan, K., Wu, M., Lakowicz, J.R., Geddes, C.D. 2007. Metal enhanced fluorescence solution-based sensing platform 2: Fluorescent core-shell Ag@SiO₂ nanoballs, *J. Fluoresc.*, 17:127-131.
21. ASTM Standard E2456. 2006. Standard terminology relating to Nanotechnology. ASTM International, West Conshohocken, PA, 2006, DOI: 10.1520/E2456-06.

22. Asz, J., Asz, D., Moushey, R., Seigel, J., Mallory, S.B., Foglia, R.P. 2006. Treatment of toxic epidermal necrolysis in a pediatric patient with a nanocrystalline silver dressing, *J. Pediatric Surg.*, 41:e9-12.
23. ATSDR. Agency for Toxic Substances and Disease Registry. 1990. Toxicological profile for silver. Atlanta, GA: US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry (TP-90-24).
24. Aymonier, C., Schlotterbeck, U., Antonietti, L., Zacharias, P., Thomann, R., Tiller, J.C., Mecking, S. 2002. Hybrids of silver nanoparticles with amphiphilic hyperbranched macromolecules exhibiting antimicrobial properties. *Chem. Commun.* 2002, 3018-3019.
25. Baalousha, M., Lead, J.R. 2007. Characterization of Natural Aquatic Colloids (<5 nm) by Flow-Field Flow Fractionation and Atomic Force Microscopy. *Environ. Sci. Technol.* 41:1111-1117.
26. Baalousha, M., von der Kammer, F., Motelica-Heino, M., Le Coustumer, P. Natural sample fractionation by FIFFF-MALLSTEM: Sample stabilization, preparation, pre-concentration and fractionation, *J. Chromatogr., A*, 2005a, 1093(1–2), 156.
27. Baalousha, M., von der Kammer, F., Motelica-Heino, M., Le Coustumer, P. 3D characterization of natural colloids by FIFFFMALLS-TEM, *Anal. Bioanal. Chem.*, 2005b, 383(4), 549.
28. Baalousha, M., von der Kammer, F., Motelica-Heino, M., Hilal, H.S., Le Coustumer, P. Size fractionation and characterization of natural colloids by flow-field flow fractionation coupled to multi-angle laser light scattering, *J. Chromatogr., A*, 2006a, 1104(1–2), 272.
29. Baalousha, M., von der Kammer, F., Motelica-Heino, M., Baborowski, M., Hofmeister, C., Le Coustumer, P. Size-based speciation of natural colloidal particles by flow field flow fractionation, inductively coupled plasma-mass spectroscopy, and transmission electron microscopy/X-ray energy dispersive spectroscopy: Colloids-trace element interaction, *Environ. Sci. Technol.*, 2006b, 40(7), 2156.
30. Baker, C., Pradhan, A., Pakstis, L., Pochan, D.J., Shah, S.I. 2005. Synthesis and antibacterial properties of silver nanoparticles. *J. Nanosci. Nanotechnol.* 5, 244-249.

31. Bakshi, M.S., Sharma, P., Banipal, T.S. Au and Au–Ag bimetallic nanoparticles synthesized by using 12-3-12 cationic gemini surfactant as template. *Mater. Lett.* 2007, 61, 5004-5009.
32. Balaguera-Gelves, M.D.R.. Detection of nitroexplosives by surface enhanced Raman spectroscopy on colloidal metal nanoparticles. 2006, Master thesis, University of Puerto Rico, Mayaguez Campus.
33. Balan, L., Malval, J., Schneider, R., Burget, D. 2007. Silver nanoparticles: new synthesis, characterization and photophysical properties, *Mater. Chem. Phys.*, 104: 417–421.
34. Balogh, L., Swanson, D.R., Tomalia, D.A., Hagnauer, G.L., McManus, A.T. Dendrimer–silver complexes and nanocomposites as antimicrobial agents. *Nano Lett.* 2001, 1, 18-21.
35. Basavaraja, S., Balaji, S.D., Lagashetty, A., Rajasab, A.H., Venkataraman, A. Extracellular biosynthesis of silver nanoparticles using the fungus *fusarium semitectum*. *Mater. Res. Bull.* 2008, 43, 1164-1170.
36. Bauer, C., Buchgeister, J., Hischier, R., Poganietz, W.R., Schebek, L., Warsen, J. Towards a framework for life cycle thinking in the assessment of nanotechnology. *J. Cleaner Prod.* 2008, 16 (8-9), 910–926.
37. Baumann, H., Tillman, A. *The Hitch Hiker’s Guide to LCA: An Orientation in Life Cycle Assessment Methodology and Application*; Studentlitteratur: Sweden, 2004.
38. Bayston, R., Ashraf, W., Fisher, L. 2007. Prevention of infection in neurosurgery: Role of ‘antimicrobial’ catheters, *J. Hosp. Infect.* 65:39-42.
39. Benn, T.M., Westerhoff, P. Nanoparticle silver released into water from commercially available sock fabrics. *Environ. Sci. Technol.*, 2008, 42:4133–4139. Erratum in: *Environ. Sci. Technol.*, 42:7025-7026.
40. Bhainsa, K.C., D’Souza, S.F. 2006. Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus*, *Colloids Surf B: Biointerfaces*, 47:160-164.
41. Bhol, K.C., Alroy, J., Schechter, P.J. 2004. Anti-inflammatory effect of topical nanocrystalline silver cream on allergic contact dermatitis in a guinea pig model. *Clin. Exp. Dermatol.* 29:282-287.
42. Bhol, K.C., Schechter, P.J. 2005. Topical nanocrystalline silver cream suppresses inflammatory cytokines and induces apoptosis of inflammatory cells in a murine model of allergic contact dermatitis. *Br. J. Dermatol.*, 152:1235-1242.

43. Bhol, K.C., Schechter, P.J. 2007. Effects of nanocrystalline silver (NPI 32101) in a rat model of ulcerative colitis, *Digestive Dis. Sci.* 52:2732-2742.
44. Blaser, S.A., Scheringer, M., MacLeod, M., Hungerbuhler, K. 2008. Estimation of cumulative aquatic exposure and risk due to silver: contribution of nano-functionalized plastics and textiles, *Sci. Tot. Environ.*, 390:396–409.
45. Bogle, K.A., Dhole, S.D., Bhoraskar, V.N. Silver nanoparticles: synthesis and size control by electron irradiation. *Nanotechnology.* 2006, 17, 3204-3208.
46. Bolea, E., Gorriz, M.P., Bouby, M., Laborda, F., Castillo, J.R., Geckeis, H. Multielement characterization of metal-humic substances complexation by size exclusion chromatography, asymmetrical flow field-flow fractionation, ultrafiltration and inductively coupled plasma-mass spectrometry detection: A comparative approach, *J. Chromatogr., A*, 2006, 1129(2), 236.
47. Bootz, A., Russ, T., Gores, F., Karas, M., Kreuter, J. Molecular weights of poly(butyl cyanoacrylate) nanoparticles determined by mass spectrometry and size exclusion chromatography, *Eur. J. Pharm. Biopharm.*, 2005, 60(3), 391.
48. Borm, P.J., Robbins, D., Haubold, S., Kuhlbusch, T., Fissan, H., Donaldson, K., Schins, R., Stone, V., Kreyling, W., Lademann, J., Krutmann, J., Warheit, D., Oberdorster, E. The potential risks of nanomaterials: a review carried out for ECETOC. Part Fibre Toxicol 3: 11, 2006.
49. Bossalis, M.G., McCall, J.T., Ahrenholz, D.H., Solem, L.D., McClain, C.J. 1987. Serum and urinary silver levels in thermal injury patients. *Surgery* 101, 40-43.
50. Bouwmeester, H., Dekkers, S., Noordam, M., Hagens, W., Bulder, A., De Heer, C., Ten Voorde, S., Wijnhoven, S., Sips, A. 2007. Health impact of nanotechnologies in food production. RIKILT/RIVM Report 2007.014. Accessed March 2010 from the website: <http://www.rikilt.wur.nl/NR/rdonlyres/BDEEDD31-F58C-47EB-A0AA-23CB9956CE18/54352/R2007014.pdf>
51. Boxall, A.B.A., Chaudhry, Q., Sinclair, C., Jones, A., Aitken, R., Jefferson, B., Watts, C. 2007. Current and future predicted environmental exposure to engineered nanoparticles. Sand Hutton, York, UK: Central Science Laboratory.
52. Bragg, P.D., Rainnie, D.J. 1974. The effect of silver ions on the respiratory chain of *Escherichia coli*. *Can. J. Microbiol.* 20, 883-889.
53. Braydich-Stolle, L.; Hussain, S.; Schlager, J. J.; Hofmann, M. C. In vitro cytotoxicity of nanoparticles in mammalian germline stem cells. *Toxicol. Sci.* 2005, 88, 412–419.

54. Bregado-Gutierrez, J., Saldivar-Garcia, A.J., Lopez, H.F. Synthesis of Silver Nanocrystals by a Modified Polyol Method. *J. Appl. Polym. Sci.* 2008, 107, 45-53.
55. Britanica. 2010. Nanotechnology. <http://search.eb.com.zorac.aub.aau.dk/eb/article-9384821?query=nanotechnology&ct=>.
56. Britz, D.A., Glatkowski, P.J. 2010. Patent application title: Deposition of metals onto nanotube transparent conductors. Website <http://www.fags.org/patents/app/20090068241>. Last accessed May, 2010.
57. Brook, R.D., Franklin, B., Cascio, W., Hong, Y. Howard, G., Lipsett, M., Luepker, R., Mittleman, M., Samet, J., Smith, S.C. Air Pollution and Cardiovascular Disease. *Circulation.* 2004, 109: 2655-2671
58. Brooks, W.E. 2010. Silver. <http://minerals.usgs.gov/minerals/pubs/commodity/silver/silvemyb04.pdf>. Date accessed Feb-2010.
59. Bruchert, W., Bettmer, J. On-line coupling of gel electrophoresis and inductively coupled plasma-sector field-mass spectrometry for the determination of dsDNA fragments, *Anal. Chem.*, 2005, 77(15), 5072.
60. Bryan, G., Langston, W. 1992. Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: A review. *Environ. Pollut.* 76: 89-131.
61. Burd, A., Kwok, C.H., Hung, S.C., Chan, H.S., Gu, H., Lam, W.K., Huang, L. 2007. A comparative study of the cytotoxicity of silver-based dressings in monolayer cell, tissue explant, and animal models. *Wound Repair Regen* 15:94–104.
62. Burrell, R.E. 1997. The in vitro and in vivo antimicrobial potency of a new silver coated dressing for wound care. 51OK K955453, Washington DC.
63. Burrell, R.E., Heggors, J.P., Davis, G.J., Wright, J.B. 1999. Efficacy of silver-coated dressings as bacterial barriers in a rodent burn sepsis model, *Wounds*, 11:64-71.
64. Buzea, C., Pacheco, I.I., Robbie, K. Nanomaterials and nanoparticles: Sources and toxicity, *Biointerphases*, 2(4): MR17-MR71, 2007.
65. Caffee, H.H., Bingham, H.G. Leukopenia and silver sulfadiazine. *J. Trauma* 22, 586-587, 1982.

66. Card, J.W.; Zeldin, D.C., Bonner, J.C., Nestmann, E.R. Pulmonary applications and toxicity of engineered nanoparticles, *Am. J. Physiol. Lung Cell Mol. Physiol.*, 295:L400-L411, 2008.
67. Carlson, C. 2006. *In vitro* toxicity assessment of silver nanoparticles in rat alveolar macrophages. Master thesis, Wright State University.
68. Carlson, C., Hussain, S.M., Schrand, A.M., Braydich-Stolle, L.K., Hess, K.L., Jones, R.L., Schlager, J.J., 2008. Unique cellular interaction of silver nanoparticles: size dependent generation of reactive oxygen species. *J. Phys. Chem. B* 112 (43), 13608–13619.
69. Cassagneau, T., Fendler, J.H. Preparation and layer-by-layer self-assembly of silver nanoparticles capped by graphite oxide nanosheets. *J. Phys. Chem. B.* 1999, 103, 1789-1793.
70. Cataleya, Carlson, 2006. *In Vitro* Toxicity Assessment of Silver Nanoparticles in Rat Alveolar Macrophages. Master thesis, Wright State University.
71. Catsakis, L.H., Sulica, V.I. 1978. Allergy to silver amalgams. *Oral. Surg.* 46:371-375.
72. Cerjan-Stefanovi, S., Katelan-Macan, M. 1991. Separation of silver from waste waters by ion-exchange resins and concentration by microbial cells. *Fresenius J Anal Chem.*, 339: 636 - 639.
73. Chalupa, D.C., Morrow, P.E., Oberdorster, G., Utell, M.J., Frampton, M.W. Ultrafine particle deposition in subjects with asthma. *Environ Health Perspect* 112: 879–882, 2004.
74. Chandran, S.P., Chaudhary, M., Pasricha, R., Ahmad, A., Sastry, M. Synthesis of gold nanotriangles and silver nanoparticles using aloe vera plant extract. *Biotechnol. Prog.* 2006, 22, 577-583.
75. Chaudhari, V.R., Haram, S.K., Kulshreshtha, S.K., Bellare, J.R., Hassan, P.A. Micelle assisted morphological evolution of silver nanoparticles. *Colloids Surfaces A.* 2007, 301, 475-480.
76. Chen, B.L., Beckett, R. Development of SdFFF-ETAAS for characterising soil and sediment colloids, *Analyst*, 2001, 126(9), 1588–1593.
77. Chen, D., Li, L., Liu, J., Qi, S., Tang, F., Ren, X., Wu, W., Ren, J., Zhang, L. Synthesis and self-assembly of monodisperse silver-nanocrystal-doped silica particles. *J. Colloid Inter.Sci.* 2007d, 308, 351-355.

78. Chen, H.W., Su, S.F., Chien, C.T., Lin, W.H., Yu, S.L., Chou, C.C., Chen, J.J., Yang, P.C. Titanium dioxide nanoparticles induce emphysema-like lung injury in mice, *The FASEB Journal*, vol. 20, no. 13, pp. 2393–2395, 2006.
79. Chen, J., Tan, M., Nemmar, A., Song, W., Dong, M., Zhang, G., Li, Y. Quantification of extrapulmonary translocation of intratracheal-instilled particles in vivo in rats: effect of lipopolysaccharide, *Toxicol.*, 2006, 222(3):195–201.
80. Chen, J., Wang, J., Zhang, X., Jin, Y. Microwave-assisted green synthesis of silver nanoparticles by carboxymethyl cellulose sodium and silver nitrate. *Mater. Chem. Phys.* 2008c, 108, 421-424.
81. Chen, K.L., Elimelech, M. 2006. Aggregation and Deposition Kinetics of Fullerene (C) Nanoparticles. *Langmuir*, 22, 10994-11001.
82. Chen, M., Feng, Y., Wang, X., Li, T., Zhang, J., Qian, D. Silver nanoparticles capped by oleylamine: formation, growth, and self-organization. *Langmuir*. 2007c, 23, 5296-5304.
83. Chen, P., Song, L., Liu, Y., Fang, Y. Synthesis of silver nanoparticles by γ -ray irradiation in acetic water solution containing chitosan. *Radiat. Phys. Chem.* 2007b, 76, 1165-1168.
84. Chen, W., Liu, Y., Courtney, H.S., Bettenga, M., Agrawal, C.M., Bumgardner, J.D., Ong, J.L. 2006. In vitro anti-bacterial and biological properties of magnetron co-sputtered silver-containing hydroxyapatite coating, *Biomaterials*, 27:5512-5517.
85. Chen, X., Schluesener, H.J. 2008. Nanosilver: A nanoparticle in medical application. *Toxicol Lett* 176:1-12.
86. Chen, Y., Wang, X. Novel phase-transfer preparation of monodisperse silver and gold nanoparticles at room temperature. *Mater. Lett.* 2008b, 62, 2215-2218.
87. Chepuri, R.K., Trivedi, D.C. 2006. Biphasic synthesis of fatty acids stabilized silver nanoparticles: Role of experimental conditions on particle size. *Materials Chemistry and Physics*, 99: 354–360.
88. Choi, N.K., Seo, D.S., Lee, J.K. Preparation and Stabilization of Silver Colloids Protected by Surfactant. *Mater. Forum.* 2005, 29, 394-396.
89. Church, D., Elsayed, S., Reid, O., Winston, B., Lindsay, R. 2006. Burn wound infections, *Clin. Microbiol. Rev.*:403 - 19:434.
90. Chopra, I. 2007. The increasing use of silver-based products as antimicrobial agents: A useful development or a cause for concern? *J. Antimicrob. Chemother.* 59:587-590.

91. Clement, J.L., Jarrett, P.S. 1994. Antibacterial Silver Metal Based Drugs, 1, 5-6. *Appl. Environ. Microbiol.*, 73:1712–20.
92. Coelho, S., Amarelo, M., Ryan, S., Reddy, M., Sibbald, R.G. 2004. Rheumatoid arthritis-associated inflammatory leg ulcers: A new treatment for recalcitrant wounds, *Int. Wound J.*, 1:81-84.
93. Cohen, M.S., Stern, J.M., Vanni, A.J., Kelley, R.S., Baumgart, E., Field, D., Libertino, J.A., Summerhayes, I.C. 2007. *In vitro* analysis of a nanocrystalline silver-coated surgical mesh, *Surg. Infect. (Larchmt.)*, 8:397-403.
94. Coombs, C.J., Wan, A.T., Masterston, J.P., Conyers, R.A., Pedersen, J., Chia, Y.T. 1992. Do burn patients have a silver lining? *Burns* 18, 179-184.
95. Cosgrove, T. *Colloid Science: principles, methods and applications*. Blackwell Publishing: UK, 2005.
96. Courrol, L.C., de Oliveira Silva, F.R., Gomes, L. A simple method to synthesize silver nanoparticles by photo-reduction. *Colloids Surfaces A*. 2007, 305, 54-57.
97. Cumberland, S.A., Lead, J.R. 2009. Particle size distributions of silver nanoparticles at environmentally relevant conditions, *J. Chrom. A*, 1216(52):9099-9105.
98. Curtis, J., Greenberg, M., Kester, J., Phillips, S., Krieger, G. Nanotechnology and nanotoxicology: a primer for clinicians, *Toxicological Reviews*, vol. 25, no. 4, pp. 245–260, 2006.
99. Dai, K., Shi, L., Fang, J., Zhang, Y. Synthesis of silver nanoparticles on functional multi-walled carbon nanotubes. *Mater. Sci. Eng. A*. 2007, 465, 283-286.
100. Daigle, C.C., Chalupa, D.C., Gibb, F.R., Morrow, P.E., Oberdorster, G., Utell, M.J., Frampton, M.W. Ultrafine particle deposition in humans during rest and exercise. *Inhal Toxicol* 15: 539–552, 2003.
101. Damle, C., Kumar, A., Sastry, M. Synthesis of Ag/Pd nanoparticles and their low-temperature alloying within thermally evaporated fatty acid films. *J. Phys. Chem. B*. 2002, 106, 297-302.
102. Danilczuk, M., Lund, A., Sadlo, J., Yamada, H., Michalik, J. 2006. Conduction electron spin resonance of small silver particles. *Spectrochim. Acta A*, 63:189-191.
103. Davis, J.M. How to assess the risks of nanotechnology: Learning from past experience. *J. Nanoscience and Nanotechnol.*, 7: 402-409, 2007.
104. Davis, J.M., Thomas, V.N. Systematic approach to evaluating trade-offs among fuel options. The lessons of MTBE. *Ann. N.Y. Acad. Sci.*, 1076: 498-515, 2006.

105. Dawn, A., Mukherjee, P., Nandi, A.K. Preparation of size-controlled, highly populated, stable, and nearly monodispersed Ag nanoparticles in an organic medium from a simple interfacial redox process using a conducting polymer. *Langmuir*. 2007, 23, 5231-5237.
106. Dekkers, S., Prud'homme de Lodder, L.C.H., De Winter, R., Sips, A.J.A.M., De Jong, W.H. 2007a. Inventory of consumer products containing nanomaterials. RIVM/SIR advisory report 11124.
107. Dekkers, S., De Heer, C., De Jong, W.H., Sips, A.J.A.M., Van Engelen, J.G.M. 2007b. Nanomaterials in consumer products. Availability on the European market and adequacy of the regulatory framework. RIVM/SIR Advisory report 11014.
108. del Rocío, M.B. 2006. Detection of nitroexplosives by surface enhanced Raman spectroscopy on colloidal metal nanoparticles. Master thesis, University of Puerto Rico, Mayaguez Campus.
109. Demling, R.H., Leslie DeSanti, M.D. 2002. The rate of reepithelialization across meshed skin grafts is increased with exposure to silver. *Burns*, 28:264-266.
110. Deng, Y., Sun, Y., Wang, P., Zhang, D., Jiao, X., Ming, H., Zhang, Q., Jiao, Y., Sun X. Nonlinear optical properties of silver colloidal solution by in situ synthesis technique. *Curr. Appl. Phys.* 2008, 8, 13-17.
111. Deshmukh R.D., Composto, R.J. Surface Segregation and formation of silver nanoparticles created in situ in Poly(methyl Methacrylate) Films. *Chem. Mater.* 2007, 19, 745-754.
112. Dilleen, J.W., Sprules, S.D., Birch, B.J., Haggett, B.D. 1998. Electrochemical determination of silver in photographic solutions using fixed-volume single-use sensors. *Analyst*, 123: 2905–2907.
113. DiRienzo, M. 2006. New Applications for Silver. The LBMA Precious Metals Conference 2006, Montreux.
114. Dockery, D.W., Luttmann-Gibson, H., Rich, D.Q., Link, M.S., Mittleman, M.A., Gold, D.R., Koutrakis, P., Schwartz, J.D., Verrier, R.L. Association of air pollution with increased incidence of ventricular tachyarrhythmias recorded by implanted cardioverter defibrillators, *Environ. Health Perspect.*, 113(6), 670-674, 2005.
115. Doering, W.E., Nie, S. 2002. Single-Molecule and Single-Nanoparticle SERS: Examining the Roles of Surface Active Sites and Chemical Enhancement. *J. Phys. Chem. B*, 106: 311-317.

116. Donaldson, K., Stone, V., Tran, C., Kreyling, W., Borm, P.J.A. *Nanotoxicology, Occup. Environ. Med.* 61(9):727-728, 2004.
117. Dong, Y., Ma, Y., Zhai, T., Shen, F., Zeng, Y., Fu, H., Yao, J. Silver Nanoparticles Stabilized by Thermoresponsive Microgel Particles: Synthesis and Evidence of an Electron Donor-Acceptor Effect. *Macromol. Rapid Commun.* 2007, 28, 2339–2345.
118. Dong, Q., Su, H., Cao, W., Han, J., Zhang, D., Guo, Q. Biogenic synthesis of hierarchical hybrid nanocomposites and patterning of silver nanoparticles. *Mater. Chem. Phys.* 2008, 110, 160-165.
119. Dorjnamjin, D., Ariunaa, M., Shim, Y.K. 2008. Synthesis of silver nanoparticles using hydroxyl functionalized ionic liquids and their antimicrobial activity. *Int. J. Mol. Sci.*, 9: 807–20.
120. Drake P.L., Hazelwood K.J. 2005. Exposure-related health effects of silver and silver compounds: A review. *Ann. Occup. Hyg.*, 49:575-585.
121. DTSC. Department of Toxic Substances Control. Personal Communication. June 2010.
122. Dubas, S.T., Pimpan, V. 2008a. Green synthesis of silver nanoparticles for ammonia sensing. *Talanta*, 76:29–33.
123. Dubas, S.T., Pimpan, V. 2008b. Humic acid assisted synthesis of silver nanoparticles and its application to herbicide detection. *Mater Lett*, 62:2661–2663.
124. Dunn, K., Edwards-Jones, V. 2004. The role of Acticoat with nanocrystalline silver in the management of burns. *Burns*, 30:S1-9.
125. Edwards, H.W., Petersen, R.P. 1936. Reflectivity of evaporated silver films. *Phys. Rev.*, 9: 871
126. Eisler, R. 1997. Silver hazards to fish, wildlife and invertebrates: A synoptic review. Washington, DC, US Department of the Interior, National Biological Service, 44 pp. (Biological Report 32 and Contaminant Hazard Reviews Report 32).
127. El-Ansary, A., Al-Daihan, S. On the toxicity of therapeutically used nanoparticles: An overview, *J. Toxicol.*, Volume 2009, 9 pages. doi:10.1155/2009/754810
128. El Badawy, A. Luxton, T., Silva, R., Scheckel, K., Suidan, M., Tolaymat, T. 2010. Impact of Environmental Conditions (pH, Ionic Strength, and Electrolyte Type) on the Surface Charge and Aggregation of Silver Nanoparticles Suspensions”, *Environ. Sci. Technol.*, 44(4):1260-1266.

129. Elder, A., Gelein, R., Silva, V., Feikert, T., Opanashuk, L., Carter, J., Potter, R., Maynard, A., Ito, Y., Finkelstein, J., Oberdörster, G. Translocation of inhaled ultrafine manganese oxide particles to the central nervous system, *Environ. Health Perspect.*, 114(8), 1172-1178, 2006.
130. Elder, A., Vidyasagar, S., DeLouise, L. Physicochemical factors that affect metal and metal oxide nanoparticle passage across epithelial barriers, *WIREs Nanomedicine and Nanobiotechnology*, 1(4), 2009. DOI: 10.1002/wnan.44. Available on-line: <http://wires.wiley.com/WileyCDA/WiresArticle/wisId-WNAN44.html>
131. Elechiguerra, J.L., Burt, J.L., Morones, J.R., Camacho-Bragado, A., Gao, X., Lara, H.H., Yacaman, M.J. 2005. Interaction of silver nanoparticles with HIV-1. *J. Nanobiotechnol.*, June 29, 3:6.
132. Elzey, S., Grassian, V.H., 2009. Agglomeration, isolation and dissolution of commercially manufactured silver nanoparticles in aqueous environments, *J. Nanoparticle Res.* doi:10.1007/s11051-009-9783-y.
133. Evanoff Jr., D.D., Chumanov, G. Size-controlled synthesis of nanoparticles. 2004. "Silver-only" aqueous suspensions via hydrogen reduction. *J. Phys. Chem. B.*, 108, 13948–13956.
134. Fabrega, J., Fawcett, S.R., Renshaw, J.C., Lead, J.R. Silver nanoparticle impact on bacterial growth: Effect of pH, concentration, and organic matter, *Environ. Sci. Technol.*, 2009, 43:7285-7290.
135. Farkas, A., Balashazy, I., Szocs, K. Characterization of regional and local deposition of inhaled aerosol drugs in the respiratory system by computational fluid and particle dynamics methods. *J Aerosol Med* 19: 329–343, 2006.
136. Faure, C., Derre, A., Neri, W. Spontaneous formation of silver nanoparticles in multilamellar vesicles. *J. Phys. Chem. B.* 2003, 107, 4738-4746.
137. Fauss, E. 2008. The Silver Nanotechnology Commercial Inventory. University of Virginia. <http://www.nanoproject.org>.
138. Feick, J.D., Velegol, D. Electrophoresis of spheroidal particles having a random distribution of zeta potential, *Langmuir*, 2000, 16(26), 10315–10321.
139. Fernandez, E.J., Barrasa, J.C., Laguna, A., Lopez de-Luzuriaga, J. M., Monge, M., Torres, C., 2008. The preparation of highly active antimicrobial silver nanoparticles by an organometallic approach. *Nanotechnology*, 19: 185602 (6pp).

140. Fleischer, T., Grunwald, A. making nanotechnology developments sustainable. A role for technology assessment. *J. Cleaner Prod.*, 2008, 16(8-9), 889-898.
141. Foldbjerg, R., Olesen, P., Hougaard, M., Dang, D.A., Hoffmann, H.J., Autrup, H. PVP-coated silver nanoparticles and silver ions induce reactive oxygen species, apoptosis and necrosis in THP-1 monocytes, *Toxicology Letters*, 190, 2009, 156-162.
142. Foot Defense. 2010. Silver: a study of this precious metal and its use in foot care. <http://www.acor.com/Downloads/ebSilverFoot.pdf>. Date accessed Feb-2010.
143. Fung, M.C., Bowen, D.L. 1996. Silver products for medical indication: Risk benefit assessment. *Clin. Toxicol.* 34:119-126.
144. Furno, F., Morley, K.S., Wong, B., Sharp, B.L., Arnold, P.L., Howdle, S.M., Bayston, R., Brown, P.D., Winship, P.D., Reid, H.J. 2004. Silver nanoparticles and polymeric medical devices: A new approach to prevention of infection? *J. Antimicrob. Chemother.*, 54:1019-1024.
145. Furst, A., Schlauder, M.C. 1978. Inactivity of two noble metals as carcinogens. *J. Environ. Pathol. Toxicol.* 1:51-57.
146. Gaiser, B.K., Fernandes, T.F., Jepson, M., Lead, J.R., Tyler, C.R., Stone, V. Assessing exposure, uptake and toxicity of silver and cerium dioxide nanoparticles from contaminated environments. *Environmental Health* 2009, 8(Suppl 1):S2 doi:10.1186/1476-069X-8-S1-S2.
147. Galiano, K., Pleifer, C., Engelhardt, K., Brossner, G., Lackner, P., Huck, C., Lass-Flörl, C., Obwegeser, A. 2007. Silver segregation and bacterial growth of intraventricular catheters impregnated with silver nanoparticles in cerebrospinal fluid drainages, *Neurol. Res. ASAP*, 30:285-287.
148. Gao, G. 2004. *Nanostructures and nanomaterials. Synthesis, Properties & Applications.* London: Imperial College Press.
149. Gao, J., Youn, S., Hovsepyan, A., Llana, V.L., Wang, Y., Bitton, G., Bonzongo, J.J. Dispersion and Toxicity of Selected Manufactured Nanomaterials in Natural River Water Samples: Effects of Water Chemical Composition, *Environ. Sci. Technol.*, 2009, 43 (9), pp 3322–3328, DOI: 10.1021/es803315v
150. Garnett, M.C., Kallinteri, P. Nanomedicines and nanotoxicology: some physiological principles,” *Occupational Medicine*, vol. 56, no. 5, pp. 307–311, 2006.

151. Gatti, A.M. Biocompatibility of micro- and nano-particles in the colon. Part II, *Biomaterials* 25, 385-392, 2004.
152. Gatti, A.M., Montanari, S., Monari, E., Gambarelli, A., Capitani, F., Parisini, B. Detection of micro- and nano-sized biocompatible particles in the blood. *J. Mater. Sci.: Mater. Med.* 15(4), 469-472, 2004.
153. Gautam, G.P., Singh, S.R. 2007. A simple polyol synthesis of silver metal nanopowder of uniform particles. *Synthetic Metals*, 157: 5-10.
154. Geranio, L., Heuberger, M., Nowack, B. The behavior of silver nanotextiles during washing, *Environ. Sci. Technol.*, 2009, 43:8113-8118.
155. Gill, Michael. 2007. Nanotechnology: What is it? *Geology 2007 Symposium*, CalEPA HQ Sacramento, CA, May 9-10, 2007.
156. Gimbert, L.J., Hamon, R.E., Casey, P.S., Worsfold, P.J. Partitioning and stability of engineered ZnO nanoparticles in soil suspensions using flow field-flow fractionation, *Environ. Chem.*, 2007, 4(1), 8-10. doi:10.1071/EN06072
157. Giusti, P., Schaumloffel, D., Encinar, J.R., Szpunar, J. Interfacing reversed-phase nanoHPLC with ICP-MS and on-line isotope dilution analysis for the accurate quantification of selenium-containing peptides in protein tryptic digests, *J. Anal. At. Spectrom.*, 2005, 20(10), 1101.
158. Green, R.J., Davies, M.C., Roberts, C.J., Tendler, S.J.B. 1999. Competitive protein adsorption as observed by surface Plasmon resonance, *Biomaterials*, 20:385-391.
159. Greenwood, N.N., Earnshaw, A. *Chemistry of the Elements*, 2nd edition, Butterworth-Heinemann, Oxford, UK, 1997.
160. Griffitt, R.J., Luo, J., Gao, J., Bonzongo, J.-C., Barber, D.S. Effects of Particle Composition and Species on Toxicity of Metallic Nanomaterials in Aquatic Organisms, *Environ. Toxicol. Chem.* 27(9):1972-1978, 2008.
161. Griffitt, R.J., Hyndman, K., Denslow, N.D., Barber, D.S. Comparison of Molecular and Histological Changes in Zebrafish Gills Exposed to Metallic Nanoparticles, *Toxicol. Sci.*, 107(2):404-415, 2009.
162. Gryczynski, Z., Lukomska, J., Lakowicz, J.R., Matveeva, E.G., Gryczynski, I. Depolarized light scattering from silver nanoparticles. *Chem. Phys. Lett.* 2006, 421,189-192.
163. Gulrajani, M.L., Gupta, D., Periyasamy, S., Muthu, S. 2008. Preparation and Application of Silver Nanoparticles on Silk for Imparting Antimicrobial Properties. *J. Appl. Polymer Sci.*, 108: 614–623.

164. Guo, L., Nie, J., Du, B., Peng, Z., Tesche, B., Kleinermanns, K. 2008. Thermoresponsive polymerstabilized silver nanoparticles. *J. Colloid Interface Sci.*, 319:175–181.
165. Gurunathan, S., Kalishwaralal, K., Vaidyanathan, R., Venkataraman, D., Ram, S., Pandian, K., Muniyandi, J., Hariharana, N., Eom, S. H. 2009. Biosynthesis, purification and characterization of silver nanoparticles using *Escherichia coli*. *Colloids and Surfaces B: Biointerfaces*, 74, (1), 328-335.
166. Hagens, W.I., Oomen, A.G., de Jong, W.H., Cassee, F.R., Sips, A.J.A.M. What do we (need to) know about the kinetic properties of nanoparticles in the body?, *Regulatory Toxicology and Pharmacology*, vol. 49, no. 3, pp. 217–229, 2007.
167. Hamal, D.B., Klabunde, K., J. Synthesis, characterization, and visible light activity of new nanoparticle photocatalysts based on silver, carbon, and sulfur-doped TiO₂. *J. Colloid Inter. Sci.* 2007, 311, 514-522.
168. Hammond, C.R. 2000. The Elements, in *Handbook of Chemistry and Physics* 81st edition. CRC press. ISBN 0849304814.
169. Hamouda, B.S., Nguyen, Q.T., Langevin, D., Chappay, C., Roudesli, S. Polyamide 12-polytetramethyleneoxide block copolymer membranes with silver nanoparticles – Synthesis and water permeation properties. *Reac. Funct. Polym.* 2007, 67, 893-904.
170. Harris, A.T., Bali, R. On the formation and extent of uptake of silver nanoparticles by live plants, *J. Nanopart. Res.*, 2008, 10:691-695.
171. Hasell, T., Yang, J., Wang, W., Brown, P.D., Howdle S.M. A facile synthetic route to aqueous dispersions of silver nanoparticles. *Mater. Lett.* 2007, 61, 4906-4910.
172. Hassellöv, M., Lyven, B., Haraldsson, C., Sirinawin, W. Determination of continuous size and trace element distribution of colloidal material in natural water by on-line coupling of flow fieldflow fractionation with ICPMS, *Anal. Chem.*, 1999, 71(16), 3497.
173. Hatchett, D.W., White, H.S. 1996. Electrochemistry of sulfur adlayers on low-index faces of silver, *J. Phys. Chem.*, 100:9854-9859.
174. Haverkamp, R.G., Marshall, A.T., van Agterveld, D. Pick your carats: nanoparticles of gold-silver-copper alloy produced *in vivo*, *J. Nanopart. Res.*, 2007, 9:697-700.
175. He, B., Zhang, S., Zhang, L., Wu, Z. Zhang, Z. Preparation of reactive surface-modified Ag nanoparticles and their assembly on a Si surface via covalent bonds. *Nanotechnology*. 2007b, 18, 1-6.

176. He, J., Kunitake, T. Formation of silver nanoparticles and nanocraters on silicon wafers. *Langmuir*. 2006, 22, 7881-7884.
177. He, P., Zhu, X. Synthesis and characterization of phospholipid-functionalized silver nanoparticles. *Mater. Res. Bull.* 2008b, 43, 625-630.
178. He, X., Chen, X., Chen, Y., Feng, J., Sun, Z. Synthesis and characterization of silver nanowires with zigzag morphology in N,N-dimethylformamide. *J. Solid St. Chem.* 2007a, 180, 2262-2267.
179. Helfrich, A., Bruchert, W., Bettmer, J. Size characterisation of Au nanoparticles by ICP-MS coupling techniques, *J. Anal. At. Spectrom.*, 2006, 21(4), 431.
180. Helmenstine, A.M. 2010. Silver Facts.
<http://chemistry.about.com/od/elementfacts/a/silver.htm>. Date accessed Feb-2010.
181. Henglein, A., Meisel, D. Spectrophotometric observations of the adsorption of organosulfur compounds on colloidal silver nanoparticles. *J. Phys. Chem. B.* 1998, 102, 8364-8366.
182. History of silver. 2010.
http://www.painreliefwellness.com/clients/2842/documents/History_of_Silver_Usage.pdf. Date accessed Feb-2010.
183. Hoet, P.H.M., Bruske-Hohlfeld, I., Salata, O.V. Nanoparticles – known and unknown health risks, *J. Nanobiotechnol*, 2(1), 12, 2004.
184. Holt, K.B., Bard, A.J. 2005. Interaction of silver (I) ions with the respiratory chain of *Escherichia coli*: An electrochemical and scanning electrochemical microscopy study of the antimicrobial mechanism of micromolar Ag⁺. *Biochemistry* 44, 13214-13223.
185. Hoppe, C.E., Lazzar, M., Pardinias-Blanco, I., Lopez-Quintela, A. One-Step Synthesis of gold and silver hydrosols using poly(n-vinyl-2-pyrrolidone) as a reducing agent. *Langmuir*. 2006, 22, 7027-7034.
186. Hsin, Y.H., Chen, C.F., Huang, S., Shih, T.S., Lai, P.S., Chueh, P.J., 2008. The apoptotic effect of nanosilver is mediated by a ROS- and JNK-dependent mechanism involving the mitochondrial pathway in NIH3T3 cells. *Toxicol. Lett.*, 179:130-139.
187. Hsu, S.L., Wu, R. Synthesis of contamination-free silver nanoparticle suspensions for micro-interconnects. *Mater. Lett.* 2007, 61, 3719-3722.

188. Hu, Z. Impact of silver nanoparticles on wastewater treatment. WERF Report U3R07. Water Environment Research Foundation. 1 April, 2011 (Expected). 50 pages. ISBN: 9781843393993.
189. Hua, L., Chen, J., Ge, L., Tan, S.N. 2007. Silver nanoparticles as matrix for laser desorption/ ionization mass spectrometry of peptides. *J. Nanopart. Res.*, 9:1133–1138.
190. Huang, H., Yang, X. Synthesis of polysaccharide-stabilized gold and silver nanoparticles: a green method. *Carbohyd. Res.* 2004, 339, 2627-2631.
191. Huang, L., Wen, T. One-step synthesis of silver nanoparticles and poly(2,5-dimethoxyaniline) in poly(styrene sulfonic acid). *Mater. Sci. Eng. A.* 2007, 445–446, 7-13.
192. Huo, S., Xue, X., Li, Q., Xu, S., Cai, W. 2006. Seeded-growth approach to fabrication of silver nanoparticle films on silicon for electrochemical surface-enhanced IR absorption spectroscopy. *J. Phys. Chem.*, 110:25721–25728.
193. Hussain, S. M.; Hess, K. L.; Gearhart, J. M.; Geiss, K. T.; Schlager, J. J. In vitro toxicity of nanoparticles in BRL 3A rat liver cells. *Toxicol. In Vitro* 2005, 19, 975–983.
194. Hussain, S. M.; Javorina, A. K.; Schrand, A. M.; Duhart, H. M.; Ali, S. F.; Schlager, J. J. The interaction of manganese nanoparticles with PC-12 cells induces dopamine depletion. *Toxicol. Sci.* 2006, 92, 456–463.
195. Huve, P., Verrecchia, T., Bazile, D., Vauthier, C., Couvreur, P. Simultaneous use of size-exclusion chromatography and photon correlation spectroscopy for the characterization of poly(lactic acid) nanoparticles, *J. Chromatogr., A*, 1994, 675(1–2), 129.
196. Hwang, E.T., Lee, J.H., Chae, Y.J., Kim, Y.S., Kim, B.C., Sang, B., Gu, M.B. 2008. Analysis of the toxic mode of action of silver nanoparticles using stress-specific bioluminescent bacteria, *Small*, 4:746-750.
197. Information and history of silver. 2010.
<http://www.cctechnologies.com.au/PDF/Information%20and%20history%20of%20silver.pdf>. Date accessed Feb-2010
198. Innes, M.E., Umraw, N., Fish, I.S., Gomez, M., Cartotto, R.C. 2001. The use of silver coated dressing on donor site wounds: a prospective, controlled matched pair study. *Burns* 27, 621-627.

199. ISO. International Standards Organization. Environmental Management - Life Cycle Assessment - Requirements and Guidelines, Principles and Framework; ISO 14044; 2006.
200. Iwasaki, S., Yoshimura, A., Ideura, T., Koshikawa, S., Sudo, M. 1997. Elimination study of silver in a hemodialyzed burn patient treated with silver sulfadiazine cream. *Am. J. Kidney Dis.* 30, 287-290.
201. Iyer, K.S., Raston, C.L., Saunders, M. 2007. Continuous flow nano-technology: manipulating the size, shape, agglomeration, defects and phases of silver nanoparticles. *Lab Chip*, 7: 1800–1805.
202. Jacob, J.A., Kapoor, S., Biswas, N., Mukherjee, T. Size tunable synthesis of silver nanoparticles in water–ethylene glycol mixtures. *Colloids Surfaces A.* 2007; 301, 329-334.
203. Jacob, J.A., Mahal, H.S., Biswas, N., Mukherjee, T., Kapoor, S. 2008. Role of phenol derivatives in the formation of silver nanoparticles, *Langmuir*, 24:528–33.
204. Ji, J.H., Jung, J.H., Kim, S.S., Yoon, J.U., Park, J.D., Choi, B.S., Chung, Y.H., Kwon, I.H., Jeong, J., Han, B.S., Shin, J.H., Sung, J.H., Song, K.S., Yu, I.J. 2007. Twenty-eight-day inhalation toxicity study of silver nanoparticles in Sprague-Dawley rats, *Inhal. Toxicol.*, 19:857-871.
205. Jia, H., Hou, W., Wei, L., Xu, B., Liu, X. 2008. The structures and antibacterial properties of nano-SiO₂ supported silver/zinc-silver materials, *Dent. Mater.*, 24:244-249.
206. Jia, H., Xu, W., An, J., Li, D., Zhao, B. A simple method to synthesize triangular silver nanoparticles by light irradiation. *Spectrochim. Acta A.* 2006, 64, 956-960.
207. Jiang, H., Moon, K., Hua, F., Wong, C.P. Synthesis and thermal and wetting properties of tin/silver alloy nanoparticles for low melting point lead-free solders. *Chem. Mater.* 2007b, 19, 4482-4485.
208. Jiang, J., Oberdörster, G., Biswas, P. Characterization of Size, Surface Charge, and Agglomeration State of Nanoparticle Dispersions for Toxicological Studies. *J. Nanopart. Res.* 2009, 11, 77-89.
209. Jiang, X., Zeng, Q., Yu, A. Thiol-frozen shape evolution of triangular silver nanoplates. *Langmuir.* 2007a, 23, 2218-2223.
210. Jiang, Z., Liu, C., Sun, L. 2005. Catalytic properties of silver nanoparticles supported on silica spheres. *J. Phys. Chem. B*, 109:1730–5.

211. Jimenez, V.L., Leopold, M.C., Mazzitelli, C., Jorgenson, J.W., Murray, R.W.
HPLC of monolayer-protected gold nanoclusters, *Anal. Chem.*, 2003, 75(2), 199.
212. Ju-Nam, Y., Lead, J.R. 2008. "Manufactured nanoparticles: An overview of their chemistry, interactions and potential environmental implications. *Sci. Tot. Environ.*, 400: 396-414.
213. Junior, A.M., de Oliveira, M.H.P., Gehlen, M.H. Preparation of silver nanoprisms using poly(N-vinyl-2-pyrrolidone) as a colloid-stabilizing agent and the effect of silver nanoparticles on the photophysical properties of cationic dyes. *Photochem. Photobiol. Sci.* 2003, 2, 921-925.
214. Kakurai, M., Demitsu, T., Umemoto, N., Ohtsuki, M., Nakagawa, H. 2003.
Activation of mast cells by silver particles in a patient with localized argyria due to implantation of acupuncture needles. *Br J Dermatol* 148:822.
215. Kalimuthu, K., Babu, R.S., Venkataraman, D., Bilal, M., Gurnathan, S.
Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. *Colloid Surfaces B.* 2008, 65, 150-153.
216. Kalishwaralal, K., Banumathi, E., Ram Kumar Pandian, S., Deepak, V., Muniyandi, J., Hyun Eom, S., Gurnathan, S., 2009. Silver nanoparticles inhibit VEGF induced cell proliferation and migration in bovine retinal endothelial cells. *Colloids Surf. B: Bio Interf.* 73, 51–57.
217. Kashiwagi, Y., Yamamoto, M., Nakamoto, M., 2006. Facile size-regulated synthesis of silver nanoparticles by controlled thermolysis of silver alkylcarboxylates in the presence of alkylamines with different chain lengths. *J. of Colloid and Interface Science*, 300: 169–175.
218. Kawata, K., Osawa, M., Okabe, S. In vitro toxicity of silver nanoparticles at noncytotoxic doses to HepG2 human hepatoma cells, *Environ. Sci. Technol.*, 2009, 43, 6046-6051.
219. Khanna, P.K., Kulkarni, D., Beri, R.K. Synthesis and characterization of myristic acid capped silver nanoparticles. *J. Nanoparticles Res.* 2008, 10, 1059-1062.
220. Khanna, V., Bhavik, B., Lee, L.J. Life cycle energy analysis and environmental life cycle assessment of carbon nanofibres production. In *Proceedings of the 2007 IEEE International symposium on Electronics & the Environment Conference Record*, 7 – 10 May 2007, Orlando, FL, 2007, pp128-133.

221. Kim, D., Jeong, S., Moon, J. Synthesis of silver nanoparticles using the polyol process and the influence of precursor injection. *Nanotechnology*. 2006, 17, 4019-4024.
222. Kim, J.S., Kuk, E., Yu, K.N., Kim, J.H., Park, S.J., Lee, H.J., Kim, S.H., Park, Y.K., Park, Y.H., Hwang, C.Y., Kim, Y.K., Lee, Y.S., Jeong, D.H., Cho, M.H. 2007. Antimicrobial effects of silver nanoparticles, *Nanomedicine*, 3:95-101.
223. Kim, S., Choi, J.E., Choi, J., Chung, K.-H., Park, K., Yi, J., Ryu, D.-Y. Oxidative stress-dependent toxicity of silver nanoparticles in human hepatoma cells, *Toxicology in Vitro*, 23, 2009, PP1076-1084.
224. Kim, S.H., Choi, B.S., Kang, K., Choi, Y., Yang, I.Y. 2007. Low temperature synthesis and growth mechanism of Ag nanowires. *Journal of Alloys and Compounds* 433: 261–264.
225. Kim, S.J., Kim, T.G., Ah, C.S., Kim, K., Jang, D. Photolysis dynamics of benzyl phenyl sulfide adsorbed on silver nanoparticles. *J. Phys. Chem. B*. 2004, 108, 880-882.
226. Kim, W.-Y., K., Kim, J., Park, J.D., Ryu, H.Y., Yu, I.J. Histological Study of Gender Differences in Accumulation of Silver Nanoparticles in Kidneys of Fischer 344 Rats. *J. Toxicol. Environ. Health, Part A*. 72: 21-22, 2009, 1279 — 1284.
227. Kim, Y. S.; Kim, J. S.; Cho, H. S.; Rha, D. S.; Kim, J. M.; Park, J. D.; Choi, B. S.; Lim, R.; Chang, H. K.; Chung, Y. H.; Kwon, I. H.; Jeong, J.; Han, B. S.; Yu, I. J. Twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley rats. *Inhalation Toxicol*. 2008, 20, 575–583.
228. Kirsner, R.S., Orsted, H., Wright, J.B. 2001. Matrix metalloproteinases in normal and impaired wound healing: a potential role for nanocrystalline silver, *Wounds*, 13(3 Suppl):4C–12C.
229. Klaus, T., Joerger, R., Olsson, E., Granqvist, C.G. 1999. Silver-based crystalline nanoparticles, microbially fabricated, *Proc. Natl. Acad. Sci. USA*, 96:13611-13614.
230. Kloepffer, W., Curran, M.A., Frankl, P., Heijungs, R., Koehler, A., Olsen, S.I. *Nanotechnology and Life Cycle Assessment: Synthesis of Results Obtained at a Workshop in Washington, DC, 2-3 October 2006*; 2007.
231. Koh, J.H., Kim, Y.W., Park, J.T., Kim, J.H. Templated synthesis of silver nanoparticles in amphiphilic poly(vinylidene fluoride-cochlorotrifluoroethylene) comb copolymer. *J. Polym. Sci B*. 2008, 46, 702-709.

232. Konradova, V. 1968. The ultrastructure of the tracheal epithelium in rabbits following the inhalation of aerosols of colloidal solutions of heavy metals. 1. Changes in the ultrastructure of the tracheal epithelium after 2-hour inhalation of colloidal solutions of iron and silver. *Folia Morphol.*, (Praha), 16:258-264.
233. Krishnan, N., Boyd, S., somani, A., Raoux, S., Clark, D., Dornfeld, D. A hybrid life cycle inventory of nano-scale semiconductor manufacturing, *Environ, Sci. Technol.*, 2008, 42(8):3069-3075.
234. Krueger, K.M., Al-Somali, A.M., Falkner, J.C., Colvin, V.L. Characterization of nanocrystalline Cd Se by size exclusion chromatography, *Anal. Chem.*, 2005, 77(11), 3511.
235. Krutyakov, Y.A., Kudrinskiy, Y.A.; Olenin, A.Y.; Lisichkin, G.V. 2008. Synthesis and properties of silver nanoparticles: advances and prospects. *Russian Chemical Reviews*, 77 (3), 233-257.
236. Kuila, B.K., Garai, A., Nandi, A.K. Synthesis, optical, and electrical characterization of organically soluble silver nanoparticles and their poly(3-hexylthiophene) nanocomposites: enhanced luminescence property in the nanocomposite thin films. *Chem. Mater.* 2007, 19, 5443-5452.
237. Kumar, A., Vemula, P.K., Ajayan, P.M., John, G. 2008. Silver-nanoparticle-embedded antimicrobial paints based on vegetable oil. *Nature Materials*, 7, 236-241.
238. Kumar, S.A., Abyaneh, M.K., Gosavi, S.W., Kulkarni, S.K., Pasricha, R., Ahmad, A., Khan, M.I. Nitrate reductase-mediated synthesis of silver nanoparticles from AgNO₃. *Biotechnol Lett.* 2007, 29, 439-445.
239. Kuo, P., Chen, C., Yuen, S. Protection effects of hydrophile-grafted silicone copolymers on the formation of colloidal silver nanoparticles. *J. Phys. Chem. B.* 2004, 108, 5541-5546.
240. Lakowicz, J.R., Sabanayagam, C.R. Increasing the sensitivity of DNA microarrays by metal-enhanced fluorescence using surface-bound silver nanoparticles. *Nucleic Acids Res.* 2007, 35, 2-9.
241. Lam, P.K., Chan, E.S.Y., Ho, W.S., Liew, C.T. 2004. In vitro cytotoxicity testing of a nanocrystalline silver dressing (Acticoat) on cultured keratinocytes. *Br J Biomed Sci* 61(3):125-127.
242. LANL. 2010. "Silver". Los Alamos National Labs. Chemistry Division. Website: <http://www.periodic.lanl.gov/elements/47.html>. Last accessed: May, 2010.

243. Larese, F.F., D'Agostin, F., Crosera, M., Adami, G., Renzi, N., Bovenzi, M., Maina, G. 2009. Human skin penetration of silver nanoparticles through intact and damaged skin. *Toxicology* 255:33–37.
244. Lansdown, A.B. 2007. Critical observations on the neurotoxicity of silver. *Crit Rev Toxicol* 37:237-250.
245. Lee, H.J., Yeo, S.Y., Jeong, S.H. 2003. Antibacterial effect of nanosized silver colloidal solution on textile fabrics, *J. Mater. Sci.*, 38:2199-2204.
246. Lee, K.J., Lee, Y., Shim, I., Joung, J., Oh, Y.S. Direct synthesis and bonding origins of monolayer-protected silver nanocrystals from silver nitrate through in situ ligand exchange. *J. Colloid Inter. Sci.* 2006, 304, 92-97.
247. Lee, K.J., Lee, Y., Shim, I., Jun, B.H., Cho, H.J., Joung, J. Large-scale synthesis of polymer-stabilized silver nanoparticles. *Sol. St. Phen.* 2007, 124-126, 1189-1192.
248. Lee, K.J., Nallathamby, P.D., Browning, L.M., Osgood, C.J., Xu, X.N. 2007. *In vivo* imaging of transport and biocompatibility of single silver nanoparticles in early development of zebrafish embryos, *Am. Chem. Soc.* 1(2):133-143.
249. Lee, K.J., Park, J.T., Goh, J.H., Kim, J.H. Synthesis of amphiphilic graft copolymer brush and its use as template film for the preparation of silver nanoparticles. *J. Polym. Sci. A.* 2008, 46, 3911-3918.
250. Lee, P.C., Meisel, D. 1982. Adsorption and surface-enhanced Raman of dyes on silver and gold sols. *J. Phys. Chem.*, 86, 3391–3395.
251. Lee, S.J., Han, S.W., Kim, K. Perfluorocarbon-stabilized silver nanoparticles manufactured from layered silver carboxylates. *Chem. Commun.* 2002, 442-443.
252. Lei, Z., Fan, Y. Preparation of silver nanocomposites stabilized by an amphiphilic block copolymer under ultrasonic irradiation. *Mater. Lett.* 2006, 60, 2256-2260.
253. Lenntech Water Treatment Solutions. 2010.
<http://www.lenntech.com/periodic/elements/ag.htm>. Date accessed Feb-2010.
254. Leong, G.N., Jo, U.B., Ryu, H.Y., Kim, Y.S., Song, K.S., Yu, I.J. Histochemical study of intestinal mucins after administration of silver nanoparticles in Sprague-Dawley rats, *Arch. Toxicol.*, 84:63-69, 2010.
255. Lesniak, W., Bielinska, A.U., Sun, K., Janczak, K.W., Shi, X., Baker, J.R., Balogh, L.P. 2005. Silver/dendrimer nanocomposites as biomarkers: Fabrication, characterization, in vitro toxicity, and intracellular detection, *Nano. Lett.*, 5:2123-2130.

256. Li, G., Chao, K., Ye, C., Peng, H. One-step synthesis of Ag nanoparticles supported on AgVO₃ nanobelts. *Mater. Lett.* 2008, 62, 735-738.
257. Li, S., Shen, Y., Xie, A., Yu, X., Qiu, L., Zhang, L., Zhang, Q. Green synthesis of silver nanoparticles using *Capsicum annuum* L. extract. *Green Chem.* 2007a, 8, 852-858.
258. Li, T., Park, H.G., Choi, S. γ -Irradiation-induced preparation of Ag and Au nanoparticles and their characterizations. *Mater. Chem. Phys.* 2007b, 105, 325-330.
259. Li, Y., Leung, P., Yao, L., Song, Q.W., Newton, E. 2006. Antimicrobial effect of surgical masks coated with nanoparticles, *J. Hosp. Infect.*, 62:58-63.
260. Li, Y., Wu, Y., Ong, B.S. Facile Synthesis of silver nanoparticles useful for fabrication of high-conductivity elements for printed electronics. *J. Am. Chem. Soc.* 2005, 127, 3266-3267.
261. Liau, S.Y., Read, D.C., Pugh, W.J., Furr, J.R. Russell, A.D. 1997. Interaction of silver nitrate with readily identifiable groups: Relationship to the antibacterial action of silver ions. *Lett. Appl. Microbiol.* 25, 279-283.
262. Limbach, L.K., Wick, P., Manser, P., Grass, R.N., Bruinink, A., Stark, W.J. Exposure of engineered nanoparticles to human lung epithelial cells: influence of chemical composition and catalytic activity on oxidative stress, *Environ. Sci. Technol.*, vol. 41, no. 11, pp. 4158–4163, 2007.
263. Limsavarn, L., Sritaveesinsub, V., Dubas, S.T. Polyelectrolyte assisted silver nanoparticles synthesis and thin film formation. *Mater. Lett.* 2007, 61, 3048-3051.
264. Lindemann, P.A. 1997. Colloidal Silver: A closer look http://www.free-energy.ws/pdf/cs_closer_look.pdf. Date accessed Feb-2010.
265. Lippmann, M. Effects of fiber characteristics on lung deposition, retention, and disease, *Environ. Health Perspect.* 88, 311-317, 1990.
266. Lippmann, M., Frampton, M., Schwartz, J., Dockery, D., Schlesinger, R., Koutrakis, P., Froines, J., Nel, A., Finkelstein, J., Godleski, J., Kaufman, J., Koenig, J., Larson, T., Luchtel, D., Liu, L.J., Oberdorster, G., Peters, A., Sarnat, J., Sioutas, C., Suh, H., Sullivan, J., Utell, M., Wichmann, E., Zelikoff, J. The U.S. Environmental Protection Agency Particulate Matter Health Effects Research Centers Program: a midcourse report of status, progress, and plans, *Environ. Health Perspect.* 111(8), 1074-1092, 2003.

267. Lin, K.H., Chu, T.C., Liu, F.K. On-line enhancement and separation of nanoparticles using capillary electrophoresis, *J. Chromatogr., A*, 2007, 1161(1–2), 314.
268. Liu, F.K., Wei, G.T. Effect of mobile-phase additives on separation of gold nanoparticles by size-exclusion chromatography, *Chromatographia*, 2004, 59(1–2), 115–119.
269. Liu, H., Xiao, F., Wang, C., Lee, Y., Xue, Q., Chen, X., Qian, D., Hao, J., Jiang, J. One-step synthesis of silver nanoparticles at the air–water interface using different methods. *Nanotechnology*. 2008, 19, 1-9.
270. Liu, J., Wang, A., Chi, Y., Lin, H., Mou, C. Synergistic effect in an Au-Ag alloy nanocatalyst: CO oxidation. *J. Phys. Chem. B*. 2005, 109, 40-43.
271. Liu, J., Wong, H.L., Moselhy, J., Bowen, B., Wu, X.Y., Johnston, M.R. Targeting colloidal particulates to thoracic lymph nodes, *Lung Cancer* 51(3), 377-386, 2006.
272. Lloyd, S.M., Lave, L.B. Life cycle economic and environmental implications of using nanocomposites in automobiles, *Environ. Sci. Technol.*, 2003, 37(15):3458-3466.
273. Lloyd, S.M., Lave, L.B., Matthews, H.S. Life cycle benefits of using nanotechnology to stabilize platinum-group metal particles in automotive catalysts. *Environ. Sci. Technol.* 2005, 39(5), 1384–1392.
274. Lockart, S.P., Rushworth, A., Azmy, A.A., Raine, P.A. 1983. Topical silver sulphadiazine: side effects and urinary excretion. *Burns Incl. Therm. Inj.* 10, 9-12.
275. Logar, M., Suvorov, D., Jancar, B., Kostanjsek, R. *In situ* synthesis of Ag nanoparticles in polyelectrolyte multilayers. *Nanotechnology*. 2007, 18, 1-7.
276. Lok, C.-N., Ho, C.-M., Chen, R., He, Q.-Y., Yu, W.-Y., Sun, H., Tam, P.K.-H., Chiu, J.-F., Che, C.-M. 2006. Proteomic analysis of the mode of antibacterial action of silver nanoparticles. *J. Proteome Res.* 5, 916-924.
277. Lok, C.-N., Ho, C.-M., Chen, R., He, Q.-Y., Yu, W.-Y., Sun, H., Tam, P.K., Chiu, J.F., Che, C.-M. 2007. Silver nanoparticles: Partial oxidation and antibacterial activities. *J Biol Inorg Chem* 12:527-534.
278. Long, D., Wu, G., Chen, S. Preparation of oligochitosan stabilized silver nanoparticles by gamma irradiation. *Rad. Phys .Chem.* 2007, 76, 1126-1131.
279. Lovern, S.B., Klaper, R. 2006. *Daphnia magna* mortality when exposed to titanium dioxide and fullerene (C60) nanoparticles. *Environ. Toxicol. Chem.* 25, 1132–1137.

280. Lu, W., Senapati, D., Wang, S., Tovmachenko, O., Yu, H., Ray, P.C. Shape dependent cellular uptake and toxic effects of silver nanomaterials on human skin HaCaT keratinocytes. *J. Am. Chem. Soc.* 2009; Submitted.
281. Lu, Y., Mei, W., Schrunner, M., Ballauff, M., Moller, M.W., Breu, J. *In situ* formation of Ag nanoparticles in spherical polyacrylic acid brushes by UV irradiation. *J. Phys. Chem. C.* 2007, 111, 7676-7681.
282. Luoma, S.N. 2008. Silver nanotechnologies and the environment: old problems or new challenges? Project on Emerging Nanotechnologies, The Pew Charitable Trusts.
283. Luo, W., Hu, W., Xiao, S. 2008. Size effect on the thermodynamic properties of silver nanoparticles. *J. Phys. Chem. C*, 112:2359–2369.
284. Lyven, B., Hasselov, M., Turner, D.R., Haraldsson, C., Anderson, K. 2003. Competition between iron- and carbon-based colloidal carriers for trace metals in a freshwater assessed using flow field-flow fractionation coupled to ICPMS. *Geochimica et Cosmochimica Acta*, 67, 20, 3791–3802.
285. Mandal, S., Arumugam, S.K., Pasricha, R., Sastry, M. Silver nanoparticles of variable morphology synthesized in aqueous foams as novel templates. *Bull. Mater. Sci.* 2005, 28, 503-510.
286. Martens, D.W., Servizi, J.A. 1993. Suspended sediment particles inside gills and spleens of juvenile pacific salmon (*Oncorhynchus* spp.). *Can. J. Fisheries Aquat. Sci.* 50, 586–590.
287. Maynard, A.D., Aitken, R.J. Assessing exposure to airborne nanomaterials: current abilities and future requirements. *Nanotoxicology* 1: 26–41, 2007.
288. McKenna, S.R., Latenser, B.A., Jones, L.M., Barrette, R.R., Sherman, H.F., Varcelotti, J.R. 1995. Serious silver sulphadiazine and mafenide acetate dermatitis. *Burns* 21, 310-312.
289. McLellan, J.M., Siekkinen, A., Chen, J., Xia, Y. Comparison of the surface-enhanced Raman scattering on sharp and truncated silver nanocubes. *Chem. Phys. Lett.* 2006, 427, 122-126.
290. Melaiye, A., Sun, Z., Hindi, K., Milsted, A., Ely, D., Reneker, D.H., Tessier, C.A., Youngs, W.J. 2005. Silver(I)-imidazole cyclophane gem-diol complexes encapsulated by electrospun terephthalic nanofibers: Formation of nanosilver particles and antimicrobial activity. *J. Am. Chem. Soc.* 127, 2285-2291.

291. Merga, G., Cass, L.C., Chipman, D.M., Meisel, D. Probing silver nanoparticles during catalytic H₂ evolution. *J. Am. Chem. Soc.* 2008, 130, 7067-7076.
292. Merga, G., Wilson, R., Lynn, G., Milosavljevic, B.H., Meisel, D. Redox catalysis on “naked” silver nanoparticles. *J. Phys. Chem. C* 2007, 111, 12220-12226.
293. Metreveli, G., Kaulisch, E.M., Frimmel, F.H. Coupling of a column system with ICP-MS for the characterisation of colloidmediated metal(loid) transport in porous media, *Acta Hydrochim. Hydrobiol.*, 2005, 33(4), 337.
294. Meyer, D.E., Curran, M.A., Gonzalez, M.A. 2009. An examination of existing data for the industrial manufacture and use of nanocomponents and their role in the life cycle impact of nanoproducs, *Environ. Sci. Technol.*, 43(5):1256-1263.
295. Mirsattari, S.M., Hammond, R.R., Sharpe, M.D., Leung, F.Y., Young, G.B. 2004. Myoclonic status epilepticus following repeated oral ingestion of colloidal silver, *Neurology*, 62:1408-1410.
296. Mishra, Y.K., Mohapatra, S., Kabiraj, D., Mohanta, B., Lalla, N.P., Pivin, J.C., Avasthi, D.K. 2007. Synthesis and characterization of Ag nanoparticles in silica matrix by atom beam sputtering. *Scr Mater*, 56:629–632.
297. Moghimi, S.M., Hunter, A.C., Murray, J.C. Nanomedicine: current status and future prospects, *FASEB Journal*, vol. 19, no. 3, pp. 311–330, 2005.
298. Mohanpuria, P., Rana, N.K., Yadav, S.K. 2008. Biosynthesis of nanoparticles: Technological concepts and future applications, *J. Nanoparticle Res.*, 10:507-517.
299. Moller, W., Felten, K., Sommerer, K., Scheuch, G., Meyer, G., Meyer, P., Haussinger, K., Kreyling, W.G. Deposition, retention, and translocation of ultrafine particles from the central airways and lung periphery. *Am J Respir Crit Care Med* 177: 426–432, 2008.
300. Moore, M.N. 2006. Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? *Environ. Int.* 32, 967–976.
301. Moores, A., Goettmann, F. 2006. The plasmon band in noble metal nanoparticles: An introduction to theory and applications, *New J. Chem.*, 30:1121-1132.
302. Morgan, T.P., Grosell, M., Playle, R.C., Wood, C.M. 2004. The time course of silver accumulation in rainbow trout during static exposure to silver nitrate: Physiological regulation or an artifact of the exposure conditions? *Aquat. Toxicol.* 66, 55-72.
303. Morley, K.S., Webb, P.B., Tokareva, N.V., Krasnov, A.P., Popov, V.K., Zhang, J., Roberts, C.J., Howdle, S.M. Synthesis and characterization of advanced

- UHMWPE/silver nanocomposites for biomedical applications. *Eur. Polym. J.* 2007, 43, 307-314.
304. Morones, J.R., Elechiguerra, J.L., Camacho, A., Holt, K., Kouri, J.B., Ramírez, J.T., Yacaman, M.J. 2005. The bactericidal effect of silver nanoparticles, *Nanotechnology*, 16:2346-2353.
305. Morones, J.R., Frey, W. Environmentally sensitive silver nanoparticles of controlled size synthesized with PNIPAM as a nucleating and capping agent. *Langmuir*. 2007, 23, 8180-8186.
306. Moss, A.P., Sugar, A., Hargett, N.A., Atkin, A., Wolkstein, M., Rosenman, K.D. The ocular manifestations and functional effects of occupational argyrosis, *Arch Ophthalmol.*, 97:906-908, 1979.
307. Muangman, P., Chuntrasakul, C., Silthram, S., Suvanchote, S., Benjathanung, R., Kittidacha, S., Rueksomtawin, S. Comparison of efficacy of 1% silver sulfadiazine and Acticoat™ for treatment of partial-thickness burn wounds, *Journal of the Medical Association of Thailand*, vol. 89, no. 7, pp. 953–958, 2006.
308. Mueller, N.C., Nowack, B. Exposure modeling of engineered nanoparticles in the environment, *Environ. Sci. Technol.*, 2008, 42(12):4447-4453.
309. Mühling, M., Bradford, A., Readman, J.W., Somerfield, P.J., Handy, R.D. An investigation into the effects of silver nanoparticles on antibiotic resistance of naturally occurring bacteria in an estuarine sediment, *Marine Environ. Res.*, 2009, 68(5):278-283.
310. Mukherjee, P., Ahmad, A., Mandal, D., Senapati, S., Sainkar, S.R., Khan, M.I., Parishcha, R., Ajaykumar, P.V., Alam, M., Kumar, R., Sastry, M. 2001. Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: A novel biological approach to nanoparticle synthesis, *Nano. Lett.*, 1:515-519.
311. Mukherjee, P., Roy, M., Mandal, B.P., Dey, G.K., Mukherjee, P.K., Ghatak, J., Tyagi, A.K., Kale, S.P. 2008. Green synthesis of highly stabilized nanocrystalline silver particles by a nonpathogenic and agriculturally important fungus *T. asperellum*, *Nanotechnology*, 19:075103 (7pp), doi: 10.1088/0957-4484/19/7/075103.
312. Murata, T., Kanao-Koshikawa, M., Takamatsu, T. 2005. Effects of Pb, Cu, Sb, In and Ag contamination on the proliferation of soil bacterial colonies, soil

- dehydrogenase activity, and phospholipid fatty acid profiles of soil microbial communities, *Water Air Soil Pollut.*, 164:103-118.
313. Murthy, P.S.K., Mohan, M., Varaprasad, K., Sreedhar, B., Raju, K.M. First successful design of semi-IPN hydrogel–silver nanocomposites: A facile approach for antibacterial application. *J. Colloid Inter. Sci.* 2008, 318, 217-224.
 314. Muthuswamy, E., Ramadev, S.S., Vasana, H.N., Garcia, C., Noe, L., Verelst, M. Highly stable Ag nanoparticles in agar-agar matrix as inorganic–organic hybrid. *J. Nanoparticle Res.* 2007, 9, 561-567.
 315. Nadagouda, M.N., Varma, R.S. Green synthesis of Ag and Pd nanospheres, nanowires, and nanorods using vitamin b2: catalytic polymerisation of aniline and pyrrole. *J. Nanomater.* 2008, Article ID 782358.
 316. Nadworny, P.L., Wang, J., Tredget, E.E., Burrell, R.E., Anti-inflammatory activity of nanocrystalline silver-derived solutions in porcine contact dermatitis. *Journal of Inflammation*, 2010, 7:13.
 317. Nano Health Solutions. http://www.fulvic.org/html/nano_silver.html. Accessed May, 2010.
 318. Nanotech Plc. 2010. http://www.jrnanotech.com/acatalog/More_Info.html. Accessed May, 2010.
 319. Navaladian, S., Viswanathan, B., Varadarajan, T.K., Viswanath, R.P. 2008. Microwave-assisted rapid synthesis of anisotropic Ag nanoparticles by solid state transformation. *Nanotechnology*, 19:1–7.
 320. Navarro, E., Piccapietra, F., Wagner, B., Marconi, F., Kaegi, R., Odzak, N., Sigg, L. & Bhra, R. Toxicity of silver nanoparticles to *Chlamydomonas reinhardtii*. *Environ. Sci. Technol.* 42, 8959–8964 (2008).
 321. Nel, A., Xia, T., Madler, L., Li, N., 2006. Toxic potential of materials at the nanolevel. *Science* 311 (5761), 622–627.
 322. Nikolaj, L., Kildeby, O.Z., Andersen, R.E., Røge, T.L., Petersen, R., Riis, J.F. 2006. Silver Nanoparticles. P3 Project, Institute for Physics and Nanotechnology - Aalborg University.
 323. Nino-Martinez, N., Martinez-Castanon, G.A., Aragon-Pina, A., Martinez-Gutierrez, F., Martinez-Mendoza, J.R., Ruiz, F. 2008. Characterization of silver nanoparticles synthesized on titanium dioxide fine particles. *Nanotechnology*, 19:1–8.
 324. Nowack, B., Bucheli, T.D. Occurrence, behavior and effects of nanoparticles in the environment. *Environ. Pollut.* 2007, 150(1):5-22.

325. NRC. National Research Council. Risk Assessment in the Federal Government: Managing the Process, National Research Council, National Academy Press, Washington, DC (1983), <http://books.nap.edu/books/PODI15/html/index.html>
326. Oberdörster, G. Lung clearance of inhaled insoluble and soluble particles, *J. Aerosol Med* 1, 289-330, 1988.
327. Oberdörster, G. Pulmonary effects of inhaled ultrafine particles, *Int. Arch. Occup. Environ. Health*, 74(1), 1-8, 2001.
328. Oberdörster, G., Oberdörster, E., Oberdörster, J., 2005a. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environ. Health Perspect.* 113 (7), 823–839.
329. 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., Yang, H. 2005b. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. *Particle Fibre Toxicol.*, 2:8-43.
330. Oberdörster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Kreyling, W., Cox, C. Translocation of inhaled ultrafine particles to the brain, *Inhalation Toxicol.* 16(6-7), 437-445, 2004.
331. Oberdörster, G., Stone, V., Donaldson, K. Toxicology of nanoparticles: a historical perspective. *Nanotoxicology* 1: 2–25, 2007.
332. OECD. Organization for Economic Cooperation and Development. 1993. Test guideline 412, Repeated dose inhalation toxicity: 28/14-day. In OECD guidelines for the testing of chemicals. Paris:OECD.
333. Olenin, A.Y., Krutyakov, Y.A., Kudrinskii, A.A., Lisichkin, G.V. 2008. Formation of surface layers on silver nanoparticles in aqueous and water-organic media. *Colloid J*, 70:71–76.
334. Osterwalder, N., Capello, C., Hungerbühler, K., Stark, W. Energy consumption during nanoparticle production: How economic is dry synthesis. *J. Nanopart. Res.* 2006, 8 (1), 1–9.
335. Ott, M., Gogvadze, V., Orrenius, S., Zhivotovsky, B., 2007. Mitochondria, oxidative stress and cell death. *Apoptosis*. 12 (5), 913–922.
336. Paddle-Ledinek, J.E., Nasa, Z., Cleland, H.J. Effect of different wound dressings on cell viability and proliferation, *Plastic and Reconstructive Surgery*, vol. 117, supplement 7, pp. 110S–118S, 2006.

337. Pal, S., Tak, Y.K., Song, J.M. 2007. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium *Escherichia coli*. *Appl. Environ. Microbiol.*, 73:1712-1720.
338. Pala, G., Fronterre, A., Scafa, F., Scelsi, M., Ceccuzzi, R., Gentile, E., Candura, S.M. 2008. Case Study: Ocular Argryrosis in a Silver Craftsman, *J. Occup. Health*, 50:521-524.
339. Panacek, A., Kvitek, L., Pucek, R., Kolar, M., Vecerova, R., Pizurova, N., Sharma, V.K., Nevecna, T., Zboril, R. 2006. Silver colloid nanoparticles: Synthesis, characterization, and their antibacterial activity. *J. Phys. Chem. B*, 110:16248-16253.
340. Pande, S., Ghosh, S.K., Praharaj, S., Panigrahi, S., Basu, S., Jana, S., et al. 2007. Synthesis of normal and inverted gold-silver core-shell architectures in β -Cyclodextrin and their applications in SERS. *J. Phys. Chem. C*, 111:10806–10813.
341. Panigrahi, S., Kundu, S., Ghosh, S.K., Nath, S., Pal, T. Sugar assisted evolution of mono- and bimetallic nanoparticles. *Colloid Surfaces A*. 2005, 264, 133-138.
342. Panyala, N.R., Pena-Mendez, E., Havel, J. Silver or silver nanoparticles: a hazardous threat to the environment and human health? *J. Appl. Biomed.* 2008 (6), 117-129.
343. Paquin, P., Gorsuch, J., Wu, K.B., Mathew, R., Santore, R., Di Toro, D. Overview of the silver research and development program (1991-2006): Advancing the science of the fate and effects of silver in the environment. International Imaging Industry Association (I3A), White Plains, NY, 2007.
344. Parfenov, A., Gryczynski, I., Malicka, J., Geddes, C.D., Lakowicz, J.R. Enhanced fluorescence from fluorophores on fractal silver surfaces. *J. Phys. Chem. B*. 2003, 107, 8829-8833.
345. Park, J., Kwak, B.K., Bae, E., Lee, J., Kim, Y., Choi, K., Yi, J. Characterization of exposure to silver nanoparticles in a manufacturing facility, *J. Nanoparticle Res.*, 2009, 11(7):1705-1712.
346. Park, S., Lee, Y.K., Jung, M., Kim, K.H., Eun-Kyung Ahn, N.C., Lim, Y., Lee, K.H. 2007. Cellular toxicity of various inhalable metal nanoparticles on human alveolar epithelial cells. *Inhalat Toxicol* 19(Suppl. 1):59-65.
347. Pastoriza-Santos, I., Liz-Marzan, L.M. 2008. Colloidal silver nanoplates. State of the art and future challenges. *J. Mater. Chem.*, 18: 1724–1737.

348. Patakfalvi, R., Diaz, D., Santiago-Jacinto, P., Rodriguez-Gattorno, G., Sato-Berru, R. Anchoring of silver nanoparticles on graphite and isomorphous lattices. *J. Phys. Chem. C*. 2007, 111, 5331-5336.
349. Patakfalvi, R., Diaz, D., Velasco-Arias, D., Rodriguez-Gattorno, G., Santiago-Jacinto, P. Synthesis and direct interactions of silver colloidal nanoparticles with pollutant gases. *Colloid Polym. Sci.* 2008, 286, 67-77.
350. Peabody, N. 2006.
http://www.nixonpeabody.com/publications_detail3.asp?ID=1609. Date accessed Feb-2010.
351. Percival, S.L., Bowler, P.G., Dolman, J. 2007. Antimicrobial activity of silver-containing dressings on wound microorganisms using an in vitro biofilm model. *Int. Wound. J.*, 4:186-191.
352. Pergolese, B., Muniz-Miranda, M., Bigotto, A. Study of the adsorption of 1,2,3-Triazole on silver and gold colloidal nanoparticles by means of surface enhanced Raman scattering. *J. Phys. Chem. B*. 2004, 108, 5698-5702.
353. Peters, A. Particulate matter and heart disease: evidence from epidemiological studies, *Toxicol. Appl. Pharmacol.*, 207(2 Suppl), S477-S482, 2005.
354. Peters, A., Dockery, D.W., Muller, J.E., Mittleman, M.A. Increased particulate air pollution and the triggering of myocardial infarction, *Circulation*, 103(23), 2810-2815, 2001.
355. Peters, A., Veronesi, B., Calderón-Garcidueñas, L., Gehr, P., Chen, L.C., Geiser, M., Reed, W., Rothen-Rutishauser, B., Schürch, S., Schulz, H. Translocation and potential neurological effects of fine and ultrafine particles a critical update, Part. *Fibre Toxicol.* 3, 13, 2006.
356. Peters, A., Wichmann, H.E., Tuch, T., Heinrich, J., Heyder, J. Respiratory effects are associated with the number of ultrafine particles, *Am. J. Respir. Crit. Care Med.* 155(4), 1376-1383, 1997.
357. Pietropaoli, A.P., Frampton, M.W., Hyde, R.W., Morrow, P.E., Oberdörster, G., Cox, C., Speers, D.M., Frasier, L.M., Chalupa, D.C., Huang, L.S., Utell, M.J. Pulmonary function, diffusing capacity, and inflammation in healthy and asthmatic subjects exposed to ultrafine particles. *Inhal Toxicol* 16, Suppl 1: 59-72, 2004.
358. Podsiadlo, P., Paternel, S., Rouillard, J.M., Zhang, Z., Lee, J., Lee, J.W., Gulari, E., Kotov, N.A. 2005. Layer-by-layer assembly of nacre-like nanostructured composites with antimicrobial properties, *Langmuir*, 21:11915-11921.

359. Pohle, D., Damm, C., Neuhofer, J., Rösch, A., Münstedt, H. 2007. Antimicrobial properties of orthopaedic textiles after *in-situ* deposition of silver nanoparticles, *Poly. Poly. Comp.*, 15:357-363.
360. Poon, V.K., Burd, A. 2004. In vitro cytotoxicity of silver: Implication for clinical wound care. *Burns*, 30:140-147.
361. Pyatenko, A., Yamaguchi, M., Suzuki, M. Synthesis of spherical silver nanoparticles with controllable sizes in aqueous solutions. *J. Phys. Chem. C*. 2007, 111, 7910-7917.
362. Radziuk, D., Shchukin, D.G., Skirtach, A., Mohwald, H., Sukhorukov, G. 2007. Synthesis of silver nanoparticles for remote opening of polyelectrolyte microcapsules, *Langmuir*, 23:4612-4617.
363. Ranville, J. 2009. Nanoparticle Stability in Natural Waters and Its Implication for Metal Toxicity to Water Column and Benthic Organisms. Interagency Nanotechnology Implications Grantees Workshop– EPA, NSF, NIH/NIEHS, NIOSH, and DOE, Las Vegas, NV November 9-10, 2009.
364. Rao, C.R.K., Trivedi, D.C. Biphasic synthesis of fatty acids stabilized silver nanoparticles: Role of experimental conditions on particle size. *Mater. Chem. Phys.* 2006, 99, 354-360.
365. Rashid M.H., Mandal, T.K. Synthesis and catalytic application of nanostructured silver dendrites. *J. Phys. Chem. C* 2007, 111, 16750-16760.
366. Ray, P.C., Yu, H., Fu, P.P. Toxicity and Environmental Risks of Nanomaterials: Challenges and Future Needs, *J. Environ. Sci. and Health, Part C*, 27:1, pp1-35. DOI: 10.1080/10590500802708267.
367. Rebitzer, G., Ekvall, T., Frischknecht, R., Hunkeler, D., Norris, G., Rydberg, T., Schmidt, W.-P., Suh, S., Weidema, B. P., Pennington, D. W. Life cycle assessment: Part 1: Framework, goal and scope definition, inventory analysis, and applications. *Environ. Int.* 2004, 30 (5), 701–720.
368. Reddy, K.R., Lee, K., Lee, Y., Gopalan, A.I. Facile synthesis of conducting polymer–metal hybrid nanocomposite by in situ chemical oxidative polymerization with negatively charged metal nanoparticles. *Mater. Lett.* 2008, 62, 1815-1818.
369. Remita, S., Fontaine, P., Lacaze, E., Borensztein, Y., Sellame, H., Farha, R., Rochas, C., Goldmann, M. X-ray radiolysis induced formation of silver nanoparticles: A SAXS and UV–visible absorption spectroscopy study. *Nucl. Instrum. Meth. B*. 2007, 263, 436-440.

370. Roduner, E. Size matters: why nanomaterials are different, *Chem. Soc. Rev.* 35, 583-592, 2006.
371. Roes, A., Marsili, E., Nieuwlaar, E., Patel, M.K. Environmental and cost assessment of a polypropylene nanocomposite. *J. Polym. Environ.* 2007, 15 (3), 212–226.
372. Roldan, M.V., Frattini, A., de Sanctis, O., Troiani, C., Pellegrini, N. 2007. Characterization and applications of Ag nanoparticles in waveguides. *Applied Surface Science* 254: 281–285.
373. Rosalind, V. 2009. EPA Has Safely Regulated Nanosilver for Decades <http://nanotech.lawbc.com/uploads/file/00054380.PDF>. Date accessed March 2010.
374. Romans, I. 1954. Silver Compounds & Oligodynamic Metals in Antiseptics, Disinfectants, Fungicides and Chemical and Physical Sterilization, G. Reddish, ed., Philadelphia: Lea & Febiger, 380-428.
375. Rosas-Hernandez, H., Jiminez-Badillo, S., Martinez-Cuevas, P.P., Gracia-Espino, E., Terrones, H., Terrones, M., Hussain, S.M., Ali, S.F., Gonzalez, C. Effects of 45-nm silver nanoparticles on coronary endothelial cells and isolated aortic rings, *Toxicology Letters*, 191, 2009, pp305-313.
376. Rosenman, K.D., Moss, A., Kon, S. 1979. Argyria: Clinical implications of exposure to silver nitrate and silver oxide. *J. Occup. Med.*, 21:430-435.
377. Rosenman, K.D., Seixas, N., Jacobs, I. 1987. Potential nephrotoxic effects of exposure to silver. *Br. J. Ind. Med.*, 44:267-272.
378. Russell, A.D., Hugo, W.B. 1994. Antimicrobial activity and action of silver. *Prog Med Chem* 31:351-370.
379. Russell, A.D., Russell, N.J. 1995. "Biocides: Activity, Action and Resistance." Fifty Years of Antimicrobials: Past Perspectives and Future Trends, P.A. Hunter, G.K. Darby, and N.J. Russell, eds., Cambridge University Press, Cambridge.
380. Rustogi, R., Mill, J., Fraser, J.F., Kimble, R.M. 2005. The use of Acticoat™ in neonatal burns, *Burns*, 31:878-882.
381. Saint, S., Elmore, J.G., Sullivan, S.D., Emerson, S.S., Koepsell, T.D. 1998. The efficacy of silver alloy-coated urinary catheters in preventing urinary tract infection: a meta-analysis. *American Journal of Medicine* 105 (3): 236–241. doi:10.1016/S0002-9343(98)00240-X.
382. Saleh, N., Kim, H., Phenrat, T., Maytyjaszewski, K., Tilton, R.D., Lowry, G.V. Ionic Strength and Composition Affect the Mobility of Surface-Modified FeO

- Nanoparticles in Water-Saturated Sand Columns. *Environ. Sci. Technol.* 2008, 42, 3349-3355.
383. Samberg, M.E., Oldenburg, S.J., Monteiro-Riviere, N.A. 2010. Evaluation of silver nanoparticle toxicity in skin in vivo and keratinocytes in vitro, *Environ. Health Perspect.* 118(3):407-413.
384. Sanderson, S., Stebar, M., Ackermann, K., Jones, S., Batjakas, I.I., Kaufman, L. 1996. Mucus entrapment of particles by a suspension-feeding tilapia (Pisces: Cichlidae). *J. Exp. Biol.* 199, 1743–1756.
385. Sangermano, M., Yagci, Y., Rizza, G. In situ synthesis of silver-epoxy nanocomposites by photoinduced electron transfer and cationic polymerization processes. *Macromolecules.* 2007, 40, 8827-8829.
386. Sanjay, S., Meddings, J.A., Calfee, D., Kowalski, C.P., Krein, S.L. Catheter-Associated Urinary Tract Infection and the Medicare Rule Changes. *Annals of Internal Medicine*, 2009 (150), 877-884.
387. Saravanan, P., Raju, M.P., Alam, S. A study on synthesis and properties of Ag nanoparticles immobilized polyacrylamide hydrogel composites. *Mater. Chem. Phys.* 2007, 103, 278-282.
388. Sardar, R., Park, J., Shumaker-Parry, J.S. Polymer-induced synthesis of stable gold and silver nanoparticles and subsequent ligand exchange in water. *Langmuir.* 2007, 23, 11883-11889.
389. Saridara, C., Mitra, S. Chromatography on self-assembled carbon nanotubes, *Anal. Chem.*, 2005, 77(21), 7094.
390. Sayes, C.M., Ivanov, I. Comparative study of predictive computational models for nano-particle induced cytotoxicity, *Risk Analysis*, Accepted. 2010.
391. SCENIHR. Scientific Committee on Emerging and Newly Identified Health Risk. 2006. Opinion on: The appropriateness of existing methodologies to assess the potential risk associated with engineered and adventitious products of nanotechnologies. European Commission Health & Consumer Protection Directorate-General. Directorate C-Public Health and Risk Assessment C7- Risk Assessment.
392. Schaff, H.V., Carrel, T.P., Jamieson, W.R.E., Jones, K.W., Ruffilanchas, J.J., Cooley, D.A., Hetzer, R., Stumpe, F., Duveau, D., Moseley, P., Van Boven, W.J., Grunkemeier, G.L., Kennard, E.D., Holubkov, R. 2002. Paravalvular leak and other

- events in silzone-coated mechanical heart valves: A report from AVERT, *Ann. Thorac. Surg.*, 73:785-792.
393. Schierholz, J.M., Lucas, L.J., Rump, A., Pulverer, G. 1998. Efficacy of silver-coated medical devices, *J. Hosp. Infect.*, 40:257-262.
394. Schmaehl, D., Steinhoff, D. 1960. Studies on cancer induction with colloidal silver and gold solutions in rats. *Z Krebsforsch* 63:586-591.
395. Schmitt-Kopplin, P., Junkers, J. Capillary zone electrophoresis of natural organic matter, *J. Chromatogr., A*, 2003, 998(1-2), 1.
396. Schnabel, U., Fischer, C.H., Kenndler, E. Characterization of colloidal gold nanoparticles according to size by capillary zone electrophoresis, *J. Microcolumn Sep.*, 1997, 9(7), 529-534.
397. Schrand, A.M., Braydich-Stolle, L.K., Schlager, J.J., Dai, L., Hussain, S.M. 2008. Can silver nanoparticles be useful as potential biological labels? *Nanotechnology* 19:235104.
398. Semmler, M., Seitz, J., Mayer, P., Heyder, J., Oberdörster, G., Kreyling, W.G. Long-term clearance kinetics of inhaled ultrafine insoluble iridium particles from the rat lung, including transient translocation into secondary organs. *Inhalation Toxicol.* 16(6-7), 453-459, 2004.
399. Senjen, R. 2007. Nanosilver – a threat to soil, water and human health? *Friends of the Earth Australia*, March 2007.
400. Seo, D., Yoon, W., Park, S., Kim, J., Kim, J. The preparation of hydrophobic silver nanoparticles via solvent exchange method. *Colloids Surfaces A*. 2006, 313-314, 158-161.
401. Setua, P., Chakraborty, A., Seth, D., Bhatta, M.U., Satyam, P.V., Sarkar N. Synthesis, optical properties, and surface enhanced raman scattering of silver nanoparticles in nonaqueous methanol reverse micelles. *J. Phys. Chem. C*. 2007, 111, 3901-3907.
402. Shah, C.P. *Public Health and preventive medicine in Canada*, University of Toronto Press, Toronto, Canada. 2007.
403. Shahverdi, A.R., Fakhimi, A., Shahverdi, H.R., Minaian, S. 2007. Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*, *Nanomedicine*, 3:168-171.

404. Shahverdi, A.R., Minaeian, S., Shahverdi, H.R., Jamalifar, H., Nohi, A. Rapid synthesis of silver nanoparticles using culture supernatants of Enterobacteria: A novel biological approach. *Process Biochem.* 2007, 42, 919-923.
405. Shankar, S.S., Ahmad, A., Sastry, M. Geranium leaf assisted biosynthesis of silver nanoparticles. *Biotechnol. Prog.* 2003, 19, 1627-1631.
406. Sharma, S., Thakur, M., Deb, K. Synthesis of silver nanoparticles using N1,N2-diphenylbenzamidine by microwave irradiation method. *J. Exp. Nanoscience.* 2007, 2, 251-256.
407. Sharma, V.K., Yngard, R.A, Lin, Y. Silver nanoparticles: green synthesis and antimicrobial activities. *Adv. Colloid Interface Sci.* 2009, 145:83–96.
408. Shen, Q., Sun, J., Wei, H., Zhou, Y., Su, Y., Wang, D. Fabrication of silver nanorods controlled by a Segmented Copolymer. *J. Phys. Chem. C.* 2007, 111, 13673-13678.
409. Shervani, Z., Ikushima, Y., Yokoyama, T., Sato, M., Hakuta, Y., Takako, N., Kunieda, H., Aramaki, K. Size controlled synthesis of Ag and Cu nanocrystals in F-AOT/ η -butanol/SC CO₂ microemulsions. *Colloids Surfaces A.* 2007, 303, 159-165.
410. Shim, I., Lee, K.J., Lee, Y., Joung, J. An organometallic route to highly monodispersed silver nanoparticles and their application to ink-jet printing. *Mater. Chem. Phy.* 2008, 110, 316-321.
411. Shin, S.H., Ye, M.K., Kim, H.S., Kang, H.S. 2007. The effects of nanosilver on the proliferation and cytokine expression by peripheral blood mononuclear cells. *Int Immunopharmacol.* 7:1813-1818.
412. Shrivastava, S., Bera, T., Roy, A., Singh, G., Ramachandrarao, P., Dash, D. 2007. Characterization of enhanced antibacterial effects of novel silver nanoparticles. *Nanotechnology* 18:225103. (9pp). doi: 10.1088/0957-4484/18/22/225103.
413. Si, S., Mandal, T.K. 2007. Tryptophan-based peptides to synthesize gold and silver nanoparticles: a mechanistic and kinetic study. *Chem. Eur. J.*, 13:3160–3168.
414. Sibbald, R.G., Browne, A.C., Coutts, P., Queen, D. 2001. Screening evaluation of an ionized nanocrystalline silver dressing in chronic wound care, *Ostomy. Wound Manage.*, 47:38-43.
415. Sibbald, R.G., Contreras-Ruiz, J., Coutts, P., Fierheller, M., Rothman, A., Woo, K. 2007. Bacteriology, inflammation, and healing: A study of nanocrystalline silver dressings in chronic venous leg ulcers, *Adv. Skin Wound Care*, 20:549-558.

416. Siepmann, R., von der Kammer, F., Forstner, U. Colloidal transport and agglomeration in column studies for advanced runoff filtration facilities—particle size and time resolved monitoring of effluents with flow-field-flow-fractionation, *Water Sci. Technol.*, 2004, 50(12), 95–102.
417. Silver, S. 2003. Bacterial silver resistance: Molecular biology and uses and misuses of silver compounds. *FEMS Microbiol. Rev.* 27:341-353.
418. Silver Mining. 2010. <http://www.infomine.com/commodities/silver.asp>. Date accessed Feb-2010.
419. Sivamohan, R., Takahashi, H., Kasuya, A., Tohji, K., Tsunekawa, S., Ito, S., Jeyadevan, B. Liquid chromatography used to size separate the amphiphilic-molecules stabilized nano-particles of CdS in the 1–10nm range, *Nanostruct. Mater.*, 1999, 12(1–4), 89–94.
420. Skebo, J.E., Grabinski, C.M., Schrand, A.M., Schlager, J.J., Hussain, S.M. 2007. Assessment of metal nanoparticle agglomeration, uptake, and interaction using high-illuminating system. *Int J Toxicol* 26:135-141.
421. Skirtach, A.G., Antipov, A.A., Shchukin, D.G., Sukhorukov, G.B. 2004. Remote activation of capsules containing Ag nanoparticles and IR dye by laser light, *Langmuir*, 20:6988-6992.
422. Skirtach, A.G., Oz, J.A., Kreft, O., Hler, K., Piera Alberola, A., Hwald, H., Parak, W.J., Sukhorukov, G.B. 2006. Laser-induced release of encapsulated materials inside living cells. *Angew. Chem. Int. Ed. Engl.* 45:4612-4617.
423. Sławinski, G.W., Zamborini, F.P. Synthesis and alignment of silver nanorods and nanowires and the formation of Pt, Pd, and core/shell structures by galvanic exchange directly on surfaces. *Langmuir*. 2007, 23, 10357-10365.
424. Smith, I., Carson, B. 1977. Trace metals in the environment. Volume 2. Silver. Ann Arbor, MI, Ann Arbor Science Publishers, 469 pp.
425. SNWG. 2009. “Evaluation of Hazard and Exposure Associated with Nanosilver and Other Nanometal Oxide Pesticide Products”, Presentation to Scientific Advisory Panel (November 4th, 2009).
<http://www.regulations.gov/search/Regs/contentStreamer?objectId=0900006480a52512&disposition=attachment&contentType=pdf>. Date accessed Feb-2010.
426. Solomon, S.D., Bahadory, M., Jeyarajasingam, A.V., Rutkowsky, S.A., Boritz, C. Synthesis and study of silver nanoparticles. *J. Chem. Educ.* 2007, 84, 322-325.

427. Sondi, I., Goia, D.V., Matijevic, E. Preparation of highly concentrated stable dispersions of uniform silver nanoparticles. *J. Colloid Inter. Sci.* 2003, 260, 75-81.
428. Sondi, I., Salopek-Sondi, B. 2004. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. *J. Colloid Interface Sci.* 275, 177-182.
429. Song, Y., Jimenez, V., McKinney, C., Donkers, R., Murray, R.W. Estimation of size for 1–2 nm nanoparticles using an HPLC electrochemical detector of double layer charging, *Anal. Chem.*, 2003, 75(19), 5088.
430. Song, Y., Heien, M.L.A.V., Jimenez, V., Wightman, R.M., Murray, R.W. Voltammetric detection of metal nanoparticles separated by liquid chromatography, *Anal. Chem.*, 2004, 76(17), 4911.
431. Song, Y., Yanga, W., King, M. Shape controlled synthesis of sub-3 nm Ag nanoparticles and their localized surface plasmonic properties. *Chem. Phys. Lett.* 2008, 455, 218-224.
432. Soto, K.F., Carrasco, A., Powell, T.G., Garza, K.M., Murr, L.E.. Comparative in vitro cytotoxicity assessment of some manufactured nano particulate materials characterized by transmission electron microscopy, *J. Nanopart. Res.* 7, 145-169, 2005.
433. Soto, K., Garza, K.M., Murr, L.E. 2007. Cytotoxic effects of aggregated nanomaterials. *Acta Biomater* 3:351-358.
434. Stolpe, B., Hassellöv, M., Andersson, K., Turner, D.R. High resolution ICPMS as an on-line detector for flow field-flow fractionation; multi-element determination of colloidal size distributions in a natural water sample, *Anal. Chim. Acta*, 2005, 535(1–2), 109-121.
435. Sun, R.W., Chen, R., Chung, N.P., Ho, C.M., Lin, C.L., Che, C.M. 2005. Silver nanoparticles fabricated in HEPES buffer exhibit cytoprotective activities toward HIV-1 infected cells, *Chem. Commun. (Camb.)*:5059-5061.
436. Sun, X., Luo, Y. Preparation and size control of silver nanoparticles by a thermal method. *Mater. Lett.* 2005, 59, 3847-3850.
437. Sun, Y., Guizhe, Z, Liu, Y., Zhang, Q. Effects of hyperbranched poly(amido-amine)s structures on synthesis of Ag particles. *J. Appl. Polym. Sci.* 2008, 107, 9-13.
438. Sung, J.H., Ji, J.H., Yoon, J.U., Kim, D.S., Song, M.Y., Jeong, J., Han, B.S., Han, J.H., Chung, Y.H., Kim, J., Kim, T.S., Chang, H.K., Lee, E.J., Lee, J.H., Yu, I.J.

2008. Lung function changes in Sprague-Dawley rats after prolonged inhalation exposure to silver nanoparticles. *Inhal. Toxicol.*, 20:567-574.
439. Supp, A.P., Neely, A.N., Supp, D.M., Warden, G.D., Boyce, S.T. Evaluation of cytotoxicity and antimicrobial activity of Acticoat® burn dressing for management of microbial contamination in cultured skin substitutes grafted to athymic mice, *Journal of Burn Care & Rehabilitation*, vol. 26, no. 3, pp. 238–246, 2005.
440. Suzuki, H., Toyooka, T., Ibuki, Y. 2007. Simple and easy method to evaluate uptake potential of nanoparticles in mammalian cells using a flow cytometric light scatter analysis. *Environ Sci Technol* 41:3018-3024.
441. Swami, A., Selvakannan, P., Pasricha, R., Sastry, M. One-step synthesis of ordered two-dimensional assemblies of silver nanoparticles by the spontaneous reduction of silver ions by pentadecylphenol langmuir monolayers. *J. Phys. Chem. B.* 2004, 108, 19269-19275.
442. Sweeney, S.F., Woehrle, G.H., Hutchison, E. 2006. Rapid purification and size separation of gold nanoparticles via diafiltration. *J. Am. Chem. Soc.* 128: 3190-3197.
443. Tai, S.P., Wu, Y., Shieh, B.D., Chen, L.J., Lin, K.J., Yu, C.H., Chu, S.W., Chang, C.H., Shi, X.Y., Wen, Y.C., Lin, K.H., Liu, T.M., Sun, C.K. 2007. Molecular imaging of cancer cells using plasmonresonant-enhanced third-harmonic-generation in silver nanoparticles, *Adv. Mater.*, 19:4520-4523.
444. Takenaka, S., Karg, E., Roth, C., Schulz, H., Ziesenis, A., Heinzmann, U., Schramel, P., Heyder, J. 2001. Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. *Environ. Health Perspect.* 109(Suppl. 4):547-551.
445. Tan, S., Erol, M., Attygalle, A., Du, H., Sukhishvili, S. 2007. Synthesis of Positively Charged Silver Nanoparticles via photoreduction of AgNO₃ in branched polyethyleneimine/HEPES solutions. *Langmuir*, 23, 9836–9843.
446. Tan, Y., Jiang, L., Li, Y., Zhu, D. One Dimensional aggregates of silver nanoparticles induced by the stabilizer 2-Mercaptobenzimidazole. *J. Phys. Chem. B.* 2002, 106, 3131-3138.
447. Tang, J., Xiong, L., Wang, S., Wang, J., Liu, L., Li, J., Yuan, F., Xi, T. Distribution, Translocation and Accumulation of Silver Nanoparticles in Rats. *Journal of Nanoscience and Nanotechnology*, Volume 9, Number 8, August 2009, pp. 4924-4932(9).

448. Tao, S., Liu, C., Dawson, R., Cao, J., Li, B. 1999. Uptake of particulate lead via the gills of fish (*Carassius auratus*). *Arch. Environ. Contam. Toxicol.* 37, 352–357.
449. Tao, S., Long, A., Dawson, R.W., Xu, F., Li, B., Cao, J., Fang, J. 2002. Copper speciation and accumulation in the gill microenvironment of carp (*Cyprinus carpio*) in the presence of kaolin particles. *Arch. Environ. Contam. Toxicol.* 42, 325–331.
450. Thomas, J. 2007. Treating a non-healing diabetic foot ulcer using Acticoat Moisture Control and the Lean improvement technique, *Wounds UK*, 3:136-138.
451. Thomas, S., Nair, S.K., Jamal, E.M.A., Al Harthi, S.H., Varma, M.R., Anantharaman, M.R. 2008. Size-dependent surface Plasmon resonance in silver silica nanocomposites, *Nanotechnology*, 19:075710. (7pp). doi:10.1088/0957-4484/19/7/075710.
452. Thomas, V., Yallabu, M.M., Sreedhar, B., Bajpai, S.K. A versatile strategy to fabricate hydrogel–silver nanocomposites and investigation of their antimicrobial activity. *J. Colloid Inter. Sci.* 2007, 315, 389-395.
453. Thompson, D.G., Enright, A., Faulds, K., Smith, W.E., Graham, D. Ultrasensitive DNA detection using oligonucleotide–silver nanoparticle conjugates. *Anal. Chem.* 2008, 80, 2805-2810.
454. Tian, J., Wong, K.K., Ho, C.M., Lok, C.N., Yu, W.Y., Che, C.M., Chiu, J.F., Tam, P.K. 2007. Topical delivery of silver nanoparticles promotes wound healing, *Chem. Med. Chem.*, 2:129-136.
455. Tiede, K., Boxall, A.B.A., Tiede, D., Tear, S.P., David, H., Lewis, J. A robust size-characterization methodology for studying nanoparticle behaviour in ‘real’ environmental samples, using hydrodynamic chromatography coupled to ICP-MS, *J. Anal. At. Spectrom.*, 2009, 24, 964-972.
456. Tielemans, M., Roose, P., De Groote, P., Vanovervelt, J. Colloidal Stability of Surfactant-free Radiation Curable Polyurethane Dispersions. *Prog. Org. Coat.* 2006, 55, 128-136.
457. Tiraferri, A., Sethi, R. Enhanced Transport of Zerovalent Iron Nanoparticles in Saturated Porous Media by Guar Gum. *J. Nanopart. Res.* 2009, 11, 635-645.
458. Tolaymat, T., El Badawy, A., Genaidy, A., Scheckel, K., Luxton, T., Suidan, M. 2010. An evidence-based environmental perspective of manufactured silver nanoparticle in syntheses and applications: A systematic review and critical appraisal of peer-reviewed scientific papers. *Sci. Tot. Environ.*, (408)5:999-1006.

459. Tong, M.C., Chen, W., Sun, J., Ghosh, D., Chen, S. Dithiocarbamate-capped silver nanoparticles. *J. Phys. Chem. B.* 2006, 110, 19238-19242.
460. Toshima, N., Kanemaru, M., Shiraishi, Y., Koga, Y. Spontaneous Formation of core/shell bimetallic nanoparticles: a calorimetric study. *J. Phys. Chem. B.* 2005, 109, 16326-16326.
461. Tredget, E.E., Shankowsky, H.A., Groeneveld, A., Burrell, R. 1998. A matched pair, randomized study evaluating the efficacy and safety of Acticoat silvercoated dressing for the treatment of burn wounds. *J Burn Care Rehabil.* 19, 531-537.
462. Trop, M., Novak, M., Rodl, S., Hellbom, B., Kroell, W. Goessler, W. 2006. Silver-coated dressing Acticoat caused raised liver enzymes and argyria-like symptoms in burn patient. *J. Trauma* 60, 648-652.
463. Tsai, S.-J., Ada, E., Isaacs, J.A., Ellenbecker, M.J. 2009. Airborne nanoparticle exposures associated with the manual handling of nanoalumina and nanosilver in fume hoods, *J. Nanopart. Res.*, 11:147-161.
464. Turkevich, J., Stevenson, P., Hillier, J. 1951. A study of the nucleation and growth processes in the synthesis of colloidal gold. *Discuss. Faraday Soc.*, 55-75.
465. Ueda, S., Masutani, H., Nakamura, H., Tanaka, T., Ueno, M., Yodoi, J., 2002. Redox control of cell death. *Antioxid. Redox. Signal.* 4 (3), 405-414.
466. U.S. EPA. Integrated Risk Information System (IRIS); Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment: Cincinnati, OH, 1994.
467. U.S. EPA. Life Cycle Assessment: Inventory Guidelines and Principles, EPA/600/R-92/245, Office of Research and Development, 1993.
468. U.S. EPA. Nanomaterial Case Studies: Nanoscale Titanium Dioxide (External Review Draft). U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-09/057, 2009.
469. U.S. EPA. Nanomaterial Case Study: Nanoscale Silver in Disinfectant Spray (Interagency Review Draft). U.S. Environmental Protection Agency, Washington, DC, 2010.
470. U.S. EPA. Science Policy Council. Nanotechnology White Paper, EPA100/B-07/001, February 2007.
471. U.S. FDA. 2010. US food and Drug Administration.
<http://www.fda.gov/ScienceResearch/SpecialTopics/Nanotechnology/ucm153723.htm>. Date accessed Feb-2010.

472. USGS. 2010. Science for a changing world. Silver Statistics and Information. <http://minerals.usgs.gov/minerals/pubs/commodity/silver/>. Date accessed Feb-2010
473. Vega-Villa, K.R., Takemoto, J.K., Yáñez, J.A., Remsberg, C.M., Forrest, M.L., Davies, N.M. Clinical toxicities of nanocarrier systems, *Advanced Drug Delivery Reviews*, vol. 60, no. 8, pp. 929–938, 2008.
474. Venugopal, B., Luckey, T.D. 1978. Metal toxicity in mammals. In: *Chemical toxicology of metals and metalloids*, Venugopal, B., Luckey, T.D. (Eds.), New York: Academic Press. pp 32-36.
475. Vermylen, J., Nemmar, A., Nemery, B., Hoylaerts, F. Ambient air pollution and acute myocardial infarction, *J. Thromb. Haemost.* 3, 1955-1961, 2005.
476. Vigneshwaran, N., Ashtaputre, N.M., Varadarajan, P.V., Nachane, R.P., Paralikar, K.M., Balasubramanya, R.H. Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*. *Mater. Lett.* 2007a, 61,1413-1418.
477. Vigneshwaran, N., Kathe, A.A., Varadarajan, P.V., Nachane, R.P., Alasubramanya, A. 2007b. Silver-Protein (Core-Shell) Nanoparticle Production Using Spent Mushroom Substrate. *Langmuir*, 23: 7113-7117.
478. Vigneshwaran, N., Kathe, A.A., Varadarajan, P.V., Nachane, R.P., Balasubramanya, R.H. Biomimetics of silver nanoparticles by white rot fungus, *Phaenerochaete chrysosporium*. *Colloids Surfaces B.* 2006a, 53, 55-59.
479. Vigneshwaran, N., Nachane, R.P., Balasubramanya, R.H., Varadarajan, P.V. A novel one-pot ‘green’ synthesis of stable silver nanoparticles using soluble starch. *Carbohydr. Res.* 2006b, 341, 2012-2018.
480. Vilchis-Nestor, A.R., Sánchez-Mendieta, V., Camacho-López, M.A., Gómez-Espinosa, R.M., Camacho-López, M.A., Arenas-Alatorre J.A. Solventless synthesis and optical properties of Au and Ag nanoparticles using *Camellia sinensis* extract. *Mater. Lett.* 2008, 62 (17-18), 3103-3105.
481. Vlachou, E., Chipp, E., Shale, E., Wilson, Y.T., Papini, R., Moiemmen, N.S. 2007. The safety of nanocrystalline silver dressings on burns: A study of systemic silver absorption. *Burns*, 33: 979-985.
482. Volpe, R., Delattre, J., Height, M. 2009. Before the United States Environmental Protection Agency Office of Pesticides Programs. Letter from the Silver Nanotechnology Working Group Following the FIFRA Scientific Advisory Panel.
483. von der Kammer, F., Baborowski, M., Tadjiki, S., Von Tumpling, W. Colloidal particles in sediment pore waters: Particle size distributions and associated element

- size distribution in anoxic and re-oxidized samples, obtained by FFF-ICP-MS coupling, *Acta Hydrochim. Hydrobiol.*, 2004, 31(4–5), 400–410.
484. von der Kammer, F., Baborowski, M., Friese, K. Application of a high-performance liquid chromatography fluorescence detector as a nephelometric turbidity detector following field-flow fractionation to analyse size distributions of environmental colloids, *J. Chromatogr., A*, 2005a, 1100(1), 81.
485. von der Kammer, F., Baborowski, M., Friese, K. Field-flow fractionation coupled to multi-angle laser light scattering detectors: Applicability and analytical benefits for the analysis of environmental colloids, *Anal. Chim. Acta*, 2005b, 552(1–2), 166.
486. von Gleich, A., Steinfeldt, M., Petschow, U. A suggested three-tiered approach to assessing the implications of nanotechnology and influencing its development. *J. Cleaner Prod.* 2008, 16 (8-9), 899–909.
487. Voronov, A., Kohut, A., Peukert, W. Synthesis of amphiphilic silver nanoparticles in nanoreactors from invertible polyester. *Langmuir*. 2007, 23, 360-363.
488. Wadhera, A., Fung, M.
http://dermatology.cdlib.org/111/case_reports/argyria/c11.jpg. Accessed March 2010.
489. Walt, D.R. 2005. Miniature analytical methods for medical diagnostics, *Science*, 308:217-219.
490. Wan, A.T., Conyers, R.A., Coombs, C.J., Masterton, J.P. 1991. Determination of silver in blood, urine and tissues of volunteers and burn patients. *Clin. Chem.* 37:1683-1687.
491. Wang, D., Xie, T., Peng, Q., Li, Y. Ag, Ag₂S, and Ag₂Se Nanocrystals: synthesis, assembly, and construction of mesoporous structures. *J. Am. Chem. Soc.* 2008a, 130, 4016-4022.
492. Wang, G., Shi, C., Zhao, N., Du, X. Synthesis and characterization of Ag nanoparticles assembled in ordered array pores of porous anodic alumina by chemical deposition. *Mater. Lett.* 2007, 61, 3795-3797.
493. Wang, H., Law, N., Pearson, G., van Dongen, B.E., Jarvis, R.M., Goodacre, R., Lloyd, J.R. Impact of silver (I) on the Metabolism of *Shewanella oneidensis*, *J. Bacteriology*, 192(4):1143-1150, 2010.
494. Wang, P., Wang, D., Xie, T., Li, H., Yang, M., Wei, X. Preparation of monodisperse Ag/Anatase TiO₂ core-shell nanoparticles. *Mater. Chem. Phys.* 2008b, 109, 181-183.

495. Wang, M.F., Dykstra, T.E., Lou, X.D., Salvador, M.R., Scholes, G.D., Winnik, M.A. Colloidal Cd-Se nanocrystals passivated by a dye labeled multidentate polymer: Quantitative analysis by size-exclusion chromatography, *Angew. Chem., Int. Ed.*, 2006, 45(14), 2221.
496. Wang, W., Chen, X., Efrima, S. Silver nanoparticles capped by long-chain unsaturated carboxylates. *J. Phys. Chem. B.* 1999a, 103, 7238-7246.
497. Wang, W., Efrima, S., Regev, O. Directing silver nanoparticles into colloid-surfactant lyotropic lamellar systems. *J. Phys. Chem. B.* 1999b, 103, 5613-5621.
498. Warheit, D.B., Borm, P.J., Hennes, C., Lademann, J. Testing strategies to establish the safety of nanomaterials: conclusions of an ECETOC workshop. *Inhal. Toxicol.*, 19: 631–643, 2007.
499. Wei, G.T., Liu, F.K. Separation of nanometer gold particles by size exclusion chromatography, *J. Chromatogr., A*, 1999, 836(2), 253.
500. Weisbarth, R.E., Gabriel, M.M., George, M., Rappon, J., Miller, M., Chalmers. R., Winterton, L. 2007. Creating antimicrobial surfaces and materials for contact lenses and lens cases, *Eye and Contact Lens*, 33:426-429.
501. Wen, H. C.; Lin, Y. N.; Jian, S. R.; Tseng, S. C.; Weng, M. X.; Liu, Y. P.; Lee, P. T.; Chen, P. Y.; Hsu, R. Q.; Wu, W. F.; Chou, C. P. Observation of growth of human fibroblasts on silver nanoparticles. *J. Phys. (Paris)* 2007, 61, 445–449.
502. Wenseleers, W., Stellacci, F., Meyer-Friedrichsen, T., Mangel, T., Bauer, C.A., Pond, S.J.K., Marder, S.R., Perry, J.W. Five orders-of-magnitude enhancement of two-photon absorption for dyes on silver nanoparticle fractal clusters. *J. Phys. Chem. B.* 2002, 106, 6853-6863.
503. White, J.M.L., Powell, A.M., Brady, K., Russell-Jones, R. 2003. Severe generalized argyria secondary ingestion of colloidal silver protein. *Clin. Experim. Dermatol.*, 28:354-256.
504. Wijnhoven, S.W.P., Peijnenburg, W.J.G.M., Herberts, C.A., Hagens, W.I., Oomen, A.G., Heugens, E.H.W., Roszek, B., Bisschops, J., Gosens, I., van de Meent, D., Dekkers, S., de Jong, W.H., van Zijverden, M., Sips, A.J.A.M., Geertsma, R.E. Nanosilver – a review of available data and knowledge gaps in human and environmental risk assessment, *Nanotoxicology*, 2009, 3(2): 109-138.
505. Wikipedia. 2010. The free encyclopedia. <http://en.wikipedia.org/wiki/Silver>. Date accessed Feb-2010.

506. Wilcoxon, J.P., Martin, J.E., Provencio, P. Size distributions of gold nanoclusters studied by liquid chromatography, *Langmuir*, 2000, 16(25), 9912–9920.
507. Wilcoxon, J.P., Martin, J.E., Provencio, P. Optical properties of gold and silver nanoclusters investigated by liquid chromatography, *J. Chem. Phys.*, 2001, 115(2), 998.
508. Wilcoxon, J.P., Provencio, P.P. Chemical and optical properties of CdSe and CdSe/ZnS nanocrystals investigated using high performance liquid chromatography, *J. Phys. Chem. B*, 2005, 109(28), 13461.
509. Wiley, B., Sun, Y., Mayers, B., Xia, Y. 2005. Shape-controlled synthesis of metal nanostructures: The case of silver, *Chem. Eur. J.*, 11:454-463.
510. Wiley B.J., Chen Y., McLellan, J.M., Xiong, Y., Li, Z., Ginger D., et al. 2007. Synthesis and optical properties of silver nanobars and nanorice. *Nano Lett*, 7:1032–1036.
511. WHO. World Health Organization. 2002. Silver and silver compounds: Environmental aspects. (Concise international chemical assessment document; 44). International Programme on Chemical Safety II. Series. <http://www.who.int/ipcs/publications/cicad/en/cicad44.pdf>. Accessed March 2010.
512. Wood, C., Munger, S., Galvez, F., Hogstrand, C. 1994. The physiology of silver toxicity in freshwater fish. In: Andren A, Bober T, editors. Transport, fate, and effects of silver in the environment. Proceedings of the 2nd international conference. 11-14 September 1994. Madison, WI, University of Wisconsin Sea Grant Institute. pp 109-114.
513. Wright, J.B., Lam, K., Buret, A.G., Olson, M.E., Burrell, R.E. 2002. Early healing events in a porcine model contaminated wounds: effects of nanocrystalline silver on matrix metalloproteinases, cell apoptosis, and healing. *Wound Repair Regen*. 10, 141-151.
514. Wright, J.B., Lam, K., Hansen, D., Burrell, R.E. 1999. Efficacy of topical silver against fungal burn wound pathogens. *Am. J. Infect. Control*, 27:344-350.
515. Wright, J.B., Lam, K., Burrell, R.E. 1998. Wound management in an era of increasing bacterial antibiotic resistance: A role for topical silver treatment. *Am. J. Infect. Control*, 26:572-577.
516. Wu, H., Fang, Y., Zhang, P.X. Fourier transform Raman and density functional theory studies on the adsorption behavior of p-hydroxybenzoic acid on silver nanoparticles. *J. Phys. Chem. B* 2005, 109, 21865-21867.

517. Wu, R., Hsu, S. Preparation of highly concentrated and stable suspensions of silver nanoparticles by an organic base catalyzed reduction reaction. *Mater. Res. Bull.* 2008, 43, 1276-1281.
518. Xiao, L., Takada, H., Gan, X.H., Miwa, N. 2006. The water-soluble fullerene derivative 'radical sponge' exerts cytoprotective action against UVA irradiation but not visible-light-catalyzed cytotoxicity in human skin keratinocytes. *Bioorg. Med. Chem. Lett.* 16, 1590–1595.
519. Xingcan, S., Qi, Y., Hong, L., Haigang, Y., Xiwen H. Hysteresis effects of the interaction between serum albumins and silver nanoparticles. *Sci. China Ser. B.* 2003, 46, 387-398.
520. Xiong, D., Chen, M., Li, H. 2008. Synthesis of para-sulfonatocalix[4]arene-modified silver nanoparticles as colorimetric histidine probes. *Chem. Commun.*, 880–882.
521. Xu, X., Brownlow, W.J., Kyriacou, S.V., Wan, Q., Viola, J.J. 2004. Real-time probing of membrane transport in living microbial cells using single nanoparticle optics and living cell imaging. *Biochem.*, 43:10400–10413.
522. Xu, Z., Hou, Y., Sun, S. 2007. Magnetic core/shell Fe₃O₄/Au and Fe₃O₄/Au/Ag nanoparticles with tunable plasmonic properties, *J. Am. Chem. Soc.*, 129:8698-8699.
523. Yamamoto, M., Kashiwagi, Y., Nakamoto, M. Size-controlled synthesis of monodispersed silver nanoparticles capped by long-chain alkyl carboxylates from silver carboxylate and tertiary amine. *Langmuir.* 2006, 22, 8581-8586.
524. Yan, W., Wang, R., Xu, Z., Xu, J., Lin, L., Shen, Z., Zhoua, Y. 2006. A novel, practical and green synthesis of Ag nanoparticles catalyst and its application in three-component coupling of aldehyde, alkyne, and amine. *Journal of Molecular Catalysis A: Chemical* 255: 81-85.
525. Yang, G., Li, H. Sonochemical synthesis of highly monodispersed and size controllable Ag nanoparticles in ethanol solution. *Mater. Lett.* 2008, 62, 2193-2195.
526. Yang, J., Lee, J.Y., Too, H. A general phase transfer protocol for synthesizing alkylamine-stabilized nanoparticles of noble metals. *Anal. Chem. Acta.* 2007a, 588, 34-41.
527. Yang, J., Lee, J.Y., Too, H. Core-Shell Ag-Au nanoparticles from replacement reaction in organic medium. *J. Phys. Chem. B.* 2005, 109, 19208-19212.

528. Yang, J., Lu, L., Wang, H., Shi, W., Zhang, H. Glycyl Glycine Templating synthesis of single-crystal silver nanoplates. *Cryst. Growth Des.* 2006b, 6, 2155-2159.
529. Yang, L., Shen, Y., Xie, A., Zhang, B. facile size-controlled synthesis of silver nanoparticles in UV-irradiated tungstosilicate acid solution. *J. Phys. Chem. C.* 2007c, 111, 5300-5308.
530. Yang, Y., Huang, H. A simple synthesis of silver nanoparticles in laponite suspensions. *Key Eng. Mater.* 2007, 334-335, 813-816.
531. Yang, Y., Matsubara, S., Xiong, L., Hayakawa, T., Nogami, M. Solvothermal synthesis of multiple shapes of silver nanoparticles and their SERS properties. *J. Phys. Chem. C.* 2007b, 111, 9095-9104.
532. Yin, H.Q., Langford, R, Burrell, RE. 1999. Comparative evaluation of the antimicrobial activity of Acticoat antimicrobial barrier dressing. *J. Burn Care Rehabil.* 20, 195-200.
533. Yu, H., Xu, X., Chen, X., Lu, T., Zhang, P., Jing, X. 2007. Preparation and antibacterial effects of PVA-PVP hydrogels containing silver nanoparticles, *J. Appl. Poly. Sci.*, 103:125-133.
534. Yu, D., Yam, V.W. Hydrothermal-induced assembly of colloidal silver spheres into various nanoparticles on the basis of HTAB-modified silver mirror reaction. *J. Phys. Chem. B.* 2005, 109, 5497-5503.
535. Zeiri, I., Bronk, B.V., Shabtai, Y., Eichler, J., Efrima, S. 2004. Surface enhanced Raman spectroscopy as a tool for probing specific biochemical components in bacteria. *Appl. Spectroscopy* 58, 33-40.
536. Zhang, G., Keita, B., Dolbecq, A., Mialane, P., Sécheresse, F., Miserque, F., Nadjio L. Green chemistry-type one-step synthesis of silver nanostructures based on Mo^{V} - Mo^{VI} mixed-valence polyoxometalates. *Chem. Mater.* 2007, 19, 5821-5823.
537. Zhang, J., Lakowicz, J.R. A model for DNA detection by metal-enhanced fluorescence from immobilized silver nanoparticles on solid substrate. *J. Phys. Chem. B* 2006a, 110, 2387-2392.
538. Zhang, L., Shen, Y., Xie, A., Li, S., Jin, B., Zhang, Q. One-step synthesis of monodisperse silver nanoparticles beneath vitamin E Langmuir monolayers. *J. Phys. Chem. B.* 2006b, 110, 6615-6620.

539. Zhang, W., Qiao, X., Chen, J., Chen, Q. Self-assembly and controlled synthesis of silver nanoparticles in SDS quaternary microemulsion. *Mater. Lett.* 2008, 62, 1689-1692.
540. ZhiLiang, J., Yuan, C., AiHui, L., HuiLin, T., NingLi, T., FuXin, Z. 2007. Silver nanoparticle labeled immunoresonance scattering spectral assay for trace fibrinogen. *Sci. China Ser B*, 50:345–350.

Product no.	Name of product	Name of manufacturer	Country of manufacturing	Country of distributors (abbreviation)	Order via internet (yes/no)	Matrix nanoparticles (fluid, solid, powder, coating, size)
116	NANOVER™ Cleansing Soap	GNS Nanogist	Korea		no	solid/creme
117	Nanbabies® Face Masks	Nanbabies®	USA		yes	solid
118	NANOVER™ Wet Wipes	GNS Nanogist	Korea		no	solid
119	Acticoat various wound dressings	Smith & Nephew	USA/UK	various countries worldwide	no	fluid/gel
120	Business Black Sock	AgActive	UK		yes	solid
121	Sport Ankle Sock	AgActive	UK		yes	solid
122	Sport Half Length Sock	AgActive	UK		yes	solid
123	Sports Long Sock	AgActive	UK		yes	solid
124	E47 SmartSilver™ Fibers and Fabrics	ARC Technologies®	USA	various countries worldwide	no	solid
125	X-System™ Base Layer Shirts	ARC Technologies®	USA	various countries worldwide	no	solid
126	X-System™ Hats--Balaclava, Head Cover, Hunting Cap, and Rad	ARC Technologies®	USA	various countries worldwide	no	solid
127	X-System™ Plantation Jacket	ARC Technologies®	USA	various countries worldwide	no	solid
128	X-System™ Scent Eliminating Boot Socks	ARC Technologies®	USA	various countries worldwide	no	solid
129	X-System™ Shooters Gloves	ARC Technologies®	USA	various countries worldwide	no	solid
130	SmartSilver™ and Dri-Lex® Shoe Lining	Faytex Corporation	USA		no	solid
131	Nano Silver Long Johns	Goodweaver Textiles Co. Ltd.	Taiwan		no	solid
132	Nano Silver Polo Shirt	Goodweaver Textiles Co. Ltd.	Taiwan		no	solid
133	Nano Silver Socks	Goodweaver Textiles Co. Ltd.	Taiwan		no	solid
134	Nano Silver Socks and Shoe Pads	Goodweaver Textiles Co. Ltd.	Taiwan		no	solid
135	SoleFresh™ Socks	JR Nanotech PLC	UK		yes	solid
136	Lexon Nano-Silver Sock	Lexon Nanotech Inc..	USA		? (website not found)	solid
137	Nanorama - Nano-silver sock	Lexon Nanotech, Inc	USA		? (website not found)	solid
138	Mipan® Magic Silver Nano	Mipan®	Korea		no	solid
139	Silver Nano Odor Free Rubber Gloves	Misian Co., Ltd.	Korea		? (website not found)	solid
140	Nanbabies® Outerwear	Nanbabies®	USA		yes	solid
141	Nanbabies® Personal Wear	Nanbabies®	USA		yes	solid
142	NanoHorizons® SmartSilver™	NanoHorizons®	USA		no	solid
143	SmartSilver Anti-Odor Nanotechnology Underwear	Pooghe Laundry	USA		no	solid
144	PuckSkin™ Odor Eliminator	PuckSkin™	Canada	USA, Canada, Denmark	no	solid
145	Antibacterial Silver Athletic and Lounging Socks	Sharper Image	USA	USA, Brazil, Mexico	yes	solid
146	Contour-Foam™ Silver Slippers	Sharper Image®	USA		yes	solid
147	I-Text® Silver Nano™ Anti Bacterial Polo-Shirt	United Textile Mills Co., Ltd.	Thailand		yes	solid
148	Nanbabies® Foot/Shoe Care	Nanbabies®	USA		yes	solid
149	Nanbabies® Sleeves and Braces	Nanbabies®	USA		yes	solid
150	100% cotton sheet set	AgActive	UK		yes	solid
151	Bath and Sports Towels	AgActive	UK		yes	solid
152	Benny the Bear Plush Toy	Pure Plushy	USA		yes	solid
153	Donny the Dog Plush Toy	Pure Plushy	USA		yes	solid



Office of Research
and Development (8101R)
Washington, DC 20460

Official Business
Penalty for Private Use
\$300

EPA/600/R-10/084
August 2010
www.epa.gov

Please make all necessary changes on the below label,
detach or copy, and return to the address in the upper
left-hand corner.

If you do not wish to receive these reports CHECK HERE ;
detach, or copy this cover, and return to the address in the
upper left-hand corner.

PRESORTED STANDARD
POSTAGE & FEES PAID
EPA PERMIT No. G-35



Recycled/Recyclable

Printed with vegetable-based ink on
paper that contains a minimum of
50% post-consumer fiber content
processed chlorine free